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A DEFECT IN THE SPLENIC T CELLS OF MICE BEARING A TUMOUR IN ITS EARLY STAGE OF DEVELOPMENT

by

DONNA ARLENE CHOW

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LIST OF ABBREVIATIONS

T cells	=	thymus derived lymphocytes
B cells	=	bone marrow derived lymphocytes
SRBC	=	shreep red blood cells
HRBC	=	horse red blood cells
POL	=	polymerized flagellin
SSS111	=	pneumococcal polysaccharide type 111
RFC	=	rosette forming cell
θ	=	theta
Ig	=	immunoglobulin
KLH	=	keyhole limpet hemocyanin
BSA	=	bovine serum albumin
Fe	=	horse spleen ferritin
FIB	=	human fibrinogen
RICA	=	reverse immune cytoadherence
FCS	=	foetal calf serum
HS	=	horse serum
MEM	=	Minimum Essential Medium
DMSO	=	dimethyl sulphoxide
Hgg	=	human gamma globulin
PFC	=	plaque forming cell
FCA	=	Freund's complete adjuvant
MAAF	=	water-soluble, lipid-free fraction from BCG
BCG	=	Bacillus Calmette - Guerin
BDB	=	bis-diazotized benzidine
ID	=	intradermally
IP	=	intraperitoneally
SC	=	subcutaneously

MCA	=	methylcholanthrene
MSV	=	Moloney sarcoma virus
LCM	=	lymphocytic choriomeningitis
MuLV	=	murine leukemia virus
FT	=	frozen and thawed
STE	=	soluble tumour cell extract
D	=	day
H	=	hour
L/TC	=	lymphoid cell to tumour cell ratio
$I^{125}UDR$	=	iodine^{125} labeled deoxyuridine
α-MIg	=	anti mouse immunoglobulin serum
α-θ	=	anti theta serum
α-L5178Y S	=	anti L5178Y serum produced in DBA/2 mice
6-HS	=	serum collected six hours after IP injection ofantigen
μCi	=	microcuries

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RATIONALE

This section is included in an effort to outline the reasoning which resulted in the origin and development of this project, as well as to clarify the format of this thesis.

"Immunological surveillance" postulates that new antigens, found on the surface of malignant cells, stimulate an immunological response which eventually eliminates the tumour cells. According to this theory, tumour cells which may constantly arise are eliminated before they become clinically detectable.

Although immune responses have been demonstrated against syngeneic tumours in the clinical stage of growth, the effectiveness of the surveillance mechanism has been seriously questioned. Thus, interference with effector mechanisms may explain the survival of a tumour after it is established, but it does not explain the failure of the body to control the growth at a much earlier stage of development.

This thesis is concerned with the examination of the initial response to a tumour in its very early stages of development. This effort took the form of a study of changes in splenic T and B cells.

Previous work in the laboratory (Paraskevas, et al, 1972b) demonstrated that, as a generalized phenomenon, an increase in Ig bearing spleen cells occurred six hours after stimulation by a wide variety of immunogens, particulate antigens alone or soluble antigens emulsified in FCA. The increase was due to the uptake of cytophilic Ig by T cells. This observation is fundamental to this project and posed the following initial questions:

(1) Are syngeneic tumour cells capable of inducing a six hour response?

(2) Are mice, bearing a tumour growing from a small inoculum of cells, capable of producing a six hour response?

The latter experiments were carried out in mice in which the natural progress of a spontaneously arising tumour was simulated by initiation of tumour growth with a very small inoculum of live cells. Results indicating that a T cell defect was observed in such tumour bearers posed two further questions:

(3) What is the mechanism involved in the observed defect?

(4) Is any aspect of the immune response correlated with the defect?

The data is presented in this thesis in a manner which corresponds with the logical sequence of the above questions.

ABSTRACT

An increase in Ig bearing cells in the spleen, six hours after IP administration of a variety of exogeneous antigens, has previously been described using a hybrid antibody rosette forming technique. This change was found to be due to the uptake of cytophilic complexes by T cells.

Administration of a large dose of L5178Y leukemia cells (10^7) into syngeneic DBA/2 mice has now been shown to produce a similar increase of Ig bearing cells, which amounts to 20% above the normal. In contrast, a low dose of tumour cells (100) produced no significant increase, but still kills 95% of mice injected. Animals bearing tumours growing from an inoculum of 100 live cells were able to produce a six hour increase of Ig bearing cells, when challenged with 10^7 frozen and thawed L5178Y cells between one and seven days after inoculation. Such animals failed also to show the six hour increase after a challenge with an unrelated antigen (5×10^8 HRBC).

These results were reproduced by replacing the inoculation of cells with treatment of animals with a tumour cell extract given for four consecutive days. Similar treatment with a soluble deaggregated preparation of Hgg was capable of producing the same results.

These findings show that continual low level stimulus such as may occur during early growth of a tumour from a low number of cells alters the properties of the T cell surface. The production of this T cell defect was found to correlate with a decrease in in vitro growth inhibiting ability of spleen cells from specifically sensitized mice.

INTRODUCTION AND LITERATURE REVIEW

For hundreds of years man has sought to understand and control the balance that exists between disease and immunity. In the fifteenth century inoculation with a small pox pustule or inhalation of dried small pox crust was used to induce a prophylactic small pox infection. Although the possibility of generalized disease was at risk, the beneficial effect of immunity to a disease by prior exposure to these agents was recognized. At the end of the eighteenth century Edward Jenner was able to demonstrate that immunity against small pox could be conferred by 'vaccination' with cow-pox, a related agent, and he proposed presenting his findings to the Royal Society in 1798 (Lechevalier and Solotorovsky, 1965).

Almost one hundred years passed before the first approach was made toward finding a general method of immunizing against infectious disease. Louis Pasteur (1880) succeeded in protecting chickens against chicken cholera by inoculation with an attenuated form of the virulent agent; in this case old cultures of the micro-organism were now called Pasteur aviseptica. Subsequently he succeeded in attenuating the virulence of anthrax bacillus for immunization of sheep and goats and he produced a vaccine for the protection of humans and dogs against rabies.

Around this time, the recognition of the universal existence of bacteria, some of which were pathogenic, suggested that a normal individual must possess a natural form of defence against bacteria. Among other observations, the finding that freshly drawn blood could kill some types of bacteria led to the belief that special agents in the blood were able to defend against bacterial infection. The classical controversy between

humoural and cellular immunity arose with the concurrent development of opposing theories which postulated antibodies and white blood cells respectively as the blood born beneficial agent.

At the turn of the twentieth century, theories concerning opsonins brought recognition of the complexity of the cell types and humoural factors involved in the immune response (Wright and Douglas, 1903). Bordet's (1898) recognition of the immune lysis of foreign red cells and Landsteiner's (1901) discovery of the ABO blood groups extended the field of immunity against infection to recognition and defense against anything 'foreign'.

The Second World War provided a major stimulus for rapid technical advance in immunology in the years 1940-1955. As a result of the practical importance of blood transfusions and plastic surgery in dealing with war casualties, the basic concept of the 'uniqueness of the individual' was emphasized over and over again. The importance of examining the distinction of 'self' from 'non-self' (or 'foreign') was evident.

Of particular significance to the study of malignant disease was the demonstration in the 1950's by several investigators that tumours arising in inbred strains of mice carried new antigens (Foley, 1953; Prehn and Main, 1957). This led to a new interpretation of the relationship between immunity and cancer.

I IMMUNE SURVEILLANCE: AN HYPOTHESIS

Thomas (1959) postulated that 'it was a universal requirement of multicellular organisms to preserve uniformity of cell type and that

the phenomenon of homograft rejection would turn out to represent a primary mechanism for natural defense against neoplasia.' This concept of immune surveillance was elaborated by Burnet (1963, 1964, 1967) through the 1960's. Basically, it is thought that throughout the lifetime of long lived animals, aberrant cells with proliferative potential arise and that a mechanism of immunological character is responsible for the regulation of these cells in such a way that the continuation of the species is assured.

The data presented herein falls under the auspices of immune surveillance as this thesis is concerned with the study of the initial immune response to a tumour, growing in mice, from a small inoculum of cells.

A prerequisite for this study is a knowledge of the immunological agents potentially involved, and their interactions. This comprises the actions of immunocytes and accessory agents, a brief outline of which follows.

II CELLS INVOLVED IN IMMUNOLOGICAL REACTIONS

i. IMMUNOCYTES

1. Functional Distinction of T and B Cells

The immunologically competent cell or immunocyte denotes those cells which are susceptible to specific stimulation by contact with the appropriate antigenic determinant (Burnet, 1969). The main response of this interaction either results in proliferation with limited differentiation to sensitized cells for cell-mediated immunity or proliferation with extensive differentiation to plasma cells which produce and secrete

antibody (Miller and Mitchell, 1969).

Thus immunologically active lymphocytes are divided into two cell types by their functions.

a. B cells, bone marrow cells or bone marrow derived cells are those small lymphocytes which give rise to antibody producing plasma cells.

b. T cells, thymus cells or thymus derived cells are those small lymphocytes which are responsible for cell mediated immune reactions such as delayed hypersensitivity, homograft rejection and the graft-versus-host reaction.

The efforts of many investigators have contributed to further distinguish these two cell types which both originate as stem cells in the bone marrow. The bone marrow is considered to contain the precursors of all lymphoid cells as it is capable of repopulating the entire lymphoid system and thus 'rescuing' an animal after lethal X-irradiation (Ford, et al, 1956; Ford and Micklem, 1963; Micklem, et al, 1966).

a. B cells

Separate pathways of differentiation have been suggested for the two basic types of immunocytes. Experiments utilizing the lymphoid system of the chicken have shown that the B cells pass through the bursa of Fabricius and then to the secondary or peripheral organs which consist of the lymph nodes, spleen, appendix, solitary follicles and Peyer's patches.

The bursa of Fabricius is a gut-associated organ which develops as an epithelial sac budding from the dorsal region of the cloaca.

Beginning at the fifteenth day after hatching lymphocytes appear and a multilobular lymphoid organ develops (Ackerman and Knouff, 1964).

The observation that this organ atrophies at sexual maturity, presumably in response to hormonal stimulation, led to experiments which demonstrated that hormonal treatment of the embryo or surgical bursectomy immediately upon hatching resulted in the loss of the birds' ability to produce antibody against antigens, without reducing its ability to reject tissue allografts (Glick, et al, 1956). The bursa is not an antibody producing organ (Dent and Good, 1965) but probably contains antibody forming cell precursors as transfer of bursal cells to irradiated birds gave them the ability to produce specific antibody in reponse to antigen challenge (Gilmour, et al, 1970). Thus the bursa is thought to be a primary lymphoid organ responsible for the differentiation of bone marrow cells into potential antibody-producing immunocytes.

The mammalian analogue of the avian bursa of Fabricius has not yet been found. Present opinion concerning it is divided between two main views:

a. The differentiation into antibody forming precursor cells is controlled by a factor produced by 'gut associated lymphoid tissue' (Miller and Mitchell, 1969), or

b. The bursa and its mammalian counterpart (possibly Peyer's patches) receive stem cells directly from the bone marrow and is responsible for their differentiation to potential antibody producing immunocytes (Cooper, et al, 1966; Good and Finstad, 1971).

Ford (1966) has also suggested that the bone marrow constitutes the bursal equivalent and is not only the ultimate source of stem cells, but

also the environment required for differentiation into precursors of antibody forming cells.

Neonatal bursectomy of chickens revealed a deficiency of germinal centres and plasma cells in secondary lymphoid organs (Cooper, et al, 1966). Germinal centres are located in the cortex of the lymph nodes and plasma cells are found in the medulla of lymph nodes and in the red pulp of spleens of normal animals.

b. T cells

The differentiation pathway followed by stem cells which seed into the thymus produces thymus cells. Those cells which depend upon thymus influence for their maturation (Osoba and Miller, 1964) or which are dependent upon the thymus for their production and subsequent seeding into the periphery are termed thymus dependent lymphocytes.

The thymus is a primary lymphoid organ which arises from the epithelium of the third and fourth pharyngeal pouches (Venzke, 1952; Hammond, 1954). Electron microscopic studies reveal that it consists of an epithelial organ densely honeycombed with packets of proliferating lymphocytes in the cortex, while the medulla is irregularly infiltrated with lymphocytes and other cells mostly associated with blood vessels (Clark, 1963). Studies of animals with radiolabelled thymuses and of chimeric or parabiotic animals having one cell population distinguished by a marker chromosome led to the basic finding that in the thymus there is a rapid turnover of cells which apart from proliferation, destruction and seeding into the periphery involves a relatively slow but continuous entry of new stem cells from extra-thymic sources (Weissman, 1967; Davies, 1969; Ford, 1966).

Unlike the lymphocytes of the spleen and lymph node, thymocytes were demonstrated to be generally not immunocompetent and any activity present was thought probably to be due to adventitious cells (van Furth, et al, 1966; Thorbecke and Cohen, 1964).

Neonatal thymectomy, a technique established by Miller and Good in the 1960's was shown to result in the generalized inability to produce cell mediated immune reactions and the inability to produce antibody to certain antigens (Miller,1963,1964;Miller et al,1962;Archer et al,1964).As thymectomy had little effect on adult animals, the demonstration was clear; the thymus was important, in the early days of life, for development of complete immune competence. Thus the thymus is considered a primary lymphoid organ, not itself engaged in the direct response to antigen, but that cells differentiated in the presence of a thymus subsequently play a part in the immunological function of the secondary lymphoid organs.

The thymus dependent areas of the lymphoid organs, as demonstrated by the homing patterns of radiolabelled thymus cells injected after thymectomy, were shown to be the area around the central arterioles in the spleen and in the mid and deep cortical areas of the lymph nodes (Parrott, et at, 1966a). Similar results were obtained by observing that these "thymus dependent" areas were depleted of lymphocytes following thymectomy, lethal irradiation and reconsitution with bone marrow (Parrott, et al, 1966b).

Thus it can be recognized that there are at least two types of cells involved in the immune response and they can be distinguished by their functional differences.

Further studies of these cell populations has led to the development of methods of distinguishing them on the basis of their surface characteristics. Although a number of surface markers have been used to characterize T and B cells, the following description will include only those markers pertinent to this study.

2. Structural Distinction of T and B Cells

a. B cells

Immunoglobulin:

A variety of techniques have been used to demonstrate that B cells have immunoglobulin molecules (Ig) on their surface. The most direct tests are those which involve labelled anti-Ig. Thus fluorescein or radioactively labelled anti-Ig have been used to show that murine cells, morphologically described as lymphocytes, bear Ig on their surface (Raff, et al, 1970; Perkins, et al, 1972). Further, the cells which stain positively consist mainly of bone marrow cells (Raff, 1970a); Bankhurst and Warner, 1971; Unanue, et al, 1971; Rabellino, et al, 1972; Perkins, et al, 1971), although under some conditions it is possible to detect T cells (Bankhurst, et al, 1971; Nossal, et al, 1972; Marchalonis, et al, 1972).

Similar observations have been made in other species including humans (Pernis, et al, 1970; Davie, et al, 1971; Bankhurst, et al, 1972; Fröland, et al, 1971).

Cell surface immunoglobulin has also been detected on a proportion of human and rabbit cells by the mixed agglutination technique. This involves the combination of anti-Ig with lymphocytes and Ig-coated erythrocytes to produce rosettes of red cells about the lymphocytes

(Coombs, et al, 1969; Chalmers, et al, 1959).

Another rosette forming technique, the reverse immune cytoadherance technique (RICA) has been used to demonstrate Ig on the surface of a proportion of cells of lymphoid organs and bone marrow from mice, guinea pigs and humans (Paraskevas, et al, 1970, 1971a, 1971b). This technique employs 5S hybrid antibody to link Ig carrying lymphocytes and protein coated erythrocytes. Thymocytes from all three species where shown to lack surface-associated-globulin by this technique (Paraskevas, et al, 1971b). As well, plasmacytoma cells and normal mature plasma cells lack surface associated immunoglobulin (Paraskevas, et al, 1970, 1971a, 1971b) as demonstrated also by others (Pernis, et al, 1970; Perkins, et al, 1972), although in disagreement with Rabellino and colleagues (1972). Using highly specific hybrid antibodies, the majority of immunoglobulin carrying mouse spleen cells were shown to be pluripotential (Paraskevas, et al, 1971b; Lee, et al, 1971) in agreement with the observations of other investigators (Bankhurst and Warner, 1971; Bankhurst, et al, 1971; Nossal, et al, 1972).

Other surface markers including the 'Fc receptor' for immunoglobulin (Paraskevas, et al, 1971b, 1972a; Basten, et al, 1972a, 1972b) and the complement receptor (Lay and Nussenzweig, 1968; Bianco, et al, 1970; Bianco and Nussenzweig, 1971) have been demonstrated on the B cell surface. These receptors have been shown to exist separately from Ig on the same cell.

b. T cells

Immunoglobulin:

Although it is generally accepted that B lymphocytes express a high density of surface Ig, the preceding description of the existence of B cell

surface immunoglobulin noted that under certain conditions it has been possible to detect Ig on the surface of T cells (Bankhurst, et al, 1971; Nossal, et al, 1972). The presence of surface Ig on T cells is controversial. Thus in mice T cell Ig is considered to be undetectable by some authors (Vitetta, et al, 1972; Raff, 1970a, Crone, et al, 1972). Others, in contrast, report that T lymphocytes express as much surface Ig as B cells (Marchalonis, et al, 1972). Several groups have demonstrated surface Ig on 'activated' T cells, T cells from stimulated animals (Bankhurst, et al, 1971; Lee and Paraskevas, 1972; Marchanolis, et al, 1972; Pernis, et al, 1974; Hudson, et al, 1974). Current investigation suggests that T cell Ig occurs on activated T cells and is of cytophilic nature (Hudson, et al, 1974; Lee and Paraskevas, 1974), obtained passively from another cell, possibly a B cell (Hudson, et al, 1974).

Theta (Θ) Determinant:

Probably the most widely used T cell surface marker is the theta antigen described by Reif and Allen (1963). Employing a dye-exclusion technique, they found that an antiserum raised against an alloantigen on thymocytes reacted in a complement dependent manner to kill not only all thymocytes, but also a proportion of the cells in the secondary lymphoid organs of the same animals. They later discovered that this antigen was under the control of a single locus with two alleles: AKR-Θ in AKR, RF and a few related substrains and C3H-Θ in most other inbred strains of mice (Reif and Allen, 1966).

The anti-Θ serum prepared by immunization of AKR with C3H thymocytes

or vice versa has been shown to detect only Θ activity (Raff, 1971). It has been used to demonstrate that thymocytes have more Θ antigen on their surface than do peripheral lymphocytes (Aoki, et al, 1969). While all thymocytes are killed by this serum, only a proportion of spleen and lymph node lymphocytes are killed (Raff, 1969) and a number of authors have demonstrated the marked decrease in Θ bearing cells in the peripheral lymphoid organs of animals depleted of T cells by neonatal thymectomy (Schlesinger and Yron, 1970; Raff and Wortis, 1970), adult thymectomy, lethal irradiation and reconstitution with bone marrow (Raff and Wortis, 1970), ALS treatment (Schlesinger and Yron, 1969; Raff, 1969) or in nude mice (Raff and Wortis, 1970). That such treatment of mice depletes the thymus dependent areas of secondary lymphoid organs has been described previously.

These surface markers which distinguish T and B cells have been used in the study of the involvement of these cell populations in the immune response.

ii. PHAGOCYTIC CELLS

Next to immunocytes, the macrophage is probably the cell most often implicated in the immune response. The macrophage is distinguished from immunocytes by its ability to engulf and digest foreign particles (Metchnikoff, 1892). They can be separated from immunocytes to a large extent on a functional basis as they adhere to glass (Nelson, 1969), while lymphocytes, for the most part, do not attach to glass.

Cells of the monocyte-macrophage system were relegated to an ancillary role in immunological process when the plasma cell series was recognized

as the major producer of antibody (Fagraeus, 1948). Nevertheless, it must be noted that cells of this series:

a) are present in organs which produce antibody (Nelson, 1969)

b) are found to contain antigen after a suitable injection of radio-labelled antigen (Nossal and Abbot, 1968)

c) have been shown in in vitro incubation with antigen to give rise to a material with greater immunogenic power than the original antigen (Fishman, 1961; Fishman and Adler, 1963; Gallily and Feldman, 1966; Askonas and Rhodes, 1965).

Further discussion of the immune involvement of the macrophage will be presented in the next section dealing with the actions of immunocytes in the immune response.

III INTERACTION OF IMMUNOCYTES IN IMMUNE PHENOMENA

The division of antigen reactive lymphocytes into two cell types has been described previously. Their separation was originally made on the basis of the functionally different roles which they performed in immune phenomena. As knowledge about the immune system expands, it is becoming evident that these cells also interact together, often in a synergistic way and probably with ancillary cells as well to produce the many manifestations of immunity.

Probably the most common measure of immune response has been the production of specific immunoglobulin.

i. HUMOURAL IMMUNE RESPONSE

Exposure of the immune system to antigen results in a series of complex interactions some of which result in the production of antibody.

Although it is recognized that the cells which secrete antibody are members of the lymphoid system, the relationship of the cells which respond to the antigen to those which produce the antibody is not defined.

1. Primary Response

Although it was possible through neonatal thymectomy to decrease the cell mediated immune responses, it was found that antibody production to antigens was also severely depressed with some notable exceptions, including pneumococcal polysaccharide (SSSIII) and haemocyanin (Humphrey, et al, 1964). There is evidence that both of these antigens are thymus-independent (Fahey, et al, 1965; Howard, et al, 1971), although the degree of thymus dependency of KLH is controversial and some work suggests that it is thymus-dependent (Unanue, 1970; Kruger and Gershon, 1971). The implication of the interference of thymectomy in the humoural response was that T cells were also required for the production of antibody to most antigens. Subsequently, Mitchell and Miller(1968) and Nossal and colleagues (1968) employing anti-H-2 isoantisera and the T6 chromosome marker respectively to identify cell populations transferred into X-irradiated recipients, showed that the interaction of thymus dependent cells was necessary to allow the appearance of antibody producing cells. The cells producing antibody were identified as non-thymic in origin. The need for viable syngeneic thymus cells, rather than X-irradiated or sonicated cells was later demonstrated (Claman, et al, 1968).

In vitro interaction of T and B cells has been studied by employing tissue culture techniques (Mishell and Dutton, 1967; Marbrook, 1967). Once again it was demonstrated that both T and B cells were required for production of antibody.

2. Secondary Response

The need for the interaction between T and B cells for the production of memory, characterized by an enhanced secondary response, has also been described. A double transfer system was employed to demonstrate that thymus cells, specifically activated in the primary host, were able to interact with normal bone marrow cells to produce a significant response (Shearer and Cudkowicz, 1969). Anti-Θ serum and complement treatment or specific elimination of T cells from immune spleens resulted in the loss of ability of such cells to transfer a secondary response to a thymus dependent antigen (Mitchell, et al, 1972; Takahashi, et al, 1970). Similarly in vitro, the depression of immune response of normal mouse spleen cells due to anti-Θ serum plus complement treatment was returned to normal levels by the addition of educated thymus cells (Chan, et al, 1970).

B cell memory was demonstrated by using as donors of primed or unprimed cells, congenic mice differing only at the loci coding for immunoglobulin type. All of the IgG anti-SRBC plaque forming cells found in the adoptively transferred secondary response were of the primed cell allotype (Jacobson, et al, 1970). Elimination of B cells from an immune spleen results in significant depression of the secondary response (Cheers, et al, 1971). The secondary response of primed T cells in irradiated recipients was enhanced by the addition of specifically primed spleen cells and unchanged by normal B cells (Miller and Sprent, 1971a).

Thus both T and B cells are capable of expressing specific and probably distinct memory and both T and B cells are required for the production of humoural responses to most antigens.

3. The 'Carrier Effect'

Mitchison (1969) demonstrated the 'carrier effect' using the adoptive transfer of separately primed cell populations into irradiated recipients. He was able to show that different cells were responding to the carrier rather than the haptenic determinants of the stimulating antigen. The carrier specific cells were revealed to be T cells by their sensitivity to anti-θ serum and complement (Raff, 1970b).

4. Role of Macrophages

In recent years it became recognized that the functional role of the macrophage in immune responses was unaffected by exposure to X-irradiation (Roseman, 1969). Subsequently, it has been demonstrated that macrophages were essential for the production of anti-hapten antibody responses (Shortman, et al, 1970; Feldman and Palmer, 1971; Shortman and Palmer, 1971) but not for antibody responses to thymus-independent antigens (Feldman, 1972; Feldman and Palmer, 1971; Shortman and Palmer, 1971).

ii. CELL MEDIATED RESPONSE

The primary role of thymus derived cells in cell mediated immune response has been demonstrated by the drastic depression of those responses in animals deprived of thymus cells, as previously described.

The involvement of T cells in cell mediated immunity in vitro has been demonstrated by the inhibition of the cytotoxicity of sensitized spleen cells following treatment with anti-θ serum and complement (Cerrottini, et al, 1970).

Some evidence for interaction of T and B cells in a cell mediated response has been reported by Globerson and Auerbach (1967). They

observed that the in vitro graft-versus-host reaction occurred only in the presence of both bone marrow cells and thymocytes (or humoural factors released from thymocytes) but the interaction between T cells has been suggested as more typical of cell mediated immune reactions (Asofsky, et al, 1971).

iii. IMMUNOLOGICAL TOLERANCE

The classical definition of immunological tolerance denotes the central failure of responsiveness attributable to the selective elimination or inactivation of clones of lymphocytes (Howard and Mitchison, 1975).

The ability to render both B and T cells tolerant, as measured by specific antibody production, has been demonstrated. The dose of tolerogen required and the kinetics of induction were found to be quite different for the two cell types (Chiller, et al, 1971). Basically, T cells are tolerized rapidly and for a long period of time after exposure to relatively small doses of antigen, whereas B cells require larger amounts of antigen, a longer time to develop tolerance and recover more rapidly than do T cells.

Neither bone marrow or thymus from unresponsive donors was capable of demonstrating synergism when transferred with their normal counterpart into lethally irradiated recipients for the production of antibody (Chiller, et al, 1970). Using cell transfer experiments, Chiller and Weigle (1973) further demonstrated that a population of B cells in the spleen entered a state of unresponsiveness more quickly than a population of B cells in the bone marrow, possibly reflecting the state of maturity of B cells in the two organs.

Elkins (1973), using a transplantation system to study the abrogation of tolerance by transfer of normal lymphocytes, found that thymectomy of the tolerized recipient prevented the return of the ability of the host-type lymphocytes to participate in a specific graft-versus-host reaction.

Thus it is evident that the production of antibody requires the interaction of immunologically sound T and B cells and that at least functional T cells are required for the occurrence of the graft-versus-host reaction.

iv. ANTIGENIC COMPETITION

Radovich and Talmage (1967), examining the phenomenon of 'antigenic competition', observed that the injection of heterologous erythrocytes, preceded 1-10 days by a similar injection of a different heterologous erythrocyte, produced a markedly inhibited antibody response to the second injection. This inhibition was maximum when the antigens were given 4 days apart.

Other investigators have shown that pretreatment with large daily doses of soluble proteins for 8-10 days (Liacopoulos and Neveu, 1964) or simultaneous injection of two antigens (Neveu, 1964) led to inhibition of induction of delayed hypersensitivity to the challenging antigen. Similarly, the repeated injection of large doses of antigen led to a significant prolongation of homograft survival (Terino, et al, 1964; Liacopoulos, 1965).

Subsequently, Gershon and Kondo (1971) demonstrated that the inhibition of the production of specific antibody following antigenic challenge in animals previously exposed to an unrelated antigen is a

thymus-dependent process.

Thus interactions among thymus derived cells, bone marrow derived cells and possibly accessory cells are required for the production of many immune phenomena. The next section is an outline of a number of models which have been suggested for the mechanism which initiates immune responses.

IV PROPOSED MECHANISMS OF INDUCTION OF IMMUNE RESPONSE

i. INTERACTIONS OF IMMUNOCYTES AND ANTIGEN

1. Antigen Binding By Cells

Basically, all of the models, at some point, depend upon the recognition of antigen by antigen sensitive cells. Exposure of the immune system to antigen is considered to result in the activation of immunocompetent or antigen sensitive cells through binding of antigen to these cells. The information concerning the binding of antigen to immunocytes has been obtained through surface localization and detection of radioactively or fluorescently labelled antigens or antigens such as enzymes or bacteria. Briefly, the results suggest that a very small proportion of cells are involved in binding any individual antigen. These cells, morphologically distinguished as lymphocytes, are considered to bind antigen in a specific, unipotential manner (Sulitzeanu and Naor, 1969; Naor and Sulitzeanu, 1967; Byrt and Ada, 1969).

The failure of most preparations of thymus cells to bind antigen compared with spleen cells suggests that only B cells bind antigen (Byrt and Ada, 1969; Humphrey and Keller, 1970). Nevertheless, some evidence exists that T cells have receptors for antigen. Raff (1971) found that a proportion of antigen binding cells for thymus dependent

antigens were sensitive to anti-Θ serum plus complement and Dwyer and colleagues (1972), were able to demonstrate antigen binding cells in foetal and mature thymocytes.

The demonstration that antigen binding cells play a functional role has been carried out employing antigen coated columns (Wigzell and Andersson, 1969; Wigzell and Mäkelä, 1970) or the antigen suicide technique (Ada and Byrt, 1969; Basten, et al, 1971; Golan and Borel, 1972) to remove antigen binding cells. Immune depletion of cells with the ability to bind to a specific antigen results in the specific loss or reduction in the immune reactivity of the resultant cell population. Thus the specific immunological capacity of antibody forming cells, memory cells, potential antibody forming cells (Wigzell and Mäkelä, 1970) as well as immunologically reactive cells in the bone marrow (Singhal and Wigzell, 1969) is revealed by their ability to bind antigen.

2. Mechanism of Binding

The clonal selection theory (Burnet, 1959), postulated that antigen reactive cells are exquisitely specific in their capacity to react with, and subsequently develop into a clone of antibody producing cells all secreting antibody specific for, a given antigen. Thus binding of antigen is thought to occur through specific receptors on the cell surface. Immunoglobulin, whch has been detected on B cells, and under certain conditions on T cell surfaces, as previously described, is considered to be eminently capable of providing the exquisite specificity required of these membrane receptors.

Experiments designed to examine the relationship of antigen receptors and surface Ig revealed that pretreatment of lymphocytes with

anti-Ig inhibited the binding of antigen to cells using both the antigen suicide and antigen coated column techniques (Byrt and Ada, 1969; Walters and Wigzell, 1970). The inhibition of both in vitro primary and secondary responses of cells treated with sera raised against Ig or Ig fragments demonstrated that the functional capacity of cells treated with anti Ig was severely reduced (Mitchison, 1967; Warner, et al, 1970).

Injection of anti-Ig agents in vivo resulted in a decrease in the production of Ig carrying the determinants against which the antisera were made(Manning and Jutila, 1972a, 1972b; Herzenberg, et al, 1967).

Some investigators have been successful in demonstrating that cell mediated immune reactions, graft-versus-host and delayed hypersensitivity are also susceptible to inhibition by treatment of lymphoid cells with anti-Ig, fragments of anti-Ig or antisera raised against different components of Ig (Mason and Warner, 1970; Riethmüller, et al, 1971; Cole and Maki, 1971). It must be noted, however, that other efforts to inhibit cell mediated reactions with anti-Ig sera have failed (Sternberg, 1970; Ivanyi, et al, 1970).

The binding of antigen and the production of immune responses initiated by antigen could be inhibited sterically by anti-Ig agents bound to adjacent receptors on the cell surface. The demonstration of inhibited binding of many different antigens and the observation, although only made in the rabbit system, that the interaction of anti-Ig with the cell surface, like antigen binding, results in cell transformation and mitosis (Daguillard and Richter, 1970; Sell, et al, 1965)

suggests that the receptor for antigen and surface Ig are identical.

Thus both B and T cells probably display surface immunoglobulin which can be receptors for antigen, but the density of Ig on the surface of B cells is likely much greater than on T cells (Bankhurst, et al, 1971; Nossal, et al, 1972). Possibly only activated T cells carry Ig as previously described.

The following models of immunocyte interaction suggest the wide range of mechanisms which may be capable of explaining the induction of observed immune phenomena.

ii. INTERACTION OF IMMUNOCYTES FOR PRODUCTION OF HUMOURAL RESPONSE

1. 'Antigen-focusing' Model

Mitchison (1969) and colleagues (1971) have proposed that T cells, which comprise the largest proportion of circulating lymphocytes (Miller and Sprent, 1971b) are responsible for the specific concentration and transportation of antigen for presentation to B cells. Taylor and Iverson (1971) proposed a modification of this model in which the T cell surface receptor for antigen secreted by the T cell acts via macrophages rather than directly with B cells to provide the appropriate trigger. Feldman and Nossal (1972) have subsequently suggested that activated T cells secrete monomeric IgM-antigen complexes which attach to macrophage surfaces where the antigen is then properly oriented for presentation to B cells.

2. 'Minimal Model'

A 'minimal model' for T-B cell interaction has been suggested by Bretscher and Cohn (1968, 1970) and reviewed by Bretscher (1972). This mechanism assumes that the binding of antigen to a B cell receptor induces

a tolerogenic signal unless T cell derived associative antibody is also present as part of the antigen-receptor complex to initiate B cell stimulation for antibody production.

3. T Cell Mediator Model

A third and possibly least restrictive approach to the interaction of T and B cells is through the action of soluble mediators. The reaction of B cell and antigen requires also the interaction of a T cell mediator whether specific or non specific to lead to antibody production (Dutton, et al, 1971; Miller and Mitchell, 1969; Katz and Benacerraf, 1972). In this regard lymphocytes are known to release a number of physiologically active soluble factors, among them blastogenic factors, lymphotoxin, and macrophage migration inhibiting factor (MIF) (David, 1968).

The allogeneic reaction, which occurs when lymphoid cells of different allotypes are exposed to each other in vivo has been observed to enhance antibody production. Katz and Benacerraf (1972) have suggested that although this enhancement could be due to a general proliferation of host T cells, it is most likely that rapidly acting, short lived, non-specific soluble factors are responsible for assisting antibody forming cell precursors.

A number of investigators have found that cell free supernates obtained from incubation of allogeneic spleen cells (Dutton, et al, 1971; Britton, 1972;Schimple and Wecker, 1972) or thymus cell cultures (Doria, et al, 1972; Hunter and Kettman, 1973) were able to restore or enhance the ability of T deprived lymphoid cell populations to produce antigen specific antibody. Supernates from thymus cell cultures

have also been shown capable of restoring the ability of B cells to respond to a T cell mitogen (Andersson, et al, 1972).

iii. INTERACTION OF IMMUNOCYTES FOR PRODUCTION OF CELL MEDIATED RESPONSE

The importance of cell interactions among T and B lymphoid cells in the development of humoural immune responses against certain antigens has been well described (Miller and Mitchell, 1969; Claman and Chaperon, 1969). In contrast cellular immunity is considered to be mediated by T lymphocytes possibly without an integral involvement of B cells. In keeping with this Parish (1972) has proposed an overall inverse relationship of cellular and humoural responses. He suggests that the binding of antigen by T cells produces proliferation of cells for cell mediated immunity and helper function. Binding of antigen by B cells produces B cell tolerance. The bridging of T and B cells leads to antibody production and tolerance of cell mediated immunity.

There is evidence to support the concept that a functional heterogeneity exists among T lymphocytes (Stobo, et al, 1973; Segal, et al, 1972) and as previously mentioned, Asofsky and colleagues (1971) suggested that T_1-T_2 cell interactions occur during initiation of graft-versus-host responses. Wagner and colleagues (1973) have speculated that functionally distinct T cell populations may be stimulated be different 'antigens' in the allogeneic cytotoxic responses. Precursors of 'helper' cells respond mainly against allogeneic lymphocyte defined (LD) differences, divide and produce soluble mediators which amplify the response of 'killer' cells. The 'killer' cell precursors respond against serologically defined (SD) H-2 antigens, divide and differentiate into mature cytotoxic effector cells. Thus the range of interactions is wide, encompassing

all of those possible for T-B collaboration including lymphocyte-activating factors, macrophage-activating factors and lymphocyte mitogens.

It is evident that the immune response, the host's reaction to anything 'foreign' is composed of many and complex processes. The characterization of the immune response to tumours is being actively pursued on a number of fronts and the following section is an outline of results gathered from recent investigations in this area.

V THE NATURE OF THE IMMUNE RESPONSE TO SYNGENEIC TUMOURS

The description of the immune response to tumours is presented under the basic headings of cell mediated and humoural responses, although interaction of the two occurs as well.

i. CELL MEDIATED RESPONSES

1. In Vitro

a. Cytotoxic T cells

Recently evidence has begun to accumulate which demonstrates the participation of T cells in in vitro cell mediated cytotoxic reactions against syngeneic tumour cells. Wagner and Röllinghoff (1973) demonstrated the formation of cytotoxic lymphocytes in in vitro cultures of mouse thymus cells with irradiated syngeneic plasmacytoma cells.

Leclerc and co-workers (1973), studying the nature of cytotoxic cells detected in spleen cells from mice bearing Moloney sarcoma virus-induced tumours, found that treatment of immune spleen cells with anti-Θ serum and complement abrogated their cytotoxic activity as measured by the Cr^{51} release assay. Removal of B cells or addition of anti-immunoglobulin to the test system had no effect. A sharp decline in cytotoxic lymphocytes

was found as tumour growth proceeded (Leclerc, et al, 1972). Lamon and colleagues (1973a) studying the same system using the microplate assay method, found cytotoxic T cells in spleens and lymph nodes taken just prior to tumour development and soon after regression. A non-T-cell-mediated activity was also detected. Similar observations were made using spleen cells from mice bearing syngeneic methylcholanthrene-induced sarcomas (Kearney, et al, 1974). These mice exhibited concomitant immunity and their spleen cells were specifically cytotoxic to cultured tumour cells in a micro-cytotoxic assay (Hellström and Hellström, 1970).

The presence of T and non-T effector cells has also been suggested by the studies of Perlman and colleagues (1972a), on cytotoxicity of blood lymphocytes from the bladder of carcinoma-bearing patients.

b. Antibody dependent cell mediated cytotoxicity

Conflicting results have been produced by in vitro studies of the effect of sera from tumour-bearing mice on the in vitro activity of lymphoid cells. Pretreatment of target cells with a large amount of antiserum often produced an increase in lymphocyte cytotoxicity, while lower concentrations of the same antiserum produced inhibition (Skurzak, et al, 1972). In contrast, Pollack and colleagues (1972) demonstrated antibody-dependent lysis by normal lymphocytes using much less antiserum than that required for inhibition of immune lymphocytes.

Sera from mice which had rejected primary MSV-induced tumours were found to be active in an assay for antibody dependent cell mediated cytotoxicity (Harada, et al, 1973). Similarly, sera from rats which had rejected their tumours (C58NT)D were found to contain lymphocyte dependent antibodies (Ortiz de Landazuri, et al, 1974).

c. Macrophage cytotoxicity

Macrophages have been observed to exhibit both specific and non-specific cytotoxic behaviour.

Specific:

The in vitro interaction of macrophages from immune mice and tumour cells has been examined by Granger and Weiser (1964, 1966). They found that tumour cells were killed in an immunologically specific manner requiring metabolic integrity of macrophages and adherence of macrophages to the tumour cells.

Peritoneal cells but not spleen cells from mice immunized with X-irradiated syngeneic lymphoma cells were able to specifically inhibit the growth of the same tumour cells in vitro (Evans and Alexander, 1970, 1972a, 1972b, 1972c). Normal macrophages became specifically cytotoxic after incubation with either lymphoid cells from repeatedly immunized mice or cell-free supernates from cultures consisting of in vivo or in vitro sensitized spleen cells and specific target cells. A factor produced by immune T cells appears to be responsible for this effect (Evans, et al, 1972).

Non-Specific:

Peritoneal macrophages from mice immunized by two injections of allogeneic lymphoma cells could not only inhibit tumour growth specifically in vitro but after 4 hours' incubation with specific target cells acquired the ability to inhibit the growth of susceptible target cells in a non-specific way (Evans and Alexander, 1972a).

Normal macrophages were shown to acquire non-specific cytotoxicity after exposure in vitro to various agents such as endotoxin, double-

stranded RNA isolated from fungal viruses or polyI.polyC (Alexander and Evans, 1971). Thus sensitized or "armed" macrophages once "activated" by contact with specific antigen appear to be capable of killing in a non-specific manner.

Hibbs, Lambert and Remington (1972), have demonstrated that macrophages from mice infected with Toxoplasma were capable of killing allogeneic and syngeneic tumour cells in an in vitro culture system. In this case persisting infection may be responsible for "activating" macrophages for non-specific killing.

2. In Vivo

A few studies have been carried out which were designed to reveal the nature of lymphoid cells responsible for the transfer of adoptive tumour immunity. Allison (1972) found that transfer of syngeneic lymphoid cells from specifically immunized donors prevented the development of tumours in adult-thymectomized, anti-lymphocyte serum treated mice infected with polyoma virus. Pretreatment of the lymphoid cells with anti-θ serum and complement abrogated their ability to transfer adoptive immunity. In a study by Rouse and colleagues (1972), treatment of spleen cells from mice immunized against a syngeneic plasma tumour, with anti-θ serum and complement abolished their ability to inhibit tumour growth in sublethally irradiated mice. Lymphoid cells from mice immunized with syngeneic cells which were transformed by papovirus SV40, prevented tumour growth when transferred, admixed with tumour cells, into recipients (Zarling and Tevethia, 1973a). The inhibition of tumour growth in this study was thought to be dependent on the presence of radiosensitive cells (Zarling and Tevethia, 1973b).

A number of investigators (Keller and Jones, 1971; Hibbs, et al, 1971), have found that "activated" macrophages from animals infected with a parasite were able to transfer resistance to tumour growth in syngeneic animals and were able to destroy tumour cells in vitro. In this regard, growth of syngeneic adenocarcinoma in mice was suppressed in mice infected with N. Brasiliensis (Keller, et al, 1971).

In general, it has been suggested (Allison, 1972) that to obtain macrophage activation in vivo either by immunization with tumour or parasites, cell mediated immunity is required. Thus transfer of lymphocytes confers protection (Allison, 1971) and treatment of mice with anti-lymphocyte serum abolishes the tumour-inhibiting effect conferred by N. Brasiliensis nematode infection (Keller and Jones, 1971).

ii. HUMOURAL RESPONSE

1. In Vitro

a. Antibody mediated lysis

Tumour specific antibody has been demonstrated in the serum of tumour bearing animals and animals which have rejected a primary or syngeneic transplantable tumour (Skurzak, et al, 1972; Pollack, 1972; Harada, et al, 1973; Thomson, et al, 1973; Prager, et al, 1973; Oritz de Landazuri, et al, 1974). A number of these antisersa have been demonstrated to be active in in vitro antibody dependent cell mediated cytotoxic reactions as previously noted. Prager and colleagues (1973) found that anti-lymphoma antibody from tumour bearing mice was non-cytotoxic in nature. Similarly, Goldstein and colleagues (1973) were able to produce a non-cytotoxic antiserum specific for syngeneic tumour cells by repeated immunization of syngeneic mice with mitomycin-C-treated lymphoma cells.

Although antibodies specifically cytotoxic to tumour cells in complement dependent in vitro tests have been detected in the sera of animals made resistant to Gross-virus or Moloney-virus induced lymphomas by immunization with isologous tumour grafts (Klein and Klein, 1964; Slettenmark and Klein, 1962), the inability of many investigators to demonstrate antibody which is active in a complement dependent cytotoxic reaction in syngeneic system suggests that non-cytotoxic antibodies are generally produced in the immune response to syngeneic tumours.

b. Serum blocking effects

Experimental animals and human patients with tumours often have lymphoid cells which show cytotoxicity against their own tumour cells in culture as detected by a colony inhibition technique (Hellström and Hellström , 1969a; Hellström and Hellström, 1969b). At the same time, they can exhibit serum factors which are capable of specifically blocking this cytotoxicity (Hellström and Hellström, 1969b; Hellström, et al, 1970).

Further studies have indicated that the serum blocking factors are antigen-antibody complexes (Sjögren, et al, 1971).

c. Unblocking serum factors

Yet another serum factor has been described. The serum taken from animals bled 4 days after immunization in the presence of BCG was able to reverse the inhibition by blocking factors, of specific cell mediated cytotoxicity in culture (Hellström and Hellström, 1969b; Bansal and Sjögren, 1971).

2. In Vivo

Although Ambrose and colleagues (1971) suggested that there is a transient appearance of cytostatic antibody in hamsters given SV40 virus,

tumours occur and persist and the animals die. In general, there is little evidence at present that specific antibody against spontaneous or experimental tumours has any inhibitory or destructive effect on tumour cells in vivo (Klein, 1966a; Southam, 1961; Harris and Sinkovics, 1970; Sophocles and Nader, 1971). In fact, serum blocking factors have been shown to enhance tumour growth (Hellström, et al, 1969 ; Alexander, 1968a).

In contrast, unblocking sera injected into mice and rats could bring about regressions of Moloney sarcomas and polyoma tumours under conditions such that this would seldom or never occur (Hellström and Hellström, 1969b; Bansal and Sjögren, 1971).

The fact that there is ample evidence of a diversified immune response to syngeneic tumours gives rise to the question concerning the role of immunity in defense against malignant disease. The relationship of the immune response to defense against tumours will be outlined in the following sections.

VI. IMMUNE SURVEILLANCE

The classical work by Foley (1953) and Prehn and Main (1957), previously mentioned, followed by the studies of Klein and colleagues (1960) on methylcholanthrene (MCA)-induced sarcoma in mice, demonstrated that each MCA tumour induced in a mouse has a specific tumour antigen, differing from all other antigens induced by the same agent in the same host strain. It was possible to immunize the host against the autochthonour tumour without immunizing it against any other MCA-induced sarcoma.

Similar to the early work with chemically induced tumours, Habel (1961) and Sjögren et al. (1961), demonstrated that virus-induced tumours also

had tumour specific antigens. In this case, it has been shown that tumours induced by the same virus express common antigens no matter what the histological type of cell or organ of origin or animal species in which the tumour is produced (Old and Boyse, 1965).

Isograft or autograft reactions have been demonstrated against some spontaneous tumours as well (Hellström and Hellström, 1969b; Klein, 1966b).

These observations all contributed to the foundation of the theory of immunological surveillance, a mechanism responsible for the detection and elimination of cells carrying new or 'foreign' antigens. In the years since these initial demonstrations were reported, evidence for the existence of immune surveillance of tumours has been sought and obtained through clinical observation and through manipulation of experimental systems.

i. EVIDENCE FOR IMMUNE SURVEILLANCE

1. Clinical correlates

If the concept of immune surveillance is valid, a number of clinical correlates should be observed.

a. Correlation of the incidence of malignant disease and development of the immune system

The incidence of malignant disease should increase at ages when the immune system is less efficient. Thus, many malignant diseases of childhood show an age incidence compatible with the probability that the process was initiated about the time of birth (Collins, et al, 1956; Knox and Pillers, 1958; Pollock, et al, 1960), a time of very low potential for active immunological response. Similarly, the increasing

risk of malignancy with advancing age is probably at least partially due to a diminishing ability to produce an immunological response to a new antigen (Sabine, et al, 1947).

b. Incidence of malignant disease related to impairment of the immune system

Depression of the immune system should be associated with increased occurrence of malignancy. Among evidence in this category cited in support of immune surveillance (Burnet, 1970), treatment with immunosuppressive drugs was found to be associated with the development of a carcinoma from an unsuspected primary in a kidney transplant. The tumour regressed completely when the immunosuppressive drugs were withdrawn (Woodruff, 1968). Significantly, 5 cases of reticulosarcoma were also reported in patients on immunosuppression after kidney transplant.

The immunodeficiency of ataxia-telangiectasia was associated with deficiencies of cell-mediated immunity (Peterson, et al, 1964) and often with a low level or lack of specific classes of immunoglobulin as well (Thieffry, et al, 1961; Biggar, et al, 1970). A malignancy frequency of 10-15% has been reported in studies of 42 cases of this disorder (Sedgwick and Boder, 1972). In fact, Good (1973) has concluded that patients with many different forms of primary immunodeficiency have developed cancer in a frequency in excess of that occurring at comparable age in the general population.

c. Successful treatment of tumours by techniques which probably produce incomplete eradication

Successful treatment of tumours by surgical, chemotherapeutic or radiotherapeutic procedures when it is reasonably sure that destruction

or removal of all cancer cells was incomplete, suggests that an initially inefficient immune system has been able to eradicate the remaining tumour cells after the major tumour load has been dealt with. Thus neuroblastoma and Wilm's tumour has been successfully treated by surgery (Collins, et al, 1956; Pollock, et al, 1960).

Cytotoxic drug therapy has been successful in treating choriocarcinoma (Hertz, et al, 1964) and Burkitt's lymphoma (Burkitt, 1966; Morrow, et al, 1967; Buchenal, 1968). With regard to this, Klein and colleagues (1966) have demonstrated a specific antigen related to Burkitt's lymphoma and as choriocarcinoma is basically a foetal tissue homograft, minor antigenic differences should exist between it and host cells. It has been postulated that patients with Burkitt's lymphoma are cured by chemotherapy because the remaining malignant cells are eradicated by the host's immune response (Burkitt, 1966; Burkitt, et al, 1965; Ngu, 1965).

d. Evidence for the presence of tumours which never reach the clinical stage

Everson (1964), studying the literature on tumours which disappeared, concluded that 130 of these had undergone spontaneous regression. This suggests that conditions had allowed the immune response, initially ineffective, to build up till it was able to eradicate the tumour. Similarly, Beckwith and Perrin (1963) reported that in unselected autopsies of children up to three months of age, small histologically-typical adrenal neuroblastomas were found forty to fifty times as common than would be predicted from the clinical incidence of adrenal neuroblastoma.

2. Experimental demonstrations

A number of immunological manipulations in experimental systems should produce certain predictable results if immune surveillance exists.

a. The relationship of immunosuppression and tumour incidence

Immunosuppressive agents should play a part in facilitating the appearance or transfer of tumour cells. In this regard, a number of investigators have claimed that carcinogenic hydrocarbons depress immune responses: antibody titres (Malmgren, et al, 1952) plaque forming cells (PFC) (Stjernswärd, 1966a); and rejection of skin homografts (Lindner 1962). Prehn (1963, 1964) showed that the capacity of syngeneic mice to accept tumour grafts was increased by methylcholanthrene (MCA) treatment. Stjernswärd (1966b) found that tumours appeared in a much higher percentage of mice treated with MCA compared with untreated controls when 10^3-10^6 tumour cells were given.

b. The relationship of thymectomy and tumour incidence

As immune surveillance is thought to result in a homograft-like or thymus dependent reaction, neonatal thymectomy would be expected to facilitate tumour growth. Law and colleagues (1966) have shown that C57BL mice which are wholly resistant to tumour development induced by polyoma virus were rendered susceptible by thymectomy at 3 days of age, to the extent that about 50% of the mice tested produced tumours when the virus was injected 2-4 weeks after birth. These results were typical of those obtained by other investigators (Malmgren, et al, 1964; Vandeputte, et al, 1963).

c. The relationship of tolerance and tumour growth

The probable specific immunoparalytic effect of a developed tumour

was reported by Klein (1966b and Stjernswärd (1967). Stjernswärd (1966b) showed that a mouse from which an MCA-induced tumour had been removed was more susceptible in a specific manner to its autochthonous tumour cells than were control mice.

Other examples of tolerance were found related to the appearance of tumours in systems involving oncogenic viruses which normally are transmitted neonatally. In mice susceptible to the Bittner Milk agent, a lower incidence of infection was obtained upon inoculation of adult syngeneic mice which had avoided neonatal infection than was found in vertically infected mice. The older mice were better able to resist transplantation of small numbers of the tumour cells (Attia, et al, 1965). Thus infection in foetal life seems to produce tolerance to Bittner virus antigens and related transplantation antigens.

Similarly, Klein and Klein (1965) showed that tolerance to the specific transplantation antigens associated with the Moloney leukemia virus was directly related to neonatal infection.

3. Relationship of homograft immunity to specific tumour immunity

The concept of immune surveillance of syngeneic tumours, as interpreted by Burnet (1970) is that it is a manifestation of homograft immunity similar to the rejection of an allogeneic tumour. Although other investigators have suggested that different effector cells are active in the cellular immune mechanisms involved in allograft rejection and in syngeneic tumour regression (Perlman, et al, 1972b), there is some evidence to support Burnet's view.

Hepatomas transplantable in the syngeneic host were obtained by feeding diethylnitrosamine to purebred Strain 2 guinea pigs. Partial

immunity was demonstrable by the injection of living cells intramuscularly. The immunity was shown by a typical delayed hypersensitivity response to a tumour-cell extract injected intradermally and by the reduced growth rate of subcutaneous transplants in sensitized animals compared with normal controls. Intradermal challenge of sensitized animals produced only small nodules which did not ulcerate and which regressed rapidly, compared with similar inoculation of normal animals in which papules developed, ulcerated and eventually regressed (Churchill, et al, 1968).

Thus a great number of clinical observations and experimental manipulations suggest the activity of an immunologically based defense against malignant disease.

ii. EVIDENCE AGAINST IMMUNE SURVEILLANCE

While conceding that there is "an almost overwhelming amount of evidence" pointing toward the existence of an immunological surveillance mechanism, Prehn (1971) presents an argument for a generally weak and ineffective immune surveillance of tumours. The "sneaking through" phenomenon demonstrable with highly antigenic tumours (Humphreys, et al, 1962; Old, et al, 1962; Potter, et al, 1969), the low antigenicity of "spontaneous" tumours arising in vivo or in immune free environments (Prehn, 1970) and the observation by some investigators of the lack of marked increments in tumour incidence under conditions of immune suppression (Allison, 1970a; Yohn et al, 1965; Miller 1963: all suggest that immunosurveillance is ineffective against tumours. In reappraisal of his own work, Prehn (1963) notes that while two highly immunogeneic MCA-induced sarcomas grew better in MCA treated mice, which were thought

to be immunosuppressed by MCA treatment, two relatively non-imunogeneic MCA-induced sarcomas grew worse in MCA treated mice.

The observed advantage of mild maternal immunity to growth of the foetus (Kirby, 1970; Lappé and Schalk, 1971) leads to the hypothesis that a little immunity may be good also for a tumour, perhaps even necessary. Prehn and Lappé (1971) have suggested that a weak immune response may be necessary for the nascent neoplasm to overcome non-immunologic homeostatic devices of the surrounding normal cells (Stocker, 1964; Prehn and Slemmer, 1967).

It is possible also that other mechanisms may contribute to the control of tumours; for instance, hormones have been shown to play a role in the surveillance of endocrine tissues and their tumours (Furth, 1953). Contact inhibition of individual malignant cells (Abercrombie and Ambrose, 1962) and allogeneic inhibition of cells with different surface arrangements (Bergheden and Hellström, 1966; Hellström and Hellström, 1966, Klein, 1966b) may be involved in the non-immunological surveillance of tumours. Due to phenomena such as these, aberrant cells could be eliminated before they sensitized the immune system.

The relative involvement of immunological versus non-immunological surveillance mechanisms is difficult to assess, but the existence of allophenic mice, a viable mosaic of histoincompatible cells (Mintz and Silvers, 1967) and the phenomena of persisting chimerism and tolerance, probably all immunologically based, are evidence against the activity of an efficient non-immunological surveillance mechanism. At the same time, although the immune response to tumours appears to vary with the stage of development of the tumour, there is an extremely large number

of reports implicating an immunologically based surveillance mechanism in the defense against tumours.

Given that there is "almost overwhelming evidence" supporting the claim that immune surveillance exists and that there is growing evidence that the host can mount an immune reponse to a syngeneic tumour, the fact that tumours are often able to arise and proliferate remains an unsolved enigma.

In keeping with the tenets of immune surveillance, theories governing all levels of the tumour-host relationship have been postulated for the persistence of antigenic tumours.

VII ESCAPE FROM IMMUNE SURVEILLANCE

i. TUMOUR CELL LEVEL

The mechanisms for the escape from immune surveillance at the level of the tumour cell include immunoresistance and masking.

1. Immunoresistance

The immune vulnerability of a cell would be influenced by characteristics of the cell membrane. The sensitivity of a cell to immune attack by humoural or cell-bound factors would be partially determined by the density of antigen sites on the cell surface. In many cases, antigen density, based on quantitative adsorption of serum, was found to correspond to cell sensitivity to the killing action of antibodies or cells (Fenyö, et al, 1968; Friberg, 1972; Möller and Möller, 1962 and 1967). Evidence for additional membrane characteristics influencing immunosensitivity is provided by experiments demonstrating that Moloney leukemia and sarcoma cells with very similar absorbing efficiency differed in

immunosensitivity (Chuat, et al, 1969).

Complement dependent cytotoxic sensitivity of Chinese hamster cells was found to vary during the cell cycle (Shipley, 1971). Similarly, cytotoxic susceptibility of Moloney virus-transformed lymphocytes varied during the cell cycle while the complement activity on antibody coated cells remained essentially constant (Lerner, et al, 1971).

In experimental systems, the selection of immunoresistant cells with low antigenic density from an immunosusceptible population has been demonstrated (Fenyö, et al, 1968; Friberg, 1972).

2. Masking

The possibility of masking of antigen determinants by surface localized substances has been postulated (Currie and Bagshawe, 1967; Simmons, et al, 1971). Evidence obtained through cell hybridization experiments has been interpreted to suggest that the Ehrlich ascites tumour which has been selected for progressive growth in allogeneic mice during 75 years is "dominant" for the suppression of antigen expression and this is responsible for the loss or considerable decrease in the detection of the isoantigens of various cells hybridized with the Ehrlich cell. The antigenic phenotype of several sublines was reaquired fully or in part after continued passage in vitro (Klein, et al, 1970).

Significantly, somatic hybrids involving a nonspecific tumour line, a subline of TA3 ascites, with normal fibroblasts expressed the isoantigens of both partners. This TA3 subline arose without selection and had a 50-60 fold concentration of isoantigens compared with the original. It was suggested that the nonspecific TA3 cell which lacks the ability to express its antigens fully can be restored by the

partner in the hybrid cell (Klein, 1972).

Thus there are a number of means by which the tumour cell itself could be responsible for avoiding detection or attack by the immune system.

ii. HOST CELL LEVEL

Mechanisms for escape of immune surveillance exist at the level of the host. These include immunological tolerance and immunosuppression.

1. Tolerance

Vertical transmission of nonfatal oncogenic viruses such as murine leukemia viruses or the mammary tumour virus transmitted via mother's milk or neonatal infection with leukemogenic viruses can lead to immunological non-reactivity. This is based on the assumption that hosts may be made tolerant to these antigens during early embryonic life before development of a competent immune system. Thus a proportion of such animals, when exposed to the virus or virus transformed cells, either experience a delay in development of virus neutralizing antibodies or antibodies directed at new viral determined cellular antigens, or never produce them. The animals in which tumours develop were shown to usually have no or low antibody titres (Essex, et al, 1971; Klein and Klein, 1965).

Mice, neonatally infected with Gross or Moloney murine leukemia virus, neither produced anti-viral or virus neutralizing antibodies (Axelrad, 1965; Klein and Klein, 1966).

It was shown that mice neonatally infected with Graffi (Gi)-MuLV failed to react immunologically with the next inoculum of the same Gi-MuLV when adults. This was revealed by the absence of both transplantable resistance and cytotoxic antibody production in immunized mice (Chieco-Bianchi, et al, 1967). The lack of reactivity was not due to a

general immune suppression as mice neonatally infected with Gi-MuLV produced antibodies to unrelated antigens. Further, the mice could recognize antigens determined by a similar but antigenically distinct MuLV such as the passage A Gross virus (Cheico-Bianchi, et al, 1970). The age of the host at first exposure to antigen and the dose of antigen given are important in induction of immunological tolerance. If animals were inoculated with Gi-MuLV after their third week of life a gradual appearance of reactivity occurred with increasing age (Chieco-Bianchi, et al, 1970). A very small dose of Gi-MuLV inoculated into newborn mice produced an immune response (Chieco-Bianchi, 1972).

2. Immunosuppression

Recent studies have indicated that the classical concept of immunological tolerance is not always applicable. The cat, when infected neonatally with feline leukemia virus (FeLV) has been shown to produce antibodies to one of the FeLV antigens after immunization with disrupted FeLV (De Noronha, et al, 1972). Similarly, while not detectable in the circulation, anti-lymphocytic choriomeningitis (LCM) virus antibody could be detected in kidney eluates of LCM virus-infected mice. The circulating anti-LCM virus antibody forms complexes with the viral antigen and is deposited in the glomeruli (Oldstone and Dixon, 1969). Renal immune complexes have been found in Balb/c mice infected neonatally with Moloney-murine leukemia virus (M-MuLV) (Hirsch, et al, 1969).

The AKR strain of mice which has a high incidence of spontaneous leukemias has been considered to be immunologically tolerant to the Gross-murine leukemia virus (G-MuLV). Recently, the presence of antibody

has been detected in the eluates from kidneys of AKR mice; the complement-fixing antibodies appear to be immunologically related to intraviral components and to G-cell surface antigen (Oldstone, et al, 1972).

Thus the concept of complete tolerance may have to be replaced with that of incomplete immunological tolerance (Aoki, et al, 1974) or perhaps immunosuppression.

Vertical transmission of the virus causing radiation-induced leukemia in C57Bl mice does not prevent C57Bl mice from being immunized against viral antigens or the antigens of the leukemia cells. Immunosuppression is thought to play a role in this system (Haran-Ghera, 1970). It has been suggested that a continuing immune response is involved which is related to the ever-present antigen excess due to viremia (Allison, 1970b).

Experimentally, immunosuppression can be brought about by X-irradiation (Law and Dawe, 1960) immunosuppressive drugs and by anti-lymphocyte serum. It also occurs in the natural decline in immune reactivity that comes with old age (Stjernswärd, 1966b).

The state of specific immune tolerance or immune suppression, naturally occurring or experimentally-induced, would predispose the host to allow tumour induction.

iii. HOST-TUMOUR INTERACTION LEVEL

By far the largest number of models for the escape of immune surveillance describe mechanisms occurring at the level of the tumour-host interaction. These include enhancement, immunologically privileged sites, antigenic simplification, immunosuppression by developed tumours

and the contest of time versus stimulation.

1. Enhancement

Immunological enhancement is the name given to the paradoxical finding that immune resistance to tumour was depressed by pretreatments which resembled immunization (Kaliss, 1958).

Kaliss and Molomut (1952) discovered that antitumour antiserum was capable of reproducing the state of immunological enhancement when passively transferred to normal animals.

Thus serum factors may be responsible for the progressive growth of tumours. Three levels of interaction can be suggested for this interaction.

a. At the afferent level, humoural antibodies may cover specific antigen sites on the tumour cell, preventing immunocompetent cells from becoming sensitized (Skurzak, et al, 1972).

b. At the afferent level, humoural antibodies may cover specific antigen sites on the tumour cell preventing sensitized lymphocytes from attacking the tumour target cell. Inhibition of cytotoxic lymphocytes has been demonstrated using blocking techniques involving antisera preabsorbed with various cells (Sendo, et al, 1974). Alternatively, antigen-antibody complexes (Sjögren, et al, 1971) or soluble antigens (Brawn, 1971; Currie and Basham, 1972) may block the interaction between attacking lymphocytes and target cells by reactions with the attacking cells. This hypothesis is supported by the facts that serum from tumour-bearing hosts inhibits in vitro cell mediated immunity, whereas serum from tumour regressors which rejected their tumours does not have the same effects (Hellström and Hellström, 1969a) and serum from tumour regressors eliminates the inhibiting effect of serum from tumour-bearing hosts on in vitro cell mediated immunity in the MSV induced sarcoma

and polyoma systems (Sjögren and Bansal, 1972). When transferred into tumour-bearing hosts the serum from regressors shows the same effect in vivo. However, a low concentration of regressor serum in the rat MSV-induced sarcoma system inhibits in vitro cell mediated immunity (Skurzak, et al, 1972). Thus the blocking factor hypothesis may not be tenable as a general rule.

c. Enhancement may be a central effect. Antibody may depress immunity by preventing the proliferation of immunocompetent cells through a feedback control mechanism (Hellström and Hellström, 1969b).

2. Contest of time versus stimulation

The fact that in transplantation tests it is often necessary to graft a large number of tumour cells to ensure outgrowth is an indication of host response. Small and large inocula have been observed to grow while medium populations do not (Kölsch, et al, 1973).

a. Eclipse of host immunity

The larger cell number may reach a large and unmanageable population size before significant immunity develops. When immunity does develop, there may simply be an inadequate supply of effector cells to deal with the established tumour. Youn and colleagues (1973) have reported that hosts cannot produce enough active lymphocytes to reject advanced tumours. Similarly, the immunotherapeutic effect of the passive transfer of peritoneal exudate cells from immunized animals to tumour bearing hosts is considered to be abrogated by the presence of a large tumour mass in the hosts (Wepsic, et al, 1971).

b. Sneaking through

The ability of small primary inocula to avoid rejection, to "sneak

through" a self-induced immune response may be due to a discrepancy in timing which favours the tumour rather than the host. The antigenicity of the tumour may be too low to sensitize lymphocytes. Thus the host is incapable of producing an immune response before the tumour growth has passed a critical size. The importance of the relationship of antigenic stimulation and time was demonstrated by Takeda and colleagues (1968). Their experiments with rats involved implantation of artificial metastases from primary MCA-induced sarcomas, followed by immunization by ligation of the primary tumour. The earlier the immunization the more marked was the growth suppression of the metastases. The possibility of achieving rejection of the second graft was over in a few days.

3. Antigenic simplification

Antigenic modulation has been found in two leukemia antigen systems, thymus leukemia (TL) (Boyse and Old, 1969) and Gross-cell surface antigen (GCSA) (Aoki and Johnson, 1972). This phenomenon provides a special mechanism whereby progressive tumour growth can occur when these antigen-positive leukemias are transplanted into syngeneic mice preimmunized against the antigens. No resistance to the leukemia cells is found and as long as the specific antibodies exist, the phenotype of the leukemia cells is changed from antigen-positive to antigen-negative.

4. Immunologically privileged sites

If the site of tumour proliferation is inaccessible, as in the central nervous system, immunological surveillance could play little or no role in opposing tumour development. Thus neonatal thymectomy, known to increase the incidence of Rous sarcomas in virus-inoculated mice, did not influence tumourigenesis if the virus was inoculated into the

brain (Law, et al, 1966). Involvement of the central nervous system is thought to relate to the ultimate fatal course of Burkitt's lymphoma, a tumour against which host immune defence is thought to play an important role (Klein, 1972).

5. Immunosuppression by developed tumours

The tumourous state of the animal also produces a form of immunosuppression even if sensitized cells are provided. Inhibition of tumour growth was less efficient in tumour-bearing and passively immunized guinea pigs challenged intradermally with tumour cells than in non-tumourous animals. This phenomenon was dependent on the size of the growing tumour and was demonstrable even when the tumours were antigenically unrelated (Bernstein, et al, 1971). Possibly recipient lymphocytes, which are in limited number and exhausted by the growing tumours, are required to assist the sensitized cells provided (Klein, 1972; Youn, et al, 1973).

6. Other regulatory systems

There may be other immunological regulatory systems of the host which inhibit cell mediated immunity. The sensitization of lymphocytes to chemically induced C57BL-transplanted leukemia EL4 cells was shown to be stronger in vitro than in vivo (Wunderlich, et al, 1972). It has been demonstrated many times that T cells have the ability to stimulate the humoural antibody production of B cells. In recent studies with pneumococcal polysaccharide and Ascaris antigens, T cells were shown to be capable of inhibiting antibody production as well (Baker, et al, 1973; Okumura and Tada, 1971). Similar regulatory mechanisms may be involved in escape from immune surveillance.

Thus an argument has been presented for the participation of immunological surveillance in the defence against malignant disease. A number of effector mechanisms have been described which are active in the clinical stage of tumour growth. In some cases, serum factors were found to obliterate the effector mechanisms allowing the uninhibited growth of the tumour ,or to cause antigenic modulation thus reducing the antigenic qualities of the tumour. In other cases, the presence of developed tumours was associated with general immunosuppression. Although interference with the effector mechanisms may give some explanation of the survival of an established tumour, it does not explain the failure of the body to control the growth at a much earlier stage.

Excluding cases of tumours occurring in immunologically privileged sites or in hosts which have been previously tolerized or immunosuppressed, interference with the ability of the immune system to cite, or initiate the induction of, a response against the tumour may be important. Such a mechanism would prevent the destruction of an antigenic tumour in its critical, early stage of development when it could be most susceptible to immune attack.

Thus the object of this study was to examine the immune system during the very early stages of tumour growth.

MATERIALS AND METHODS

I. MICE

Mice of the inbred strains DBA/2 and AKR were obtained from Jackson Laboratories, Bar Harbor Maine.

The DBA/2 mice used throughout this study were matched for sex and age in each experiment.

The C3H mice were obtained from Jackson laboratories or from North American Laboratory Supply, Winnipeg, Manitoba.

II. RABBITS

Outbred white rabbits, weighing about 5 lb., obtained from Canadian Breeding Laboratories, St. Constant, Quebec, were used for the preparation of rabbit anti-BSA, anti-Fe and anti-mouse Ig serums.

III. TUMOUR CELLS

The L5178Y lymphoblastic leukemia originated as a spontaneous leukemia in the DBA/2 mouse strain (Goldenberg and Thomas, 1967). It was obtained from Dr. G. J. Goldenberg and was maintained in ascites form by IP. transfer, every 5 days, of 0.1 ml of ascites (approximately 10^7 tumour cells) in the DBA/2 strain of mice. This tumour line was also cultured in Fischer's medium (GIBCO, Grand Island, N.Y.) containing 50 mcg/ml streptomycin and 50 IU/ml penicillin (GIBCO) and either 10% horse serum (HS) (GIBCO) or 10% foetal calf serum (FCS) (GIBCO). The doubling time of L5178Y cells in both of these culture systems was approximately 12 hours. The biological viability of the tumour line was assessed by recording the time of death following IP inoculation of a range of doses of L5178Y cells.

IV. FREEZING L5178Y CELLS FOR STORAGE

Following the outline suggested by Paul (1965), L5178Y ascites cells were washed once in Minimum Essential Medium (MEM) obtained from GIBCO and were resuspended at 5 x 10^6 cells/ml in MEM solution containing 10% FCS and 9% dimethylsulphoxide (DMSO) (Baker Chem. Co., Phillipsburg, N.J.). Aliquots of 1.0 ml were transferred to sterile glass ampoules which were then heat sealed. The ampoules, kept at room temperature for 10 min, then at 4°C for 10 min, were frozen slowly, at 1°/min from 4°C to -38°C, and then cooled rapidly to -65°C, prior to storage at -80°C.

V. ANTIGENS

i. MOUSE IgG AND IgF MYELOMA PROTEINS

Ascites fluid was collected via I.P. hypodermic tap of Balb/C mice carrying transplantable mineral oil induced tumours. The ascites was centrifuged at 3,000 r.p.m. for 5 min and the supernate, equilibrated in 0.005M phosphate buffer, pH 7.5, was chromatographed on a DEAE-cellulose ion-exchange column. The proteins were eluted by stepwise increase of the ionic strength of the phosphate elution buffer from 0.005M to 0.01M to 0.033M. The eluate was collected in aliquots by an automatic fraction collector. The protein content of each tube was assessed by determining the optical density at 280 nm with a Zeiss M4QIII spectrophotometer (Carl Zeiss, Oberkochen, Wuerttemberg). The aliquots in a given peak were combined, dialyzed in cellulose tubing with 0.15M borate saline buffer, pH 8.0, and concentrated using a Diaflo UM10 ultrafilter. The purified mouse globulins were subjected to immunoelectrophoresis

against rabbit anti-whole mouse serum. The presence of IgG myeloma protein was indicated in the 0.005 and 0.01M fractions, while IgF myeloma proteins were in the 0.010 and 0.033M fractions of the appropriate ascites fluid.

ii. FERRITIN

Horse spleen ferritin (Fe), twice crystallized, cadmium-free, was obtained from Nutritional Biochemical Corp., Cleveland, Ohio.

iii. BOVINE SERUM ALBUMIN

Bovine serum albumin (BSA) was obtained from Pentex, Kanakee, Illinois.

iv. HORSE RED BLOOD CELLS

Horse red blood cells (HRBC) were obtained from National Biological Laboratory, Dugald, Manitoba. The blood was collected in citrate buffer and the cells were washed three times in 0.15M saline before use.

v. HUMAN GAMMA GLOBULIN

Human gamma globulin (Hgg) fraction II was obtained from Pentex, Kankakee, Illinois. Human gamma globulin was deaggregated according to the method of Chiller and Weigle, (1970). A 7.20 g aliquot of Hgg was dissolved in 24 ml 0.15M saline (30 mg/ml). The solution was ultracentrifuged in a Ti50 rotor in an L2 preparative ultracentrifuge (Beckman Corp., Toronto, Ontario) at 45,000 r.p.m. (approximately 150,000 G) for 3 hours at 4°C. The upper third of the centrifuged solution was removed and stored at 4°C.

vi. MITOMYCIN-C-TREATED L5178Y CELLS

L5178Y cells were washed twice in Dulbecco's phosphate buffered

saline (DPBS) pH 7 and resuspended in the same DPBS containing 25 µg mitomycin-C (Nutritional Biochem. Corp., Cleveland, Ohio). The suspension was warmed at 37°C for 30 min, then washed twice and resuspended in DPBS for injection.

vii. HUMAN FIBRINOGEN

Human fibrinogen (FIB) was obtained from Connaught Medical Research Laboratories, Toronto, Ont.

VI ADJUVANT

Freund's complete adjuvant (FCA) was obtained from Difco Laboratories, Detroit, Michigan.

VII RABBIT IMMUNIZATION AND BLEEDING

Rabbits were immunized by intramuscular injection,in a rear flank, of 1.0 mg protein in 0.5 ml 0.15M saline emulsified with 0.5 ml FCA. The animals were immunized once a week for six weeks and bled by cardiac puncture one week after the final injection. The antiserum was tested for antibody activity by immunoelectrophoresis against the respective antigen. When a high antibody titre was found, 50 ml of blood was taken weekly for six weeks. The blood was allowed to clot at room temperature over a few hours and the serum was separated from the clot by two centrifugations at 1,800 r.p.m. for 20 minutes. The sera were stored at -20°C.

VIII. MOUSE IMMUNIZATION AND BLEEDING

Mice were immunized by intraperitoneal (IP) injection of the desired antigen with or without FCA. Serum, collected by cardiac puncture, was

allowed to clot at room temperature for 2-4 hours and the supernatant was separated from the clot by centrifugation at 3,000 r.p.m. for 5 minutes. The serum was frozen and stored at -20°C or stored at 4°C and used within 1-2 days.

IX DEAE-CELLULOSE ION-EXCHANGE CHROMATOGRAPHY

The method of King (1968) was followed in packing the DEAE-cellulose ion-exchange column. Dry DEAE-cellulose ion-exchanger (Carl Schleicher and Schuell Co., Keene, N.H., 0.9 meq/g dry wt.) was washed by suspending it, with continuous stirring in 0.5 M NaOH and 0.5 M NaCl at room temperature After allowing the ion exchanger to settle for 30 minutes, the cloudy supernate was decanted and the cellulose was resuspended in 1M NaCl. Resuspension and settling, followed by decanting, were repeated twice and the cellulose then was filtered by suction using a Büchner funnel and Whatman #1 filter paper (W. R. Balston Ltd., England). The damp cellulose was resuspended in 1M HCl, filtered immediately by suction and washed with distilled water until the filtrate was at neutral pH. The ion-exchanger was then equilibrated to the desired molarity and pH by resuspending and washing with the first buffer to be used on the column. The thick cellulose buffer mixture was homogenized in a Waring blender and trapped air bubbles were removed by evacuation with an oil vacuum pump. The suspension was then poured into a glass column which was partially closed with a glass wool plug above glass beads. The ion-exchanger was allowed to settle while the excess buffer slowly dropped from the outlet. As the packed bed rose, more cellulose suspension was added through a 5-8 cm column of buffer. The last 15 cm or so of the column was packed

with the aid of slight external pressure applied by pressing downward on a rubber stopper closing the top of the column. This was done to equalize the conditions of packing throughout the column as the lower parts of it had been packed with a head of buffer on it. The rubber stopper was removed by inserting a needle to prevent a vacuum forming when the stopper is removed.

X SEPHADEX G-200 AND G-100 GEL FILTRATION

Sephadex G-200 and G-100 columns were packed according to the instructions provided by Pharmacia Ltd. (Montreal, Canada). The gel was swollen in excess 0.15M borate saline buffer, pH 8.0, for three days at room temperature (or 5 hours at 60°C). In packing a G-200 column, the open space below the support disc, the small bore plastic tubing attached to the outlet and one quarter of the column all were filled with buffer. A thin slurry of gel in buffer was stirred and poured down the wall of the column till it reached the top. The outlet of the G-200 column was kept at the same level with the top of the gel slurry. When approximately 10 cm of the bed was settled, the outlet to the column was lowered 1 cm from the head of the column. When about 20% of the column was packed, the excess buffer was removed and another portion of gel slurry was added before the previous portion had settled completely in order to prevent the formation of a boundary. The outlet tubing was lowered to maintain a pressure equal to one tenth of the packed bed length. When the column was packed, a sample applicator was placed on the surface of the packed bed. In order to check the homogeneity of the column and to determine the void volume, 5 mg of blue dextran dissolved in 3 ml of buffer was passed through the column

at a rate of 5-10 ml per hour at 3-4 cm pressure.

Packing the G-100 column was carried out at atmospheric pressure. A piece of filter paper was applied to the packed bed to protect its upper surface and the column was run at a pressure greater than atmospheric pressure such that a flow rate of 30-40 ml per hour was achieved.

XI IMMUNOELECTROPHORESIS

Immunoelectrophoresis was carried out according to the method of Grabar and Burtin (1964). Glass slides (2.5 x 7.6 cm) were coated with 2 ml of a 0.5% boiling solution of melted agar (Agar-Noble, Difco) in distilled water and the slides were dried at 80°C for 4 hours. A 3 ml aliquot of 2% melted agar in 0.025M barbital buffer, pH 8.6, was layered onto the coated slides. The agar gel was allowed to solidify and the wells and troughs were cut for immunoelectrophoresis. The solutions to be tested were placed in the wells and the slides were subjected to a 14 ma. current, approximately 70-75 volts for three and one-half hours in an electrophoresis apparatus. The slides were removed and the troughs were filled with the detecting antisera. The slides were then placed in a moist chamber overnight at room temperature to allow the precipitin lines to develop. They were then washed in 0.15M saline for 24 hours, desalted in distilled water for eight hours and, after a damp filter paper was laid along the surface of the agar to promote even drying, the slides were dried overnight. The slides were stained with amido black (1 g/1000 ml sodium acetate buffer) for 10 minutes, washed in an acetic acid:methanol:water solution (20:980:300 by volume) for 10 minutes and air dried.

XII OUCHTERLONY GEL DIFFUSION

Slides coated as for immunoelectrophoresis were layered with 3 ml of a 1.5% boiling solution of agar in 0.15M saline. The gel was allowed to solidify in a moist chamber and then wells were cut with a template for diffusion. The wells were filled with antigen (1 mg/ml) or antiserum in an arrangement which would achieve the desired antigen-antibody precipitin lines and the slide was left in a moist chamber overnight to allow precipitin lines to develop. The slide was then washed and stained as for immunoelectrophoresis.

XIII ANTIBODY PURIFICATION

The production of purified antibodies was achieved by the use of immunoadsorbents containing the corresponding antigen. Two methods were used for the preparation of the immunoadsorbents.

i. CNBr-ACTIVATED SEPHAROSE 4B METHOD

A one gram aliquot of CNBr-activated Sepharose 4B was conjugated according to instructions provided by Pharmacia. The activated sepharose was washed 4 times with 40 ml of 0.001M HCl, followed by 40 ml of 0.1M $NaHCO_3$ buffer containing 0.5M NaCl, pH 8.0. The washed Sepharose, resuspended in the high salt $NaHCO_3$ buffer, was shaken continuously for 2 hours at room temperature with 15-30 mg of protein in a total volume of 5 ml. The conjugated Sepharose was washed once with the same buffer and then shaken for a further 2 hours at room temperature with a 5 ml aliquot of 0.1M ethanolamine, adjusted with concentrated HCl to pH 8. The conjugated Sepharose was then washed 6 times alternatively with high and low pH, high salt buffers to remove unreacted protein and ethanolamine. The conjugate

was packed, over glass beads and glass wool in a 10 ml plastic syringe and stored at 4°C in 0.15M borate saline buffer, pH 8.0, containing a drop of toluene.

Approximately 25 ml of fresh 0.15M borate saline buffer was passed through the conjugate before each use. A 5 ml aliquot of rabbit serum was then passed through the conjugate during 60 minutes at room temperature and the effluent collected. The conjugate was rinsed rapidly with 100 ml of 0.15M borate saline buffer, pH 8, and eluted during 15 minutes with a 5 ml aliquot of 0.1M glycine/HCl, pH 2.5, followed by 5 ml of 0.15M borate saline buffer, pH 8.0. The pH of the eluant was neutralized with 1M NaOH and the Sepharose conjugate was neutralized by rinsing with more borate saline buffer. Both the original effluent and the eluant were tested by Ouchterlony technique for specific antibody activity. The concentration of specifically precipitable protein in the eluant was found to be at least 80%.

ii. BIS-DIAZOTIZED BENZIDINE (BDB) METHOD

The stock solution was prepared as described by the method given in the Handbook of Experimental Immunology (Herbert, 1967). A two hundred and thirty mg aliquot of benzidine (Hartman-Leddon Co., Philadelphia, Pa.) was dissolved in 45 ml of 0.2M HCl and cooled in an ice bath. A one hundred and seventy-five mg aliquot of $NaNO_2$ (J. T. Baker Chemical Co., Phillipsburg, N.J.) was dissolved in 5 ml of distilled water, cooled, and added to the benzidine solution during one minute. The reaction was allowed to proceed in an ice bath for 30 minutes with stirring at 5 minute intervals. The resulting solution was aliquoted in 2 ml volumes into ampoules which were sealed. The stock solutions were frozen and

stored at -20°C.

Proteins were aggregated according to the method of Bernier and Cebra (1965). A 15 mg aliquot of the appropriate antigen was dissolved in 5 ml of 0.1M phosphate buffer, pH 6.8, and added to 5 ml of a 1:15 dilution of the stock solution described above. The mixture was allowed to stand at room temperature for 5 hours and the resulting yellow-brown coloured aggregate was washed three times with buffer, twice with 0.1M glycine-HCl buffer, pH 2.5, and neutralized with 0.2M phosphate buffer, pH 7.2. Each wash solution was separated from the aggregate by centrifugation at 3,000 r.p.m. for 5 minutes.

A 10 ml aliquot of rabbit antiserum was added to 50 mg of aggregated antigen. The suspension was left at room temperature for 2 hours and then stirred at 4°C overnight. The aggregate was removed by centrifugation, 10,000 r.p.m. for 15 min, and washed three times with 0.15M phosphate buffered saline, pH 6.4, at 4°C. After the final wash, the aggregate was suspended in 0.1M glycine-HCl buffer at pH 2.5 and left at 4°C for 1 hour, followed by centrifugation at 10,000 r.p.m. for 15 min at 4°C. The supernatant liquid was removed and neutralized immediately with 0.2M phosphate pH 7.2. The aggregate was also neutralized and the process of elution repeated to recover as much antibody as possible from the serum. The antibody activity was examined by Ouchterlony technique. The concentration of specifically precipitable protein was found to be between 80 and 90%. The antibodies thus produced were further purified by stepwise elution from a DEAE-cellulose column.

XIV PEPSIN DIGESTION OF IMMUNOGLOBULINS

Purified 7S rabbit anti-mouse immunoglobulin, anti-Fe, anti-BSA were separately digested with pepsin (Worthington Biochemical Co., Freehold, N.J.) according to the method of Nisonoff, et al (1960) and Utsumi and Karush (1965). Digestion was carried out using a pepsin to protein ratio of 2:100. After incubation for 5 hours at 37°C in 0.1M acetate buffer, pH 4.0, the digestion was stopped by neutralization to pH 8 with 1M NaOH. The $F(ab')_2$ peaks were isolated by gel filtration on Sephadex G-200. The 5S peak thus obtained was found to form a precipitin line by the Ouchterlony test with the homologous antigen.

XV COATING OF SHEEP RED BLOOD CELLS

Sheep red blood cells (National Biological Laboratory, Dugald, Manitoba) formalinized by the method of Wede (1962) were used to prepare protein-coated sheep red blood cells. The cells, collected in Alsever's solution, were washed three times with 0.15M saline, pH 7.5. Equal volumes of 8% SRBC and 3% formaldehyde, pH 7.5 (adjusted with 0.1N NaOH) were incubated at 37°C for 24 hours. The cells were washed four times in distilled water at neutral pH and stored as a 10% suspension in distilled water with 0.1% sodium azide or formalin at 4°C for up to 6 months.

According to the tanning and coating method of Herbert (1967), 2 ml aliquots of a 2% suspension of the above formalinized cells were washed three times in 0.15M phosphate buffered saline, pH 6.4. The cells were then suspended and incubated at 37°C for 30 minutes in a 0.0025% solution of tannic acid (Baker Chem. Co., Phillipsburg, N.J.) in 0.15M phosphate buffer,

pH 6.4. The cells were washed once and left at 4°C overnight. The cells were then resuspended in 2 ml of 0.15M phosphate buffer, pH 6.4, containing 0.08 to 0.14 mg/ml of the desired protein antigen Fe or BSA, and the cells were incubated at 37°C for one hour. The coated cells were then washed three times with 0.15M phosphate buffered saline, pH 6.4, suspended in 1.5 ml of the same buffer containing a drop of a 2.5% solution human serum albumin to stabilize the cells. The resulting suspension was approximately 2.5% cells by volume.

XVI HYBRID ANTIBODY PREPARATION

The method of preparation of hybrid antibody was that described by Paraskevas, et al (1970, 1971a). The purified anti-Ig, anti-Fe and anti-BSA antibodies were digested separately with pepsin as described previously. Their respective $F(ab')_2$ fragments were isolated by Sephadex G-100 filtration. Following the method of Nisonoff and Rivers (1961), the $F(ab')_2$ fragments of anti-Ig and either anti-Fe or anti-BSA were mixed in approximately equal amounts, according to the optical density at 280 nm in a 0.1M acetate buffer, pH 5.0, and then reduced with 0.015M 2-mercapto-ethylamine hydrochloride (Matheson Colman & Bell, Norwood, Ohio) under N_2 for 1 hour at 37°C to yield univalent F(ab') fragments. The sample was immediately passed through an AG-50W-X4 cation exchange (Bio-Rad Laboratories, Mississauga, Ontario) to remove the reducing agent. The eluate was re-oxidized by passing molecular oxygen through the solution of univalent anti-body fragments at room temperature for 2 hours. The divalent $F(ab')_2$ fragments were separated from any unoxidized fragments by G-100 chromatography.

In order to isolate the hybrid molecules, the reoxidized material was absorbed with a BDB-aggregated Fe or BSA conjugate. The eluant from this

aggregate was then adsorbed with a BDB-aggregated mouse Ig conjugate. Ouchterlony gel diffusion confirmed the presence of only the hybrid molecules in the eluant from the second aggregate.

Hybrid antibodies used in this study were:

i. antimouse immunoglobulin - antiferritin (α-MIg-α-Fe)

ii. antimouse immunoglobulin - antibovine serum albumin (α-MIg-α-BSA)

Following the terminology of Potter, et al (1965), the antimouse Ig used for these preparations reacted with heavy chains of both mouse IgG (7SIgG2a) and IgF(7SIgG1) globulins as well as with mouse light chains.

XVII REVERSE IMMUNE CYTOADHERENCE (RICA) TECHNIQUE

This technique employs a 5S hybrid antibody with one receptor site specific for mouse Ig and the other specific for another protein, either horse spleen ferritin or bovine serum albumin. Through the former site, the antibody reacts with mouse Ig on the surface of lymphocytes and through the latter it reacts with protein, coated on the surface of sheep red blood cells (SRBC) thus forming a rosette of the small SRBC about the larger lymphocyte.

The methods described by Paraskevas, et al (1970, 1971a) were followed in setting up and reading this test.

i. SPLEEN CELLS

Normal or treated DBA/2 mice were killed with chloroform and their spleens quickly removed. A suspension of cells was prepared by teasing the spleen with forceps in cold Hanks' balanced salt solution (Microbiological Associates Inc., Bethesda, Maryland). The suspension was sieved through a stainless steel mesh (200/inch) to remove tissue debris. The cells were

washed two times and separated by centrifugation at 800 r.p.m. for 7 minutes. A total cell count was made using 2% acetic acid in a hemocytometer. A viable cell count was made using the trypan blue exclusion technique.

When cells were incubated in serum before setting up the RICA technique, they were always washed three times after treatment to remove any traces of materials used for the treatment.

ii. TEST

A suspension of 7-10 x 10^5 washed spleen cells, 30 cmm of 2.5% Fe or BSA coated sheep red blood cells (approximately 100 SRBC per spleen cell) and 50 µg of the appropriate hybrid antibody was diluted to 300 cmm and the samples were incubated overnight at 4°C. For each experiment, a control employing normal spleen cells was prepared under similar conditions and controls corresponding to all experiments were prepared without addition of hybrid antibody.

The tests were read by counting cells and rosettes using Bellco slides (Bellco Glass Inc., Vineland, N.J.) with 20 mm square chambers. The cells were resuspended gently by light tapping and gentle pipetting and a sample was introduced onto the chamber using a Pasteur pipette. The chamber was covered by a 22 mm square glass coverslip and the cells were allowed time to settle. A count of 1000 spleen cells and the number of rosette forming cells (RFC) found in that number of spleen cells was made for each experiment using a phase contrast microscope (Carl Zeiss) and a 40 x phase objective. A rosette consisted of a lymphoid cell surrounded by a minimum of four SREC.

XVIII ANTI-THETA CYTOTOXICITY TEST

i. ANTI-THETA ANTISERUM (α-Θ)

Antiserum was prepared against C3H thymocytes in AKR mice according to the method of Reif and Allen (1964). Thymocytes (5-10 x 10^6) were injected intraperitoneally at weekly intervals for six weeks. The serum was collected by cardiac puncture 8-10 days after the last injection. It was absorbed at 4°C for 30 minutes with packed red blood cells from C3H, DBA/2 and Balb/c mice. The absorbed serum was decomplemented by heat inactivation at 56°C for 30 minutes, aliquoted into small amounts and stored at -20°C.

Antiserum thus prepared was over 90% cytotoxic to Balb/c and DBA/2 thymocytes at a dilution of 1:64 and the antiserum was not cytotoxic to bone marrow cells.

ii. TEST SYSTEM

The two-step procedure suggested by Batchelor (1967) was followed. The anti-theta serum diluted 1:6 was incubated with 2 x 10^6 spleen cells from normal or treated animals for 30 min at 37°C. The cells were washed once in the cold and then incubated for 45 min at 37°C with guinea pig complement (Pentex), diluted 1:3. The viable cells were determined by the trypan blue exclusion method. Spleen cells, incubated with anti-Θ serum, washed and then incubated with normal mouse serum, heat inactivated at 56°C for 45 min, were used as a control.

The number of theta-carrying cells was also determined after exposure of normal spleens to serums from treated mice. Cells treated with serum from normal mice were used as a control.

The percent cytotoxicity was calculated from the following formula

$$\% \text{ cytotoxicity} = 100 \times \frac{VCAs - VCAsC}{VCAs}$$

where

VCAs ≡ viable count of antiserum treated preparations

VCAsC ≡ viable count of antiserum plus complement treated preparations

XIX FREEZE THAWING

The L5178Y tumour cells were alternately frozen and thawed (FT) four or seven times to obtain an L5178Y preparation, free of viable cells for injection into DBA/2 mice.

XX ULTRAFILTRATION

Diaflo ultrafiltration membranes (Amicon Corp., Lexington, Massachusetts) are anisotropic molecular filters manufactured from synthetic polymers. These membranes will not denature proteins and are biologically inert. They retain molecules with size and dimension characteristics greater than their specified minimum and smaller molecules pass through the membrane. They can thus be used to describe broadly the molecular weight of materials which are passed through or retained by the membranes.

In this study, the UM10 and UM05 membranes with cut off levels of 10,000 and 500 MW respectively were used.

XXI PREPARATION OF SOLUBLE TUMOUR CELL EXTRACT (STE)

The procedure outlined by (Muramatsu, et al, 1973) was followed for the papain digestion of L5178Y cells. A suspension of 7-10 x 10^8 lymphoid cells was washed twice in Hanks' balanced salt solution and diluted to 4 ml volume. A 1 ml aliquot of a 0.3 M Tris HCl buffer, pH 8.4, containing 1 mg

mercury papain (Worthington Biochem. Co., Freehold, N.J.) and 0.00005 moles of cysteine HCl was added to the cell suspension. The mixture was incubated at 37°C for 35 minutes with periodic shaking. A 50 cmm aliquot of a 2.0M monoiodoacetic acid solution (pH adjusted to 4.4 with conc. NaOH) was added to the reaction mixture to stop the digestion and the pH of the resultant mixture was neutralized with 0.1N NaOH. The suspension was centrifuged in the cold at 10,000 r.p.m. for one hour and the supernatant was fractionated on a Sephadex G-200 column. The lowest molecular weight fraction to be eluted from the G-200 column was further fractionated by ultrafiltration through UM10 and UM05 Diaflo membranes.

The following cells were digested with papain as described.

1) ascites cells from DBA/2 carrying L5178Y leukemia (L5178Y cells plus red blood cells)
2) normal DBA/2 thymocytes
3) normal DBA/2 thymocytes plus red blood cells

XXII PREPARATION OF ANTI-L5178Y SERUM IN DBA/2 MICE (α-L5178Y)

DBA/2 mice were inoculated IP with 10^5 or 2 x 10^6 mitomycin-C-treated L5178Y cells from ascites or culture containing HS. At monthly intervals, a second, and in some cases a third injection of 10^5 mitomycin-C-treated L5178Y cells was given. Survivors were inoculated with 10^3 live L5178Y cells either one, two, or three times and serum was collected 8 days after the last injection.

XXIII TREATMENT OF L5178Y TUMOUR CELLS

A suspension of L5178Y tumour cells was prepared from cells maintained in culture in Fischer's medium with 10% FCS. The cells were centrifuged once

at 1,200 r.p.m. for 10 min at 4°C and resuspended in Minimum Essential Medium MEM (GIBCO) and 2 x 10^6 cells were incubated for 20 min at 37°C with the following solutions which had been preincubated for 10 min at 37°C and then cooled in an ice bath.

1) 50 cmm of DBA/2 NS + 50 cmm 0.15M borate saline buffer, pH 8
2) 50 cmm of DBA/2 α-L5178Y sera + 50 cmm 0.15M borate saline buffer 8
3) 50 cmm of DBA/2 α-L5178Y sera + 50 cmm STE of L5178Y (0.017 OD 280)
4) 50 cmm of DBA/2 α-L5178Y sera + 50 cmm PKIIIa soluble extract of DBA/2 thymocytes (0.017 OD 280)

The cells were washed three times with MEM and the RICA test was performed on them. Untreated tumour cells were used as a control with and without addition of hybrid. The decrease in RFC of L5178Y incubated in α-L5178Y serum upon addition of STE or PKIIIa G-200 papain digestion of thymocytes was calculated. Thus[(L5178Y + α-L5178Y S) - (L5178Y + NS)] - [(L5178Y + α-L5178Y + STE) - (L5178Y + NS)] was compared with [(L5178Y + α-L5178Y S) - (L5178Y + NS)] - [(L5178Y + α-L5178Y S + PKIIIa Thym. Prep.) - (L5178Y + NS)]. The anti-Θ test was also performed on untreated tumour cells taken from culture and ascites.

XXIV SPLEEN CELL SUSPENSIONS

RICA and/or anti-theta tests were performed as described in Materials and Methods, Sections XVII and XVIII upon cell suspensions prepared as described (Materials and Methods, Section XVII) from spleens taken from animals at the following times after the treatment specified.

i. 3,6,12 hours, 1,3,7,14 days after IP injection of 10^7, 4XFT L5178Y cells

ii. 6 hours after IP injection of 1: 5 x 10^8 HRBC, 2: 250mg Hgg/FCA 1:1, 3: 10^7 DBA/2 thymocytes, 4: 5 x 10^6 DBA/2 peritoneal cells.

iii. 6,30 hours, 3,7,14 days after IP injection of 1000 or 100 live L5178Y with and without challenge by 10^7, 7XFT L5178Y or 5 x 10^8 HRBC

iv. 6 hours after injection of G-200 peaks of papain digestion of 1: L5178Y ascites (including STE), 2: DBA/2 thymocytes, 3: DBA/2 thymocytes + blood cells

v. 6,30 hours, 3,7 days after 1 injection of STE with or without challenge by 10^7, 7XFT L5178Y or 5 x 10^8 HRBC

vi. 6,30 hours, 3,7,14 days after the first of daily injections of STE given for 4 consecutive days with and without challenge by 10^7, 7XFT L5178Y or 5 x 10^8 HRBC

vii. 6,30 hours, 7 days after 1 injection of deaggregated Hgg with or without challenge by 10^7, 7XFT L5178Y or 250 mg Hgg/FCA

viii. 6 hours, 7, 14 days after the first of daily injections of deaggregated Hgg given for 4 consecutive days with or without challenge by 10^7, 7XFT L5178Y or 250 mg Hgg/FCA

ix. 6 hours, 3,7 days after 1 injection of 0.45-μ Millipore filtrate of supernate of 1000 live L5178Y ascites with or without challenge by 10^7 7xFT L5178Y cells

XXV TREATMENT OF NORMAL SPLEEN CELL SUSPENSIONS

Spleen cell suspensions were prepared from normal DBA/2 mice,6-8 weeks old,in Hanks' balanced salt solution (Materials and Methods, Section XVII) and 5 x 10^6 cells in 200 cmm were incubated for 30 min at 30°C with 200 cmm of each of the following:

i. 3,6,12 hours, 1,3,7,14 day serum from mice given 10^7 4xFT L5178Y IP

ii. G-200 fractions of above 6-H serum,combinations of G-200 fractions of 6-H serum with NS or G-200 fractions thereof,with or without antigen

iii. 6,30 hour, 3,7,14 day serum from mice given 1,000, or 100 live L5178Y cells IP with and without challenge of 10^7 7XFT L5178Y or 5×10^8 HRBC

iv. 6 hour, 1,3,7 day serum from mice pretreated with one injection of STE with and without challenge of 10^7 7XFT L5178Y or 5×10^8 HRBC

v. 6,30 hour, 3,7,14 day serum from mice after the first of daily injections of STE given for 4 consecutive days with and without challenge of 10^7 7XFT L5178Y or 5×10^8 HRBC

After incubation, the cells were washed three times at 4°C in balanced salt solution and used in both RICA and anti-Θ cytotoxicity tests.

XXVI ASSAY FOR CYTOSTASIS OF L5178Y IN CULTURE

The assay system employed by Chia and Festenstein (1973) was followed and subsequently modified so that it could be carried out in microcultures.

i. MACROSYSTEM

Aliquots of L5178Y cells in log phase of culture at a concentration of 2.5×10^5/ml were incubated with an equal volume of spleen cells from normal and treated DBA/2 mice in lymphocyte to tumour cell ratios (L/TC) of 25/1, 50/1, 100/1 and, in some experiments, 200/1,in Falcon plastic culture tubes (Oxnard, California). The mixed cell cultures were incubated at 37°C in a 5-10% CO_2 in air atmosphere for 1,2 or 3 days. A 50 cmm aliquot of fresh medium containing approximately 0.1 μCi of I^{125} deoxyuridine (I^{125}_{UDR}) was added and the culture was carried on for 4 or 24 hours. The cells were then washed three times with Hanks' balanced salt solution and the radioactivity of the pellet was counted in a Series 1185 Automatic Gamma Counter (Nuclear -

Chicago, Des Plains, Illinois).

ii. MICROSYSTEM

According to the modification of Greenberg (1975), the method of Chia and Festenstein (1973) was scaled down for use in a microsystem. A 65 cmm aliquot of L5178Y cells in log phase culture resuspended at 4×10^4 cells/ml in fresh medium was exposed to an equal volume of spleen cells from normal or treated DBA/2 mice in L/TC ratios of 25/1, 50/1 and 100/1. The mixed cell cultures were incubated in Linbro (IS-FB-96-TC) microplates U and/or V, obtained from Linbro Chem. Co., New Haven, Connecticut, for 1, 2 or 3 days in a moist, 5-10% CO_2 in air atmosphere at 37°C. At the end of this time, a 90 cmm aliquot of fresh medium containing 0.5-1.0 µCi I^{125} UDR/ml was added to each culture. The microcultures were harvested 20 hours later using a multiple cell culture harvester, obtained from Skatron A.S., Heggtoppen, Norway, which employs glass fibre filters (Skatron A.S.). The filters were air dried and counted in a gamma counter as mentioned previously.

The 3 day cultures were refed with 40 cmm of fresh medium after 48 hours of culture.

The lymphoid cells incubated with the L5178Y cells in these experiments were taken from DBA/2 mice which had received the following treatment.

(1) no treatment

(2) IP injection of 10^7, 7XFT L5178Y cells 10-19 days previously

(3) IP injection of 5×10^8 HRBC 10-19 days previously

(4) IP injection of 10^7, 7XFT L5178Y cells 10-19 days previously preceded by one daily injection of STE for four consecutive days beginning at day -7

(5) IP injection of 10^7, 7XFT L5178Y 10-19 days previously preceded by one daily injection of deaggregated Hgg for four consecutive days beginning at day -7

Cultures of all of these lymphoid cells without L5178Y tumour cells were used for controls.

The growth inhibition was calculated according to the following formula.

$$\% \text{ growth inhibition} = 100 \times \left[1 - \frac{T.L_T - L_T}{T.L_N - L_N}\right]$$

where

$T.L_T \equiv$ counts per min L5178Y:treated spleen cell cultures

$T.L_N \equiv$ counts per min L5178Y:normal spleen cell cultures

$L_T \equiv$ counts per min treated spleen cell cultures

$L_N \equiv$ counts per min normal spleen cell cultures

The same calculations were performed substituting the counts per minute in the appropriate HRBC sensitized spleen cell cultures for the normal spleen cell culture (with and without L5178Y cells) in order to eliminate tumour cell growth inhibition which may be due to non-specific sensitization.

XXVII STATISTICAL ANALYSIS

All statistical analyses were carried out using a multiple T-Test program on a Control Data Corp. System 1700 computer. Differences with a probability greater than one percent that they could have occurred randomly, were not considered significant unless otherwise noted.

EXPERIMENTAL RESULTS

I. IMMUNOGLOBULIN BEARING CELLS IN NORMAL MOUSE SPLEENS

Normal mouse spleen cells which exhibit immunoglobulin on their surface are capable of forming rosettes in the presence of 5S hybrid antibody and erythrocyte indicator cells (Figure 1). (All methods used are described previously in the Materials and Methods Section.) Cells lacking immunoglobulin on their surface remain unattached. Two hydrid antibodies, α-MIg-α-Fe and α-MIg-α-BSA which reacted with all classes of mouse Ig, were used in this study. The former detected approximately 30% of normal DBA/2 spleen cells as being Ig bearing (mean $\pm$ SD of 53 mice, 302.9 $\pm$ 20.7 RFC/1000 spleen cells). The latter detected about 40% of normal spleen cells (mean $\pm$ SD of 142 mice, 394.7 $\pm$ 21.4). The difference in the two test systems is probably related to a number of variables, i.e. different charge effects and antibody affinities involved in the use of different proteins for coating sheep red blood cells. Differences in the density of the two protein coatings on SRBC could also lead to the detection of a different proportion of spleen cells. A normal control was tested with every experiment and the results which follow compare the Ig bearing spleen cells of groups of experimental animals with those of normals for which the same hybrid antibody test system was used.

II. Ig BEARING SPLEEN CELLS

SIX HOURS AFTER ADMINISTRATION OF ANTIGEN

Spleen cells of DBA/2 mice were examined for surface Ig six hours after IP administration of a variety of antigens. Heterologous erythrocytes HRBC alone and Hgg emulsified in FCA produced statistically significant increases in Ig bearing spleen cells ($p < .001$).

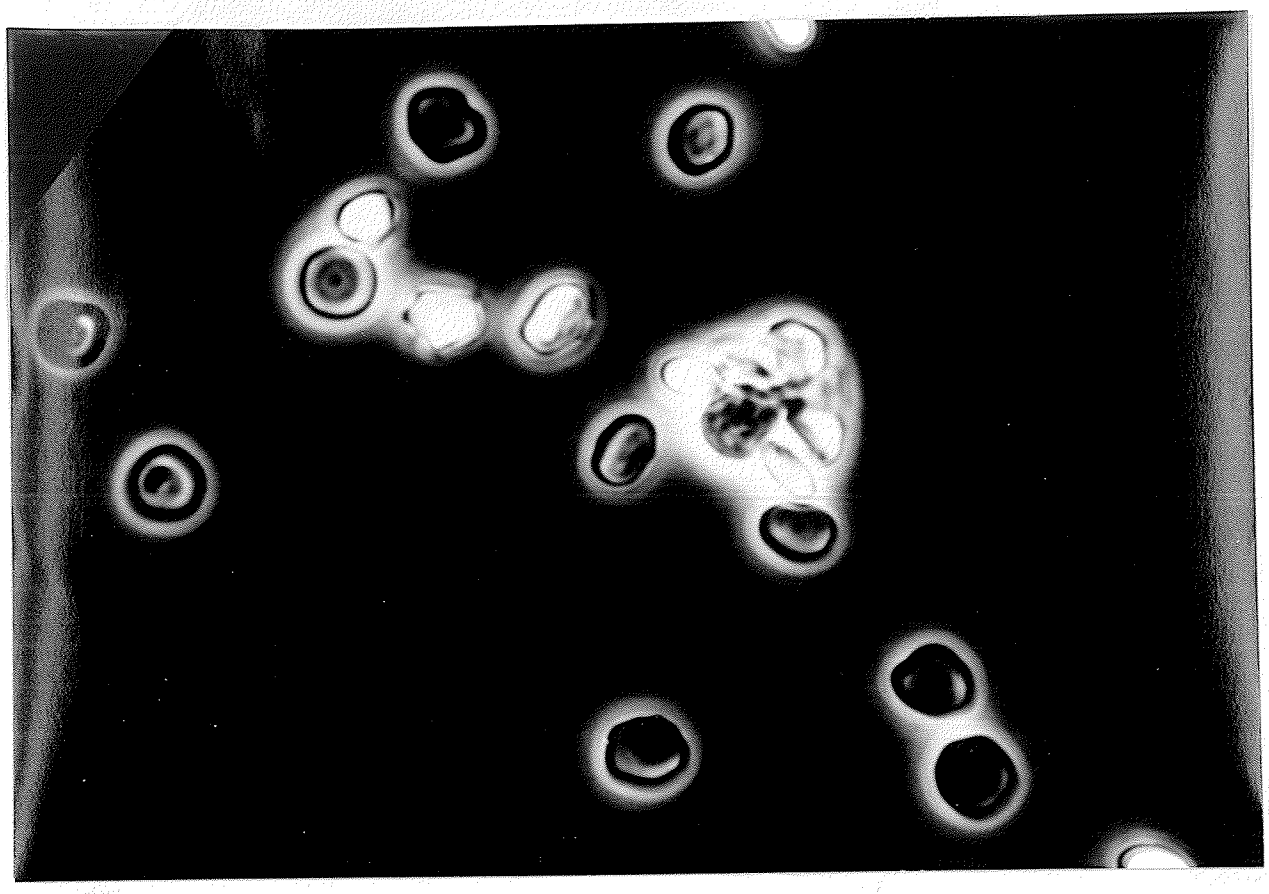

Figure 1: Rosette forming cell in normal DBA/2 mouse spleen examined by phase contrast microscopy.

A large dose of syngeneic L5178Y leukemia cells, 10^7, living or frozen and thawed (FT) produced statistically significant increases in Ig bearing spleen cells ($p < .001$) amounting to about 20% above the normal level. As the L5178Y cells were shown to carry the Θ determinant (described later) syngeneic thymocyctes as well as peritoneal cells and the supernate of 10^7 ascites cells were examined and did not produce any six hour increase in Ig bearing cells as shown in Table I.

The mean $\pm$ SD of the spleen weight of normal mice was 80.6 ± 17.1 mg compared with the mean $\pm$ SD of the spleen weight of mice which exhibited a six hour increase in Ig carrying spleen cells, 72.6 ± 15.1 mg. Similarly, the total spleen cell count of normal mice was $9.7 \pm 3.3 \times 10^7$ cells while that of mice exhibiting a six hour increase of Ig bearing cells was $7.6 \pm 2.8 \times 10^7$. Thus the increase in Ig bearing spleen cells six hours after administration of antigen was not associated with any signifigant change in spleen weight or total spleen cell count.

III. CHANGES IN SPLEEN CELL POPULATION AFTER ADMINISTRATION OF 10^7, 4xFT L5178Y CELLS

i. Ig POSITIVE CELLS

Intraperitoneal injection of 10^7, four times frozen and thawed (4 x FT), L5178Y cells, which produced a significant six hour increase in Ig bearing spleen cells produced no significant change from normal levels at the other time intervals examined. These tests were carried out for two weeks after the inoculation, as seen in Figure 2.

ii. Θ POSITIVE CELLS

Examination of the Θ bearing spleen cell population, using anti-Θ serum

TABLE I

% Change in Ig+ Spleen Cells Six Hours After Administration of Antigen

Treatment	% Change Ig+ Spleen Cells ± SD
5 x 10^6 DBA/2 Peritoneal cells	-3.0 ± 2.3 (2)[a]
10^7 DBA/2 Thymocytes	-4.2 ± 0.9 (3)
10^7 L5178Y, 4xFT	20.4 ± 5.7 (4)
10^7 L5178Y, live	21.4 ± 3.7 (3)
5 x 10^8 HRBC	21.7 ± 3.4 (3)
Supernate of 10^7 L5178Y Ascities	1.3 ± 5.2 (3)
250 µgHgg/FCA 1:1	21.0 ± 2.7 (4)
None	0.0 ± 5.4 (142)

[a]The numbers in parenthesis in all tables are the numbers of animals examined

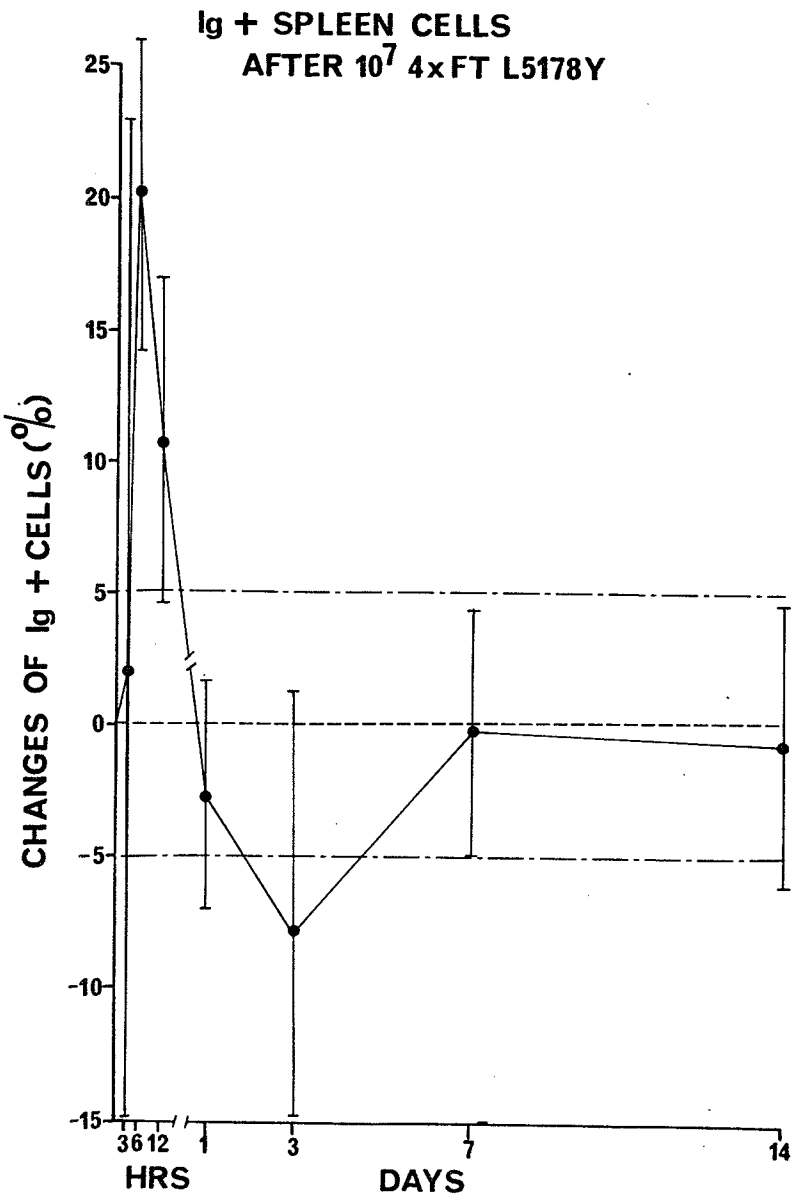

Figure 2: Changes in Ig+ spleen cells are expressed as a percentage of the normal count.

and complement revealed that concomitant with the six hour increase in Ig bearing cells, a significant decrease ($p < .001$) of approximately the same magnitude, occurred in the Θ bearing population. No other significant change from normal levels was observed in the Θ bearing spleen cell population for up to two weeks after administration of the tumour cells as seen in Figure 3.

IV. EFFECT ON NORMAL SPLEEN CELLS OF SERA TAKEN AT INTERVALS AFTER ADMINISTRATION OF 10^7, 4xFT L5178Y CELLS

i. Ig POSITIVE CELLS

Normal spleen cells were incubated for 30 minutes at 37° C in an aliquot of serum taken from mice at different time intervals after administration of 10^7, 4xFT, L5178Y cells. This treatment revealed that the resulting Ig bearing and Θ bearing spleen cell populations reproduced these resulting Ig from the spleen cell populations of the donor mice (Tables II and III). Thus a statistically significant increase in Ig bearing cells $p < .01$) was observed upon incubation of normal spleen cells in serum taken six hours after administration of 10^7 live or frozen and thawed L5178Y cells (6-HS)(Table II).

ii. Θ POSITIVE CELLS

Conversely a significant decrease ($p < .001$) was observed in the Θ bearing population of the same cells obtained after incubation of normal spleen cells in 6-HS as seen in Table III. Thus the six hour increase in Ig bearing spleen cells was thought to be due to uptake of cytophilic Ig by T cells.

As well the three hour serum produced a partial increase in Ig carrying cells concomitant with a partial decrease in the Θ bearing population ($p < .001$ for the latter).

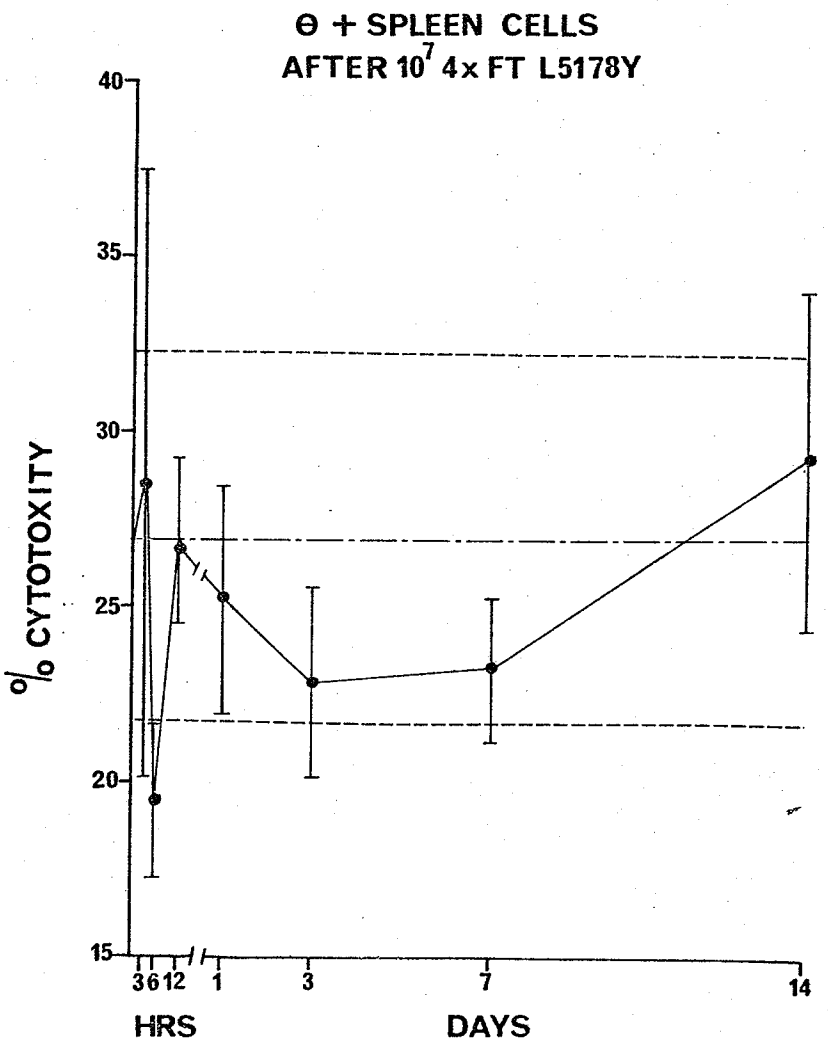

Figure 3: θ+ spleen cells are expressed as the percentage of spleen cells killed by anti-θ serum and complement.

TABLE II

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After IP Inoculation of 10^7, 4xFT, L5178Y Cells

Time Sera Taken[a]	% Change Ig+ Spleen Cells ± SD
3H	9.6 ± 5.4 (3)
6H	22.0 ± 1.2 (2)
6H[b]	18.3 ± 4.9 (3)
24H	-21.7 ±29.1 (3)
3D	-7.5 ± 5.5 (3)
7D	-9.8 ±11.6 (4)
14D	-3.4 ±11.5 (4)
NS	0.0 ± 9.5 (7)

[a]Time after injection of 10^7, 4xFT, L5178Y cells when sera were taken.

[b]Live L5178Y cells (10^7) were used in this case.

TABLE III

% Θ+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After IP Inoculation of 10^7, 4xFT L5178Y Cells

Time Sera Taken[a]	% Θ+ Spleen Cells ± SD
3H	18.3 ± 3.4 (6)
6H	16.6 ± 1.8 (4)
12H	30.0 ± 7.6 (3)
24H	20.9 ± 8.5 (3)
3D	23.3 ±10.6 (5)
7D	23.9 ± 2.8 (4)
14D	22.9 ± 5.2 (8)
NS	28.6 ± 5.7 (34)

[a]Time after injection of 10^7, 4xFT L5178Y cells when sera were taken.

V. EFFECT OF 6-HS ON θ-DEPLETED NORMAL SPLEEN CELLS

Normal spleen cells were treated with AKR anti-θ serum and complement. The base level of Ig bearing cells was consequently higher but the resulting population of cells was found incapable of producing an increase in Ig bearing spleen cells upon incubation in 6-HS. In contrast, the same normal spleen cells incubated in normal AKR serum in the presence of complement produced a population of cells quite able to produce a significant increase in Ig bearing cells ($p < .001$) upon incubation in 6-HS (Table IV).

These results corroborated the previous observations that the increase in Ig carrying spleen cells, upon incubation of normal spleen cells in 6-HS, was related to uptake of at least cytophilic Ig by T cells.

Work previously done in this laboratory had shown that six hour serum obtained following injection of a number of antigens contained a 7S fraction which reproduced all the activities of the six hour serum (Orr and Paraskevas 1973). Thus the 6-HS obtained after administration of L5178Y cells was examined for this activity.

VI. EFFECT OF G-200 FRACTIONS OF 6-HS ON NORMAL SPLEEN CELLS

Six hour serum obtained following injection of 10^7, 7xFT L5178Y cells was separated on a Sephadex G-200 column (Figure 4). Three major protein peaks were eluted, the 19S, 7S and 4S. Incubation of normal spleen cells at 37° C for 30 minutes in aliquots of the individual fractions showed that a significant increase in Ig bearing cells ($p < .01$) was obtained only with the use of the 7S fraction of 6-HS (Table V). Similarly a significant decrease in the θ bearing population ($p < .001$) was demonstrated in the case of the 7S

TABLE IV

Uptake of Cytophilic Ig by T Cells Demonstrated by Inhibition with Anti-Θ Serum and Complement Treatment.

Treatment of Spleen Cells	Number RFC/1000 Spleen Cells ± SD	% Increase Ig+ Cells Incubated in 6-HS.
AKR α-Θ + C + [DBA/2 NS]	490.3 ± 14.7 (4)	
AKR α-Θ + C + [DBA/2 6-HS]	491.3 ± 30.9 (4)	0.2 ± 6.3
AKR NS + C + [DBA/2 NS]	404.0 ± 11.5 (4)	
AKR NS + C + [DBA/2 6-HS]	478.5 ± 16.1 (4)	18.4 ± 3.4

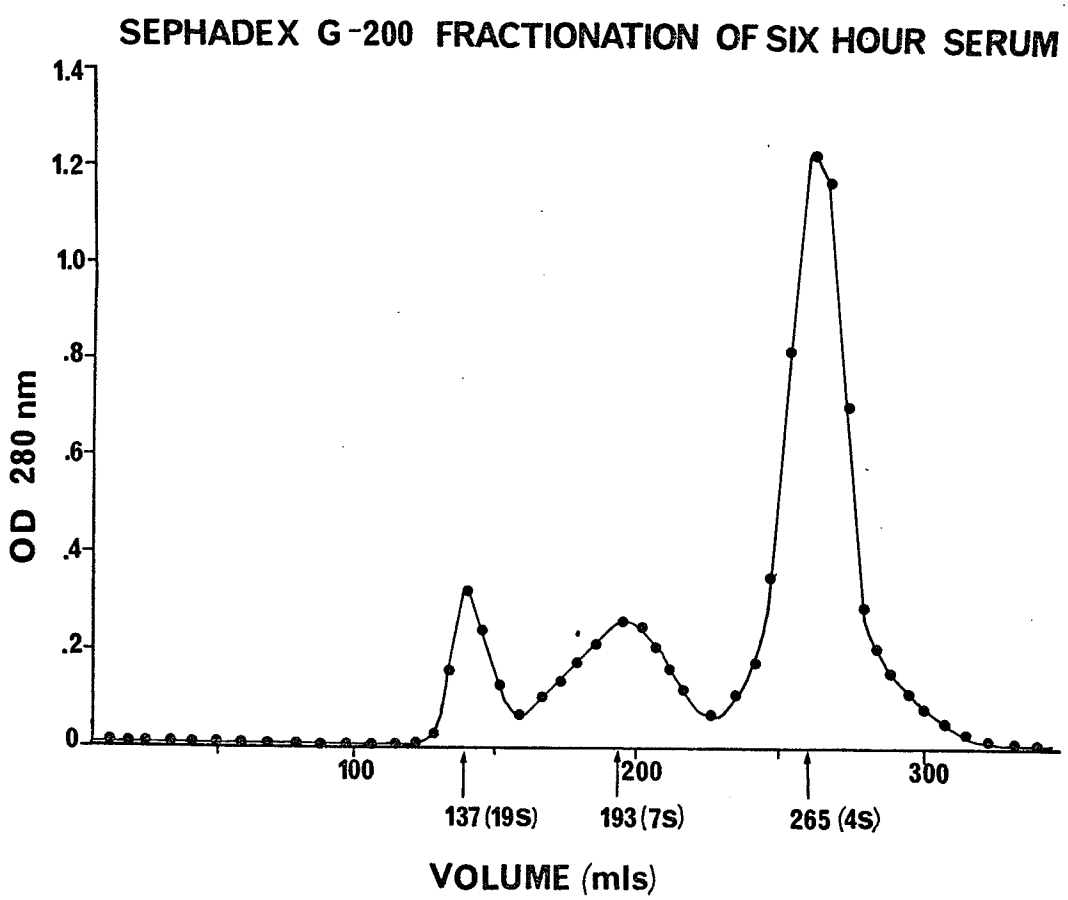
SEPHADEX G-200 FRACTIONATION OF SIX HOUR SERUM
OD 280 nm
1.4
1.2
1.0
.8
.6
.4
.2
0
100
200
300
137 (19S)
193 (7S)
265 (4S)
VOLUME (mls)

Figure 4:

TABLE V

% Change in Ig+ Spleen Cells:

Effect of Sephadex G-200 Fractions of 6-HS on Normal Spleen Cells.

Treatment	% Change in Ig+ Spleen Cells ± SD
6-H-19S	-7.5 ± 13.2 (7)
6-H- 7S	22.6 ± 16.7 (6)
6-H- 4S	4.3 ± 1.4 (6)
NS	0.0 ± 9.5 (7)

fraction of 6-HS (Table VI). Thus the 7S fraction of 6-HS appears to contain all of the activity of the 6-HS and the cytophilic Ig involved in this phenomenon appears to be a 7S Ig.

As studies by other investigators in this laboratory had revealed that the 4S fraction of six hour serum obtained after IP administration of a particulate antigen was active in the presence of antigen and normal mouse serum the 4S and 19S fractions of the L5178Y 6-HS were examined in the same manner.

VII. EFFECT OF 4S AND 19S FRACTIONS OF 6-HS ON NORMAL SPLEEN CELLS IN THE PRESENCE OF NS AND ANTIGEN

As seen in Table VII the 4S fraction of 6-HS in the presence of normal mouse serum (NS) and an unrelated soluble antigen (BSA) was capable of producing a statistically significant increase in Ig bearing spleen cells ($p < .01$). In contrast the 19S fraction did not display any such activity. Concomitant with the increase in Ig bearing cells, incubation of normal spleen cells in the 4S fraction of 6-HS in the presence of BSA produced a significant decrease ($p < .001$) in θ bearing cells while the 19S fraction had no effect (Table VIII).

Thus there appeared to be an active factor in the 4S fraction of this 6-HS which was capable of inducing the uptake of cytophilic Ig by T cells in the presence of soluble antigen.

VIII. EFFECT OF 4S FRACTION OF 6-HS ON NORMAL SPLEEN CELLS IN THE PRESENCE OF ANTIGEN AND DIFFERENT FRACTIONS OF NS

Further, similar Sephadex G-200 fractionation of normal mouse serum (NS) followed by incubation of normal spleen cells in the 4S fraction of L5178Y 6-HS plus soluble antigen with one of the three fractions of NS showed no cooperative ability in the 19S or 4S fractions of NS. Only the 7S fraction

TABLE VI

% Θ+ Spleen Cells:

Effect of Sephadex G-200 Fractions of 6-HS on Normal Spleen Cells

Treatment	% Θ+ Spleen Cells ± SD
6-H-19S	26.4 ± 4.1 (7)
6-H- 7S	16.6 ± 4.5 (6)
6-H- 4S	27.5 ± 4.6 (5)
NS	28.6 ± 5.7 (34)

TABLE VII

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sephadex G-200 Fractions 4S and 19S of 6-HS with NS and Soluble Protein Antigen

Treatment	% Change in Ig+ Spleen Cells ± SD
6-H-19S + NS	-2.8 ± 4.8 (6)
6-H-19S + NS + BSA	-7.4 ±12.6 (8)
6-H-4S + NS	-0.4 ±10.5 (6)
6-H-4S + NS + BSA	17.8 ±14.0 (7)
NS	0.0 ± 9.5 (7)

TABLE VIII

% Θ+ Spleen Cells:

Effect on Normal Spleen Cells of Sephadex G-200 Fractions 4S and 19S of 6-HS with NS and Soluble Protein Antigen

Treatment	% Θ+ Spleen Cells ± SD
6-H-19S + NS	24.9 ± 2.4 (6)
6-H-19S + NS + BSA	24.8 ± 5.0 (8)
6-H-4S + NS	25.7 ± 2.8 (6)
6-H-4S + NS + BSA	13.0 ± 5.4 (7)
NS	28.6 ± 5.7 (34)

was able to interact with the active 4S fraction of 6-HS in the presence of soluble antigen, to produce a statistically significant increase in Ig bearing cells ($p < .01$) (Table IX). Similarly only the 7S fraction of NS was active in co-operating to produce significant decreases in Θ bearing cells in the presence of the 4S fraction of 6-HS and either BSA ($p < .001$) or FIB ($p < .01$) (Table X).

Thus the observation of an increase in Ig bearing spleen cells with a concomitant decrease in the Θ bearing population six hours after administration of a large dose of L5178Y cells appeared to involve the action of an active 4S serum factor in the uptake of at least cytophilic 7S Ig by T cells. From these results it is evident that the L5178Y cells in the syngeneic DBA/2 mice express antigenic qualities similar to a wide variety of antigens previously tested in this laboratory (Paraskevas et al 1972).

As the six hour response involves large cell population changes and is one of the earliest occurrences following exposure to antigen it was considered to be a probe which might prove fruitful in detecting changes in the lymphoid system of mice bearing a growing tumour. Our interests were in studying animals with tumours at the very early stages of development. Thus it was desirable to simulate the progress of a naturally arising tumour by initiating tumour growth with a small number of cells which would still produce a high percentage of deaths. For this purpose the biological viability of our L5178Y line was assessed in DBA/2 mice.

IX. THE RELATIONSHIP OF TUMOUR CELL DOSE WITH BIOLOGICAL VIABILITY AND SIX HOUR RESPONSE

In order to determine the lowest doses of tumour cells which would kill

TABLE IX

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of 4S Fraction of 6-HS, Antigen And Sephadex G-200 Fractions of NS

Treatment	% Change in Ig+ Spleen Cells ± SD
6-H-4S + FIB. + NS-19S	0.4 ± 3.1 (4)
6-H-4S + FIB. + NS-7S	22.4 ± 1.6 (4)
6-H-4S + FIB. + NS-4S	6.2 ± 2.2 (4)
NS	0.0 ± 9.5 (7)

TABLE X

% Θ+ Spleen Cells:

Effect on Normal Spleen Cells of 4S Fraction of 6-HS, Antigen and Sephadex G-200 Fractions of NS

Treatment	% Θ+ Spleen Cells ± SD
6-H-4S + FIB + NS-19S	37.4 ± 1.6 (2)
6-H-4S + FIB + NS -7S	15.1 ± 2.3 (2)
6-H-4S + FIB + NS -4S	30.7 ± 3.5 (2)
6-H-4S + BSA + NS-19S	21.9 ± 8.9 (3)
6-H-4S + BSA + NS-7S	13.3 ± 3.9 (3)
6-H-4S + BSA + NS-4S	23.6 ± 6.5 (3)
NS	28.6 ± 5.7 (34)

a high proportion of mice, the time of death of mice was observed following an IP inoculation of the live tumour cells given at different log concentrations. As seen in Figure 5 the plot of mean survival time versus the log of the dose of tumour cells given was a relatively straight line. The least squares regression line had a slope of -2.3 and a Y intercept of 26.6.

A dose of 1000 cells or more killed 100% of animals tested.

The results of a study of the ability of the lowest doses to produce both animal death and a six hour response are shown in Table XI. An inoculum of 10 live cells produced no six hour increase in Ig bearing spleen cells but only killed 29% of the mice injected. It was not known whether the small proportion of tumour take was due to the possibility that the tumour cells injected had a low potential for initiating a progressively growing tumour. Alternatively, the host animals may have been able to produce an effective response against this small number of cells, although,other investigators have reported that peritoneal injection of from 10 to 100 cells routinely gives rise to fatal tumours in greater than 85% of the recipients (Goldstein and Manson 1975).

A 100 cell inoculum of live L5178Y cells also produced no significant six hour increase but killed 95% of mice injected. Administration of 1000 cells killed every animal thus treated and produced a partial (60%) but still statistically significant six hour response ($p < .001$).

Thus animals bearing tumours growing from inoculae of live L5178Y cells in the order of 100 and 1000 cells were considered worthwhile to examine.

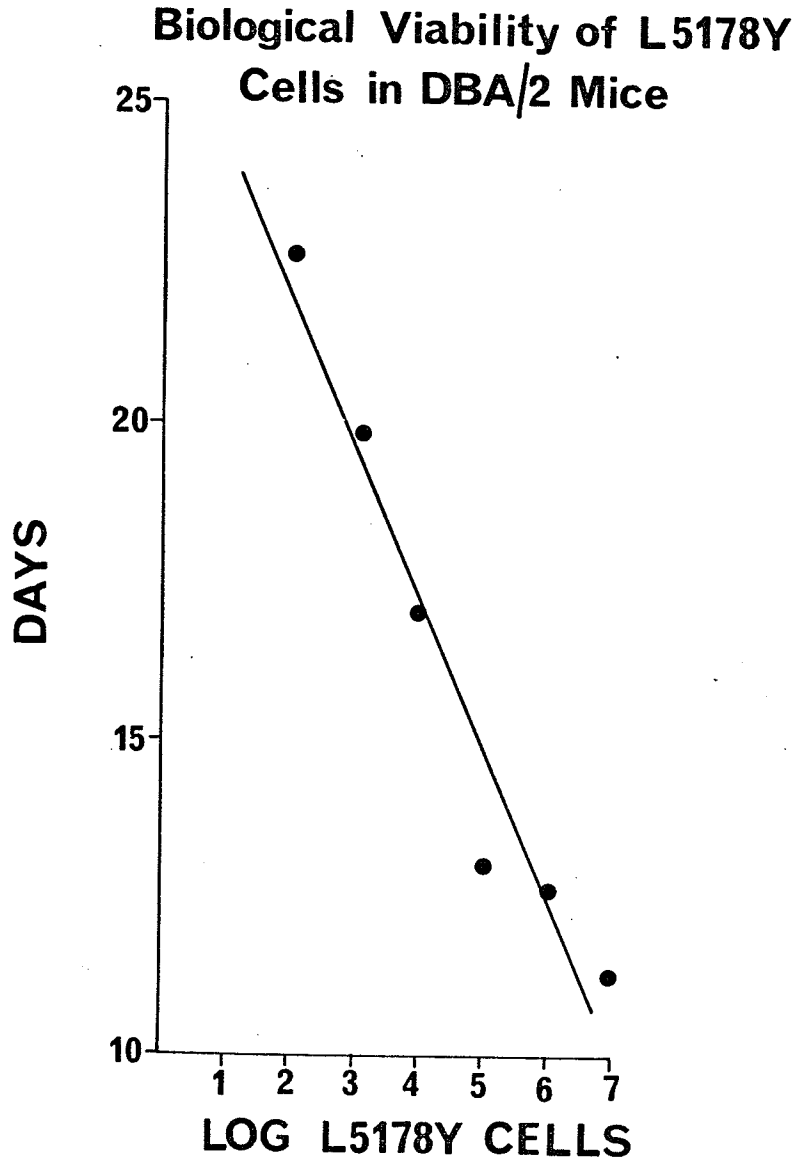

Figure 5: The number of days represents the mean survival time of mice inoculated with live L5178Y cells.

TABLE XI

Titration of Biological Viability and Six Hour Increase in Ig+ Spleen Cells with Injection of Viable L5178Y Cells.

Number L5178Y Injected	% Increase Ig+ Spleen Cells After 6H ± SD	% Animals Killed
1000	12.0 ± 8.4 (12)	100 (20)
100	3.0 ± 6.9 (9)	95 (20)
10	2.6 ± 9.9 (11)	29 (62)

X. CHANGES IN SPLEEN CELL POPULATIONS FOLLOWING INOCULATION WITH 100 LIVE L5178Y CELLS

i. Ig POSITIVE CELLS

As shown in Figure 6 the percentage of Ig bearing cells following IP inoculation of 100 live tumour cells remained in the normal range from six hours to thirty hours but on day three a significant increase above the normal was observed ($p<.001$). The level of Ig bearing cells was back in the normal range on days seven and fourteen. The ascites tumour became noticeable in these mice at approximately two and one-half weeks after initiation of tumour growth and the animals died by day thirty.

Mice bearing tumours growing from an inoculum of 100 live L5178Y cells were given an antigen challenge at different intervals after the live cell inoculum. Thus it was shown that these mice failed to produce a six hour increase in Ig bearing spleen cells in response to 10^7, 7xFT, L5178Y cells given on day one to at least day seven after initiation of tumour growth (Figure 6). Similarly no response was detected six hours after a challenging dose of HRBC ($5x10^8$) was administered at the same time intervals. In fact, at day three when a peak of Ig bearing cells occurs in these tumour bearing mice, administration of challenging doses of L5178Y cells or HRBC resulted in a decrease of Ig bearing cells which was statistically significant in the case of HRBC ($p < .005$).

By day fourteen the tumour bearing animals appeared to be capable of producing a partial although not significant six hour increase.

ii. Θ POSITIVE CELLS

Examination of the Θ bearing population of spleen cells in mice bearing

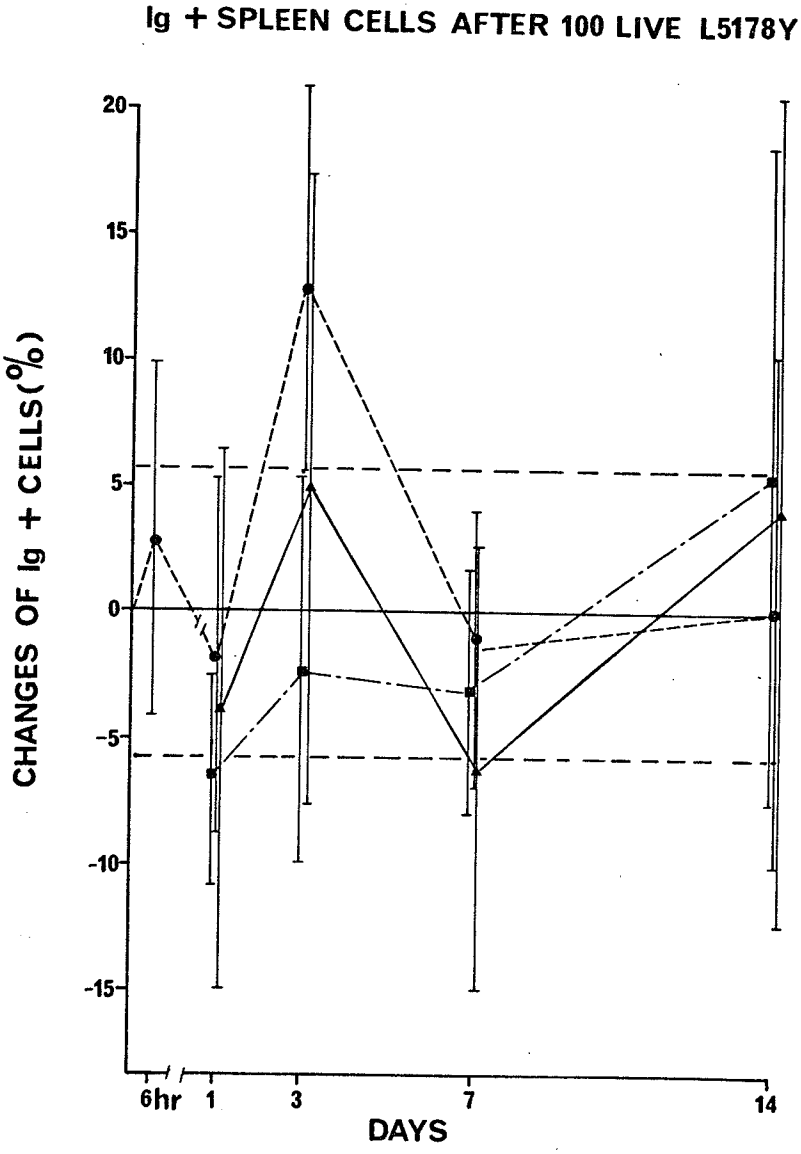

Figure 6: No challenge ●--●; 6H 10^7,7xFT L5178Y challenge ▲——▲; 6H $5x10^8$ HRBC challenge ■---■.

tumours growing from a 100 cell inoculum showed that a significant decrease in the Θ bearing population ($p < .001$) occurred on day three concomitant with the increase in Ig bearing cells (Table XII). By days seven and fourteen the level of Θ-bearing cells returned to normal. Challenge on day three with either 10^7 L5178Y or $5x10^8$ HRBC produced a significant increase in Θ-bearing cells six hours later ($p < .01$). On day seven the mice were also found to be incapable of producing a six hour decrease in Θ-bearing cells in response to challenge by either antigen.

By day fourteen administration of the same doses of L5178Y or HRBC produced at least a partial six hour decrease of Θ-bearing cells which was not signifigant.

Thus the changes in the Ig bearing spleen cell population were mirrored by changes in the Θ-bearing population and the tumour bearing animals were found to be incapable of responding to specific and non-specific antigen challenge from day one to day seven after initiation of tumour growth.

In order to determine whether the observed defect was present in the serum of the tumour bearing mice the ability of the sera from all of these mice to alter the populations of cells in normal spleens was examined.

XI. EFFECT ON NORMAL SPLEEN CELLS OF SERA FROM MICE BEARING TUMOURS GROWING FROM 100 LIVE L5178Y CELLS

i. Ig POSITIVE CELLS

Incubation of normal spleen cells in serum taken from mice six hours after inoculation of 100 live tumour cells produced a partial but still significant increase in Ig bearing cells ($p < .001$). Otherwise, incubation of normal spleen cells at 37° for 30 minutes in sera from tumour bearing mice with and without antigen challenge reproduced the same results obtained

TABLE XII

% Θ+ Spleen Cells

After Inoculation of 100 Live L5178Y Cells.

Time after 100	% Θ+ Spleen Cells ± SD		
L5178Y Given	No Challenge	L5178Y Challenge[a]	HRBC Challenge[b]
3D	13.1 ± 5.1 (4)	30.3 ± 9.0 (4)	25.4 ± 3.4 (4)
7D	23.3 ± 6.3 (6)	20.7 ± 8.6 (6)	28.1 ± 3.0 (4)
14D	25.2 ±10.0 (6)	19.7 ± 7.6 (5)	18.1 ± 2.9 (4)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT L5178Y Cells.

[b]Six hours after challenge with $5x10^8$ HRBC

from the spleen cells of the animals which provided the sera (Table XIII). No increase in Ig bearing spleen cells was produced from serums of challenged animals compared with that of tumour bearer controls taken from days one to seven after live cell inoculation. Incubation of normal spleen cells in serum from animals challenged with either L5178Y or HRBC fourteen days after initiation of tumour growth showed a significant increase in Ig bearing cells over incubation of the same cells in serum from unchallenged fourteen day tumour bearers ($p < .001$). Also a significant increase in Ig bearing cells ($p < .005$) was observed following incubation of normal spleen cells in serum taken three days after inoculation of 100 live L5178Y cells and serum from animals which were challenged on that day produced slight decreases in the Ig bearing population of normal spleen cells.

ii. Θ POSITIVE CELLS

An insignifigant decrease in the Θ-bearing population was observed upon incubation normal spleen cells in serum taken three days after live tumour cell inoculation (Table XIV). Serum from three day tumour bearers challenged with HRBC or L5178Y cells produced only slight changes in the Θ-bearing population of normal spleen cells. No significant decrease in Θ-bearing cells was observed, upon examination of normal spleen cells incubated in serum taken from animals challenged with L5178Y or HRBC on days three, seven or fourteen after initiation of tumour growth.

Thus the tumour bearer animals challenged with specific and non-specific antigen have been shown to be incapable of exhibiting a six hour response in their spleen cells or their serum from day one to at least day seven after initiation of tumour growth.

TABLE XIII

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After Inoculation with 100 Live L5178Y Cells.

Time Sera Taken[a]	% Change in Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
6H	15.4 ± 11.8 (6)		
30H	-5.2 ± 4.3 (4)	8.9 ± 20.8 (4)	3.4 ± 19.4 (4)
3D	11.7 ± 9.5 (6)	9.9 ± 9.4 (6)	10.4 ± 11.4 (6)
7D	-6.1 ± 7.5 (4)	-7.9 ± 7.5 (4)	-5.4 ± 3.1 (4)
14D	8.2 ± 7.7 (4)	26.2 ± 9.9 (4)	27.2 ± 5.7 (4)
NS	0.0 ± 7.4 (38)		

[a]Time after inoculation of 100 live cells when sera were taken.

[b]Sera from tumour bearers taken six hours after challenge with 10^7, 7xFT L5178Y cells.

[c]Sera from tumour bearers taken six hours after challenge with $5x10^8$ HRBC.

TABLE XIV

% θ+ Spleen Cells

Effect on Normal Spleen Cells of Sera Taken After Inoculation with 100 Live L5178Y Cells.

Time Sera Taken[a]	% θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
3D	22.2 ± 4.1 (4)	27.3 ± 7.2 (4)	26.6 ± 4.9 (4)
7D	31.6 ± 6.7 (4)	30.0 ± 7.8 (4)	28.0 ± 5.8 (4)
14D	28.9 ± 6.3 (4)	28.1 ± 2.8 (4)	24.8 ±10.3 (4)
NS	28.6 ± 5.7 (34)		

[a]Time after inoculation of 100 live cells when sera were taken.

[b]Sera from tumour bearers taken six hours after challenge with 10^7, 7xFT L5178Y cells.

[c]Sera from tumour bearers taken six hours after challenge with $5x10^8$ HRBC.

In order to assess the effect of tumour growth from a dose of live cells which produced 100% kill and only a partial six hour response, tumour growth was initiated with 1000 live cells as well.

XII. CHANGES IN SPLEEN CELL POPULATIONS FOLLOWING INOCULATION WITH 1000 LIVE L5178Y CELLS

i. Ig POSITIVE CELLS

As seen in Table XV, after a significant, although partial, increase in Ig bearing cells six hours after initiation of tumour growth ($p < .001$) the Ig bearing population returned to normal at thirty hours. Similar to inoculation of 100 live cells a significant increase was observed at day three ($p < .001$) and the Ig bearing spleen cell population returned to normal levels by day seven and remained there on day fourteen. These tumour bearing animals were unable to produce a six hour increase in Ig bearing cells following antigen challenge on day one to day seven after inoculation of 1000 live tumour cells. In fact on day three, challenge of mice by either L5178Y cells or HRBC resulted in a depression of the Ig bearing cell population ($p < .005$) compared with similar but unchallenged tumour bearers.

ii. θ POSITIVE CELLS

The changes in the θ population mirrored the changes in Ig bearing cells (Table XVI). Following inoculation of 1000 live L5178Y cells a decrease in the percentage of splenic θ-bearing cells ($p < .001$) was observed after three days. This returned to normal by day seven and remained there at day fourteen. On day three there was an increase in the θ-bearing population six hours after challenge with either L5178Y cells or HRBC and this increase was significant in the case of L5178Y cells ($p < .005$). The mice bearing tumours growing from

TABLE XV

% Change in Ig+ Spleen Cells

After Inoculation with 1000 Live L5178Y Cells.

Time after 1000 L5178Y Given	% Change in Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	HRBC Challenge[b]
6H	12.0 ± 8.4 (12)		
30H	0.1 ± 8.3 (6)	-0.5 ± 7.6 (6)	-12.0 ± 11.3 (4)
2D	8.9 ±10.8 (2)	-1.3 ± 1.6 (2)	- 3.1 ± 14.9 (2)
3D	21.4 ±13.6 (8)	2.0 ± 9.8 (8)	-4.9 ± 5.3 (4)
7D	-0.8 ± 7.9 (6)	-4.7 ± 3.5 (6)	-6.5 ± 9.9 (4)
14D	-4.0 ± 8.3 (6)	10.4 ± 9.6 (6)	13.0 ± 5.7 (4)
Normal	0.0 ± 5.4 (142)		

[a]Six hours after challenge with 10^7, 7xFT L5178Y cells.

[b]Six hours after challenge with $5x10^8$ HRBC.

TABLE XVI

% Θ+ Spleen Cells

After Inoculation of 1000 Live L5178Y Cells.

Time after 1000	% Θ+ Spleen Cells ± SD		
L5178Y Given	No Challenge	L5178Y Challenge[a]	HRBC Challenge[b]
3D	13.6 ± 4.5 (4)	29.6 ± 5.7 (4)	24.7 ± 11.1 (4)
7D	24.2 ± 5.6 (4)	25.9 ± 7.5 (4)	26.7 ± 7.2 (4)
14D	23.7 ± 2.3 (4)	17.1 ± 9.0 (4)	17.5 ± 7.4 (4)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT L5178Y cells.

[b]Six hours after challenge with $5x10^8$ HRBC.

1000 cells were unable to produce a six hour decrease in the splenic θ-bearing population up to at least day seven after initiation of tumour growth. On day fourteen a partial six hour decrease is demonstrated although it was not statistically significant.

XIII. EFFECT ON NORMAL SPLEEN CELLS OF SERA FROM MICE BEARING TUMOURS GROWING FROM 1000 LIVE L5178Y CELLS

i. Ig POSITIVE CELLS .

The essential features of the spleen cell changes observed in animals bearing tumours growing from an IP inoculum of 1000 live cells were reproduced upon incubation of normal spleen cells in their sera (Table XVII). A statistically significant although partial, increase in Ig bearing cells was found in the case of serum taken six hours after the injection of 1000 live tumour cells ($p < .01$). The level of Ig bearing cells also showed a significant increase upon incubation of normal spleen cells in three day sera ($p < .001$). Otherwise sera taken from mice at thirty hours and seven and fourteen days after initiation of tumour growth produced no change. Table XVII also shows that sera taken from tumour bearers challenged from thirty hours up to at least day seven were incapable of producing a six hour increase in Ig positive normal spleen cells. In fact a decrease in Ig bearing cells was found upon use of sera from three day animals which were challenged with either L5178Y cells or HRBC. The biological signifigance of this is unknown.

The sera from animals challenged with either of these same antigens on day fourteen after initiation of tumour growth showed an increase of Ig bearing cells compared with the sera of unchallenged fourteen day tumour bearers.

TABLE XVII

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After Inoculation with 1000 Live L5178Y Cells.

Time Sera Taken[a]	% change in Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
6H	12.5 ± 12.9 (3)		
30H	-13.8 ± 9.5 (4)	-17.9 ± 12.3 (4)	-5.1 ± 13.0 (4)
2D	-2.9 ± 3.1 (2)	-23.0 ± 1.1 (4)	4.4 ± 13.8 (4)
3D	16.8 ± 13.7 (6)	0.7 ± 13.0 (6)	0.9 ± 10.4 (6)
7D	-0.4 ± 11.1 (4)	3.9 ± 10.4 (4)	1.3 ± 17.8 (4)
14D	1.9 ± 9.7 (4)	15.8 ± 11.2 (4)	23.1 ± 16.7 (4)
NS	0.0 ± 7.4 (38)		

[a]Time after inoculation of 1000 live cells when sera were taken.

[b]Sera from tumour bearers taken six hours after challenge with 10^7, 7xFT, L5178Y cells.

[c]Sera from tumour bearers taken six hours after challenge with $5x10^8$ HRBC.

ii. Θ POSITIVE CELLS

The results of Θ cytotoxicity determination on normal spleen cells incubated in these same tumour bearer sera mirrored the findings for the Ig bearing population (Table XVIII). A decrease in Θ-bearing cells was observed following incubation of normal spleen cells in three day tumour bearer serum ($p < .001$). In contrast, normal levels of this population of cells were obtained by the use of sera from three day tumour bearers which had been challenged with either L5178Y cells or HRBC. Further, no decrease in the Θ-bearing population was observed following the use of sera taken from challenged tumour bearers till fourteen days after initiation of tumour growth. Even then the decreases were not marked.

Thus mice bearing tumours growing from an inoculum of 1000 live cells were also unable to produce a six hour response to antigen challenge in their spleens or sera from day one to seven following the live cell inoculum.

It was considered possible that ascites fluid injected with the live cell inoculae might contain substances active in this system. In order to assess the effect of the ascites cell supernate in these experiments involving a live cell inoculum, the course of the Ig bearing spleen cell population was followed in animals given a single injection of cell free ascites supernate corresponding to an inoculum of 1000 live L5178Y cells.

XIV. CHANGES IN Ig BEARING SPLEEN CELL POPULATIONS FOLLOWING INJECTION OF CELL-FREE ASCITES SUPERNATE CORRESPONDING TO 1000 LIVING L5178Y CELLS

Table XIX shows that injection of the supernate of 1000 live L5178Y ascites cells did not produce a six hour increase in Ig bearing cells.

TABLE XVIII

% Θ+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After Inoculation with 1000 Live L5178Y Cells.

Time Serum Taken[a]	% Θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
3D	16.5 ± 8.6 (4)	26.0 ± 9.2 (4)	30.3 ± 6.1 (4)
7D	24.0 ± 4.0 (4)	23.7 ± 8.0 (4)	21.0 ± 5.1 (4)
14D	25.2 ± 5.5 (4)	21.7 ± 7.8 (4)	22.9 ± 2.7 (4)
NS	28.6 ± 5.7 (34)		

[a]Time after inoculation of 1000 live cells when sera were taken.

[b]Sera from tumour bearers taken six hours after challenge with 10^7, 7xFT, L5178Y cells.

[c]Sera from tumour bearers taken six hours after challenge with $5x10^8$ HRBC.

TABLE XIX

% Change in Ig+ Spleen Cells

After Inoculation with Supernate of 1000 Live L5178Y Cells.

Time After Supernate Given	% Change in Ig+ Spleen Cells ± SD	
	No Challenge	L5178Y Challenge[a]
6H	-4.6 ± 4.7 (3)	
3D	5.5 ± 4.5 (3)	22.5 ± 9.1 (3)
7D	-4.5 ± 4.7 (3)	13.0 ± 6.7 (3)
Normal	0.0 ± 5.4 (142)	

[a]Six hours after challenge with 10^7, 7xFT L5178Y.

Further, this treatment did not inhibit the animals' ability to produce a six hour increase in Ig bearing cells in response to a challenging dose of L5178Y cells given on days three or seven after the supernate injection. Thus, the ascites fluid did not appear to play a role in the phenomena observed following inoculation with live tumour cells.

Thus the overall observations on mice bearing tumours growing from a low inoculum of cells (100 or 1000) reveal that at best only a partial six hour response is made to the initial injection of living syngeneic tumour cells. An increase of Ig bearing cells and a concomitant decrease in Θ-bearing cells is observed three days after the live cell inoculum. Finally, and most significantly, the tumour bearing mice are unable to produce a six hour response to a challenging dose of specific or non-specific antigen from day one till at least day seven after initiation of tumour growth.

In order to test the hypothesis that the observed effects could be related to the continuing presence of soluble tumour antigen which may be associated with tumour growth, a soluble tumour extract (STE) was prepared.

XV. PREPARATION OF SOLUBLE TUMOUR EXTRACT OF L5178Y CELLS

i. DIGESTION AND FRACTIONATION

Papain digestion of L5178Y cells was carried out according to Materials and Methods section XXI. The supernate of this digestion was fractionated on a Sephadex G-200 column (Figure 7). The final fraction, PKIV, relative elution volume 3.01, was further separated by ultrafiltration with Diaflo filters UM10 and UM05. All of the fractions were examined for their ability to produce a six hour response after a volume corresponding to 10^7 cells

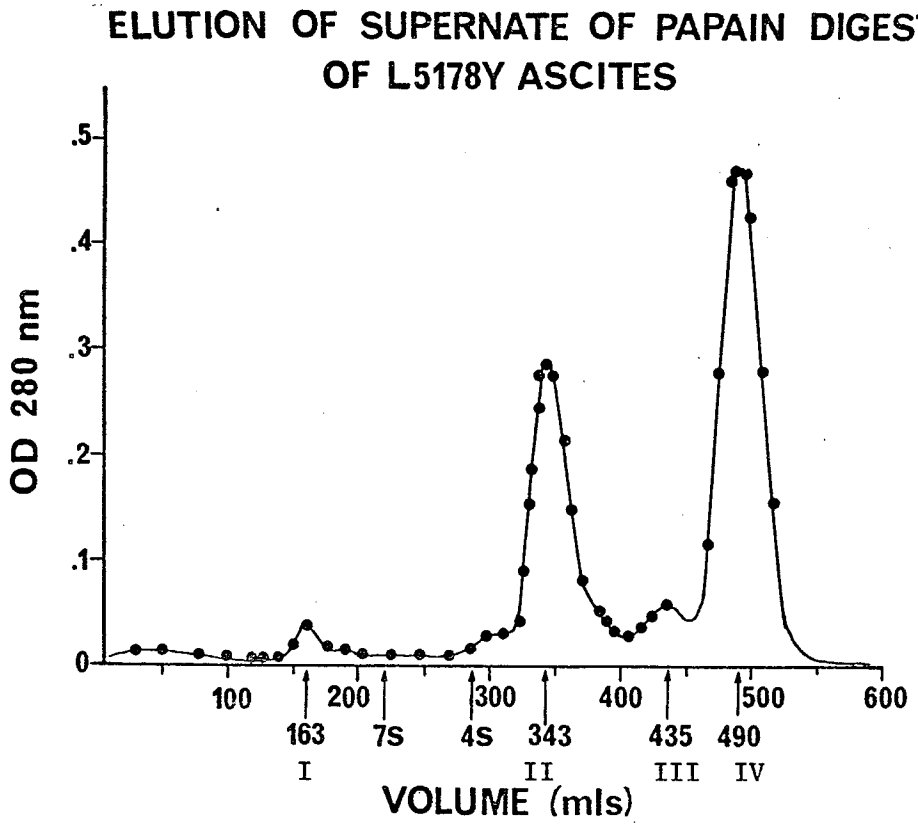

Figure 7: Elution from a Sephadex G-200 column. The relative elution ratio of the last peak is 3.01 .

was injected IP into DBA/2 mice. As can be seen in Table XX, Fraction IVa which was that component of Fraction IV (Figure 7) which would not pass through a UM10 Diaflo membrane, was able to produce a six hour increase in Ig bearing cells ($p < .001$). Correspondingly the θ-baering spleen cells of animals treated with Fraction IVa showed a concomitant six hour decrease which was signifigant ($p < .001$) (Table XXI). A single test of Fraction III, a small peak, which eluted immediately before Fraction IV also produced a marked increase in Ig-bearing cells although no change in the θ-bearing population. Consequently Fraction IVa of the supernate of the papain digestion of L5178Y ascites was used as a soluble tumour cell extract and denoted as STE.

The Fraction III of this same preparation (Figure 7) which was red in colour was considered to be a hemolysate of the red cells which comprise part of most L5178Y ascites samples as the Sephadex G-200 elution volume of this peak corresponds approximately to the molecular weight of hemoglobin, circa 65,000 daltons.

A second preparation (B) of STE resulted in the production of a similar fraction having the same relative elution volume and molecular weight characteristics as well as the same ability to induce a statistically significant six hour increase in Ig bearing spleen cells ($p < .001$) (Table XXII), and a concomitant, decrease in the θ-bearing population ($p < .001$) (Table XXIII).

Administration of different doses of STE revealed that double the amount of STE (Preparation B, 250 cmm, Table XXII) produced a significant six hour increase in spleen cells displaying surface Ig ($p < .001$). Decreasing the injection of Preparation A to one-fifth or less of the dose which corresponded to 10^7 cells resulted in no signifigant increase in the Ig bearing spleen cells six hours later (Table XXII).

TABLE XX

% Change in Ig+ Spleen Cells

Six Hours After IP Administration of Sephadex G-200 Fractions of the Supernate[a] of Papain Digestion of L5178Y Ascites.

Fraction	% Change in Ig+ Spleen Cells ± SD
I	4.8 ± 5.6 (3)
II	9.7 ± 2.1 (3)
III	33.1 (1)
IVa (STE)[b]	23.3 ± 7.2 (4)
IVb[c]	4.2 ± 3.8 (3)
IVc[d]	-4.1 ±28.3 (3)
None	0.0 ± 3.7 (3)

[a]Fractions of preparation A were given in a dose which corresponded to 10^7, L5178Y cells.

[b]The component of fraction IV which was retained by a UM 10 Diaflo membrane.

[c]The component of fraction IV which passed through a UM 10 Diaflo membrane but which was retained by a UM05 Diaflo membrane.

[d]The component of fraction IV which passed through a UM05 Diaflo membrane.

TABLE XXI

% θ+ Spleen Cells

Six Hours After IP Administration of Sephadex G-200 Fractions of the Supernate[a] of Papain Digestion of L5178Y Ascites.

Fraction	% θ+ Spleen Cells ±SD
I	22.6 ± 2.2 (2)
II	23.5 ± 1.3 (3)
III	21.9 (1)
IVa (STE)[b]	12.0 ± 4.1 (4)
IVb[c]	25.0 ± 6.4 (3)
IVc[d]	21.8 ± 2.5 (3)
None	22.8 ± 0.9 (5)

[a]Fractions of preparation A given in a dose which corresponded to 10^7 L5178Y cells.

[b]The component of fraction IV which was retained by a UM10 Diaflo membrane.

[c]The component of fraction IV which passed through a UM10 Diaflo membrane but which was retained by a UM05 Diaflo membrane.

[d]The component of fraction IV which passed through a UM05 Diaflo membrane.

TABLE XXII

% Change in Ig+ Spleen Cells

Six Hours After IP Administration of Different Doses of STE.

Volume STE Given	% Change in Ig+ Spleen Cells ± SD
Preparation A	
50 cmm[a]	23.3 ± 7.2 (4)
10 cmm	6.9 ± 5.4 (4)
5 cmm	1.5 ± 4.1 (2)
1 cmm	-3.1 ± 9.2 (8)
Preparation B	
250 cmm	19.8 ± 3.4 (6)
120 cmm[b]	20.9 ± 2.9 (3)
None	0.0 ± 5.4 (142)

[a]The volume of STE corresponding to 10^7 L5178Y cells.

[b]This volume contains the same number of OD280 units as 50 cmm of preparation A.

TABLE XXIII

% θ+ Spleen Cells

Six Hours After IP Administration of Different Doses of STE or Papain Digest of DBA/2 Thymocytes.

Treatment STE	Volume	% θ+ Spleen Cells ± SD
Preparation A	50 cmm[a]	12.0 ± 4.1 (4)
	10 cmm	22.0 ± 1.0 (2)
	1 cmm	25.2 ± 6.9 (7)
Preparation B	120 cmm[b]	12.2 ± 3.6 (7)
Thymocyte Digestion PKIIIa	250 cmm[b]	22.3 ± 5.7 (3)
None		26.8 ± 5.3 (139)

[a]This volume of STE corresponds to 10^7 L5178Y cells.

[b]This volume contains the same number of OD280 units as 50 cmm preparation A of STE.

As treatment of L5178Y leukemia cells with anti-Θ serum and complement resulted in over 90% cell death the L5178Y cells were shown to be Θ-bearing and thus probably derived from a T cell. Therefore as a control, a similar papain digestion of DBA/2 thymocytes was carried out and the supernate was separated by Sephadex G-200 gel filtration (Figure 8). The last peak eluted, which had a relative elution volume of 2.91, and thus corresponded to the STE of L5178Y ascites cells, was further fractionated by the use of UM10 (Fraction IIIa retained) and UM05 Diaflo membranes. Fractions I and IIIa were tested using doses which corresponded to at least 10^7 thymocytes and contained the same number of OD280 units as the corresponding fractions of the ascites preparations tested. Neither of these fractions was capable of producing a six hour increase in Ig bearing spleen cells (Table XXIV) and Fraction IIIa also produced no six hour decrease in the Θ-bearing spleen cell population (Table XXIII).

The thymocyte digestion did not contain any red cells. Also in contrast with the ascites preparation there was no red coloured fraction eluted between the void volume (I) and the small peak (II) preceding the final elution fraction (III) in the Sephedex G-200 separation of the thymocyte preparation, (Figure 8). Thus a papain digestion was performed on DBA/2 thymocytes and blood cells. The ratio of thymocytes to red clood cells was 1:1 as this was the approximate ratio of L5178Y cells to red blood cells found in ascites samples examined. The supernate of this digestion was separated on a Sephadex G-200 column (Figure 9) and a large, red coloured fraction (II) was found between the void volume (I) and the small peak (III) which preceded the final fraction eluted from the column (IV). The appearance of the red coloured

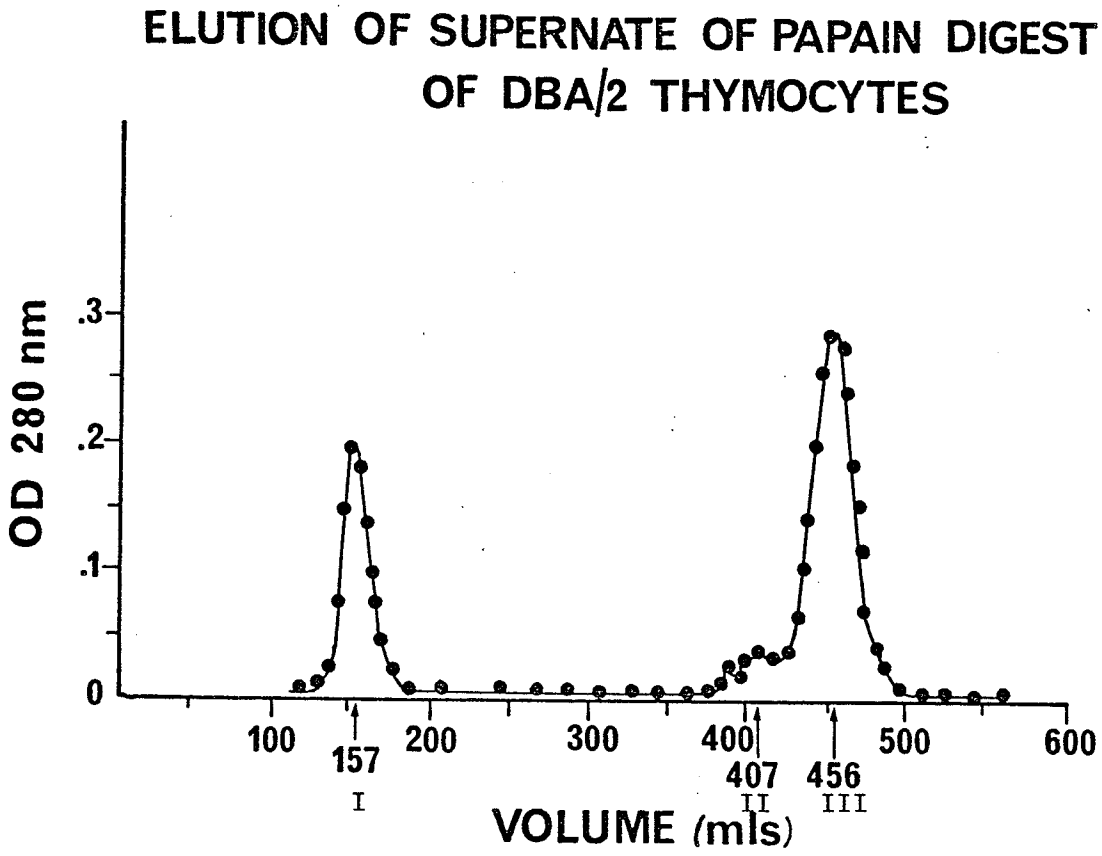

Figure 8: Elution from a Sephadex G-200 column. The relative elution ratio of the last peak is 2.91 .

TABLE XXIV

% Change in Ig+ Spleen Cells Six Hours After IP Administration of Sephadex G-200 Fractions of Papain Digestion of DBA/2 Thymocytes with and without DBA/2 Blood Cells.

Fraction	Volume	% Change Ig+ Spleen Cells ± SD
Thymocyte Digestion		
I	250 cmm	-6.43 ± 7.8 (3)
IIIa[b]	250 cmm[a]	7.3 ± 8.0 (3)
Thymocyte plus Blood Cell Digestion		
IVa[b]	100 cmm[a]	3.3 ± 2.4 (3)
None		0.0 ± 5.4 (142)

[a]This volume contains the same number OD280 units as 50 cmm of PKIVa papain digest of L5178Y ascites (STE), preparation A.

[b]This component had the same relative elution volume as STE and was retained by a UM10 Diaflo membrane.

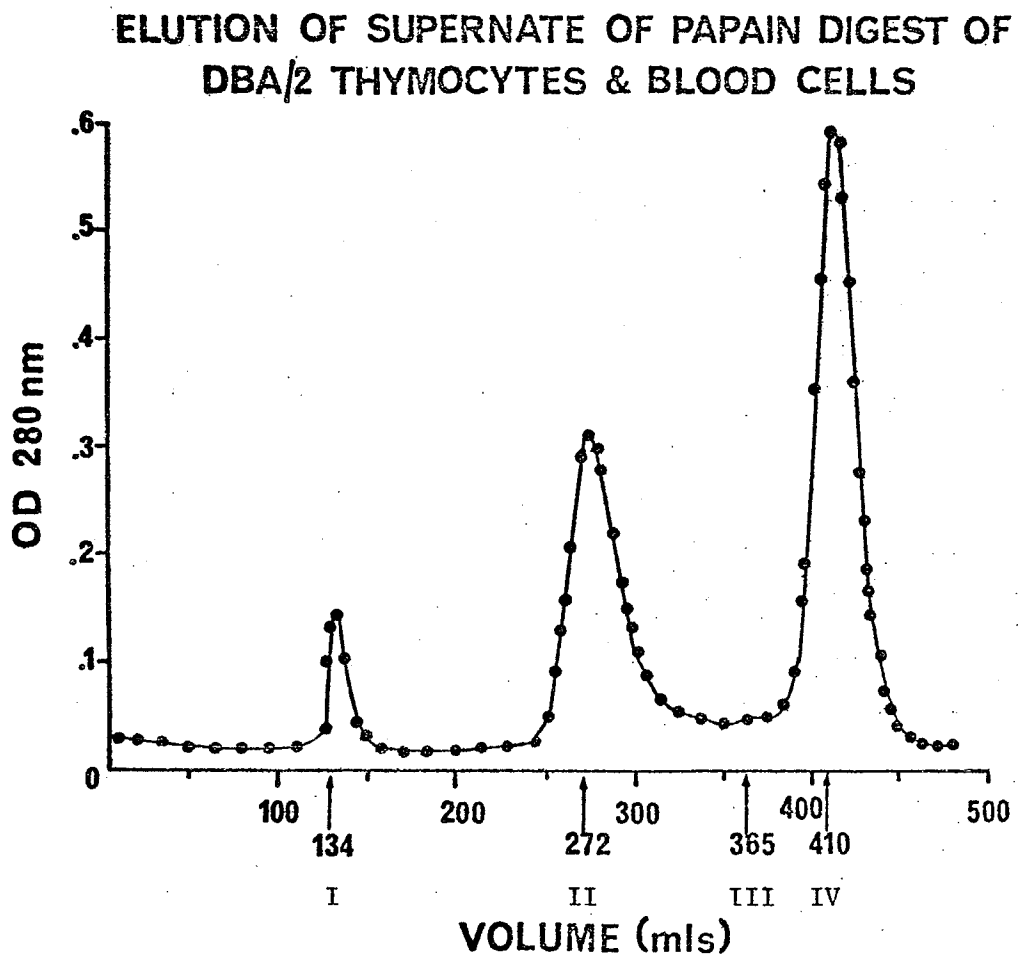

Figure 9: Elution from a Sephadex G-200 column. The relative elution ratio of the last peak is 3.05 .

peak in the thymocyte preparation following addition of red cells to the digestion was considered to be further evidence suggesting that the peak is related to the presence of red blood cells in the papain digestion.

The final peak (IV), eluted from the thymocyte red cell preparation, was further separated by filtration using a UM10 Diaflo membrane. Injection of the component of Fraction IV retained by the UM10 membrane (IVa) in a dose which corresponded to at leat 10^7 thymocytes and contained the same number of OD280 units as the STE produced no six hour increase in Ig bearing spleen cells (Table XXIV).

Thus a soluble fraction of a papain digestion of L5178Y ascites (IVa), denoted as STE, was obtained which exhibited antigenic qualities, the ability to produce a six hour response, unlike similar preparations of DBA/2 thymocytes with or without blood cells.

ii. INHIBITION OF TUMOUR ROSETTE FORMATION BY STE

Experiments were carried out to determine the effect of this soluble tumour extract (STE) on the rosette forming ability of L5178Y leukemia cells which had been incubated in serum raised in DBA/2 mice against L5178Y cells. Surface Ig was not detected on untreated L5178Y cells by the RICA technique (Table XXV). Initially some difficulty was encountered in finding suitable conditions for incubation of L5178Y cells in the anti-L5178Y serum for demonstration of Ig bearing tumour cells but following a twenty minute incubation at 37°C, relatively consistent results were obtained (Figure 10). Generally the tumour rosettes appeared larger than typical spleen cell rosettes, probably reflecting the difference in size of L5178Y cells compared with splenic lymphoid cells.

TABLE XXV

L5178Y ROSETTES

Number RFC/1000 Cells

L5178Y	L5178Y + NS	L5178Y + α-L5178YS	L5178Y + α-L5178YS + STE	L5178Y + α-L5178YS + PKIIIa Thym.[a]
45	55	141	96	191
3	30	74	34	73
35	56	111	62	115

DBA/2 Thymocytes	DBA/2 Thymocytes + α-θS	DBA/2 Thymocytes + α-L5178YS
22	304	35
25	423	34
18	335	19

[a]G-200 fraction of supernate of papain digestion of DBA/2 thymocytes which had the same relative elution volume as STE and was retained by a UM10 Diaflo membrane.

The signifigance of the decrease in L5178Y rosettes produced in the presence of STE was obtained by comparing the means ±SD of $[(L5178Y + \alpha\text{-}L5178YS)-(L5178Y + NS)] - [(L5178Y + \alpha\text{-}L5178YS + STE)-(L5178Y + NS)]$ and $[(L5178Y + \alpha\text{-}L5178YS)-(L5178Y + NS)] - [(L5178Y + \alpha L5178YS + PKIIIa\ Thym.^{a})-(L5178Y + NS)]$.

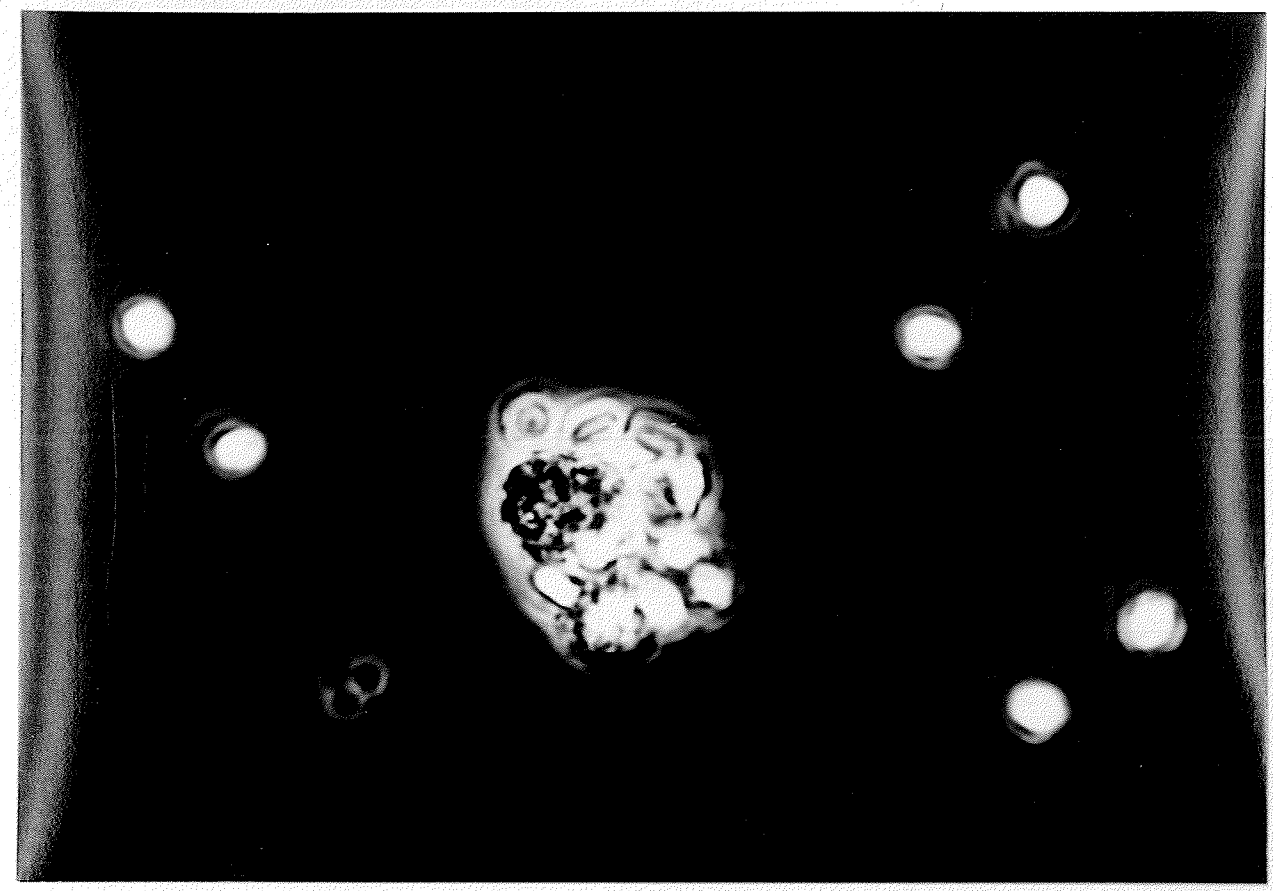

Figure 10: L5178Y tumour cell rosette. A double rosette containing two tumour cells is shown using phase contrast microscopy.

The conditions described above were used to produce the results presented in Table XXV. These three experiments show that STE was able to reduce the formation of rosettes unlike a quantity of the corresponding thymocyte preparation (IIIa) containing the same number of OD280 units. The observed reduction in the number of rosette forming cells (RFC) per 1000 L5178Y cells in the presence of anti-L5178Y serum and STE was statistically significant ($p < .01$). The anti-L5178Y serum used in these experiments was shown not to react with DBA/2 thymocytes under the conditions used above. In contrast AKR anti-C3H Θ serum, (α-θS), was shown to be able to react with DBA/2 thymocytes under the same experimental conditions (Table XXV).

This piece of evidence suggested that the STE was related to the tumour cell surface. Thus this soluble preparation was used to examine the hypothesis that soluble tumour-associated materials may be involved in the abrogation of the six hour response observed in tumour bearing animals.

XVI. CHANGES OF SPLEEN CELL POPULATIONS FOLLOWING A SINGLE INJECTION OF 1 CMM STE

i. Ig POSITIVE CELLS

Injection of 1 cmm of STE, a dose which is one-fiftieth of the volume which corresponds to 10^7 L5178Y cells produced no six hour increase in Ig bearing spleen cells and resulted in no change in the Ig bearing spleen cells on days one, three and seven following the injection (Figure 11). No six hour increase in Ig bearing spleen cells, was produced following challenge with L5178Y cells or HRBC on days one and three after injection of 1 cmm STE. On day seven after injection of 1 cmm STE, challenge by either L5178Y cells or HRBC produced a statistically significant increase in the Ig bearing

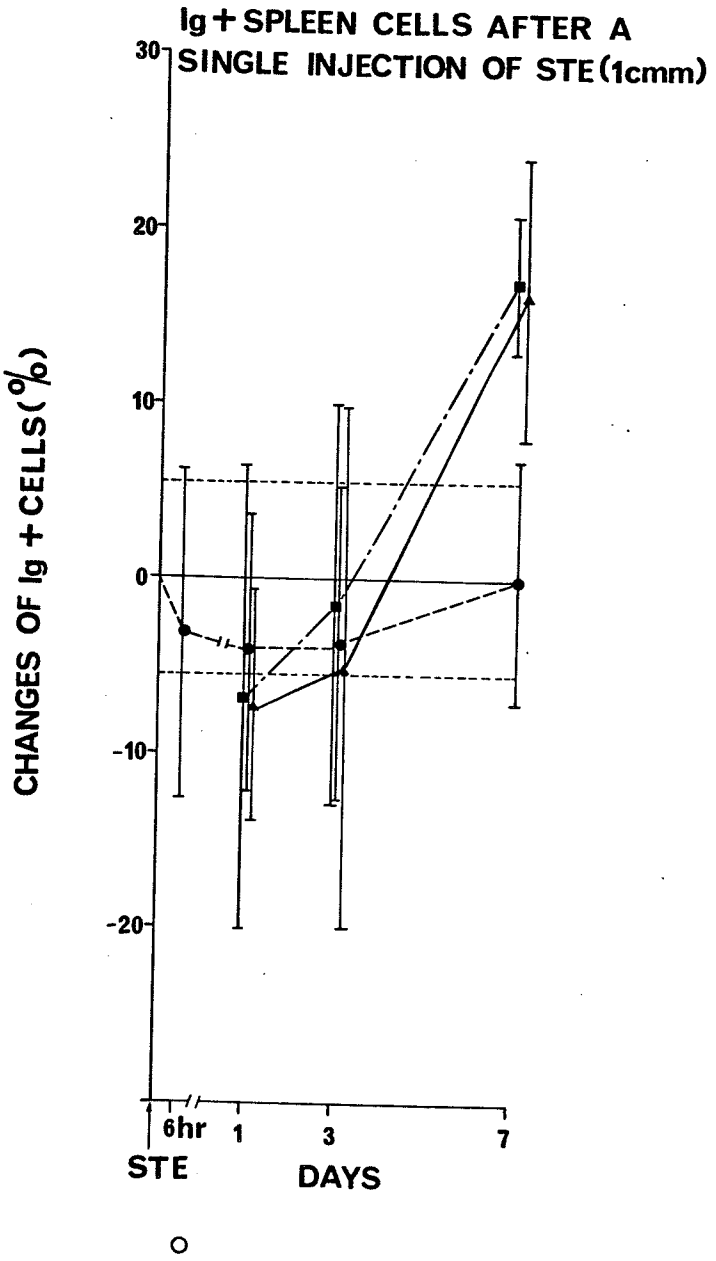

Figure 11: No challenge ●--●; 6H 10^7, 7×FT L5178Y challenge ▲——▲; 6H 5x10^8 HRBC challenge ■--■.

spleen cell population ($p < .005$ and .001 respectively).

ii. Θ POSITIVE CELLS

The results of examination of the Θ-bearing spleen cells show that no change was observed from six hours to seven days after IP injection of 1 cmm STE(Table XXVI). No six hour decrease in Θ-bearing cells following antigen challenge was observed till day seven following injection of 1 cmm STE. By this time a significant decrease was observed after challenge with both L5178Y cells and HRBC ($p < .001$).

The activity of sera from these mice was examined as well.

XVII. EFFECT ON NORMAL SPLEEN CELLS OF SERA TAKEN FROM ANIMALS GIVEN 1 CMM OF STE

Incubation of normal spleen cells in serum taken from mice given a single injection of 1 cmm STE followed by challenge with L5178Y cells or HRBC at different time intervals reproduced the observations made on the spleen cells of the mice which provided the sera. Increases in Ig bearing cells were observed following the use of serum from animals challenged with HRBC or L5178Y cells on day seven after injection of 1 cmm STE. The increase was statistically significant in the latter case ($p < .005$) (Table XXVII). The sera from animals challenged with either L5178Y cells or HRBC on day three following injection of 1 cmm STE produced a partial but not significant decrease in the Θ-bearing population of spleen cells (Table XXVIII).These results may suggest that RICA is more reproducible than α-Θ cytotoxicity.

Thus treatment of animals with a single dose of 1 cmm STE resulted in a transient inhibition of the ability of mice to produce a six hour response following challenge with specific and non-specific antigen.

TABLE XXVI

% θ+ Spleen Cells After Injection of 1 cmm STE.

Time After 1 cmm STE	% θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	HRBC Challenge[b]
6 H	25.2 ± 6.9 (7)		
30 H	25.3 ± 6.3 (6)	24.9 ± 4.5 (6)	26.4 ± 2.6 (6)
3 D	26.2 ± 4.9 (6)	25.7 ± 4.2 (6)	24.1 ± 5.7 (6)
7 D	29.3 ± 4.0 (6)	18.8 ± 3.1 (6)	18.1 ± 3.1 (6)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT, L5178Y.

[b]Six hours after challenge with $5x10^8$ HRBC.

TABLE XXVII

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After

IP Administration of 1 cmm STE.

Time Sera Taken[a]	% Change in Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
6 H	2.4 ± 7.1 (6)		
30 H	-6.5 ± 13.6 (6)	-16.1 ± 13.5 (6)	5.5 ± 11.9 (6)
3 D	-0.8 ± 7.5 (6)	6.5 ± 5.4 (6)	6.6 ± 7.7 (6)
7 D	-0.2 ± 9.4 (6)	18.1 ± 4.6 (6)	20.0 ± 17.9 (6)
NS	0.0 ± 7.4 (38)		

[a]Time after I.P. injection of 1 cmm STE when sera taken.

[b]Sera, from animals given 1 cmm STE, taken 6 hours after challenge with 10^7, 7xFT, L5178Y cells.

[c]Sera, from animals given 1 cmm STE, taken 6 hours after challenge with $5x10^8$ HRBC.

TABLE XXVIII

% θ+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken

After IP Administration of 1 cmm STE.

Time Sera Taken[a]	% Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
6 H	30.8 ± 7.1 (6)		
30 H	29.5 ± 5.3 (6)	25.7 ± 6.5 (6)	30.5 ± 7.6 (6)
3 D	28.3 ± 6.0 (6)	20.8 ± 8.5 (6)	23.8 ± 4.9 (6)
7 D	27.1 ± 3.8 (6)	22.3 ± 9.3 (6)	26.6 ± 10.3 (6)
N.S.	28.6 ± 5.7 (34)		

[a]Time after I.P. injection of 1 cmm STE when sera taken.

[b]Sera, from animals given 1 cmm STE, taken six hours after challenge with 10^7, 7xFT L5178Y cells.

[c]Sera, from animals given 1 cmm STE, taken six hours after challenge with $5x10^8$ HRBC.

As the abrogation of the six hour response observed in tumour bearing animals was observed up to at least day seven following initiation of tumour growth, it was considered probable that a regime of repeated injections of STE would be required to reproduce this defect. Thus the effect of injecting STE on consecutive days was examined.

XVIII. CHANGES IN Ig BEARING SPLEEN CELLS ON DAY SEVEN FOLLOWING REPEATED INJECTIONS OF STE

Mice were treated at three different dose levels with a daily injection of STE for four consecutive days. This pretreatment was followed by challenge with 10^7 7xFT, L5178Y cells, or $5x10^8$ HRBC, on day seven after the first injection of STE. Results, given on Table XXIX, show that such repeated treatment of mice with 1 cmm or 10 cmm of STE, abrogated the ability of mice to produce a six hour increase in Ig bearing cells in response to specific and non-specific antigen challenge given on day seven after the first pretreating injection.

A single trial with a lower dose of STE failed to interfere with the six hour response to antigen challenge.

Experiments were thus carried out to study the changes in spleen cell populations at different intervals following daily injection of 1 cmm STE for four consecutive days.

XIX. CHANGES IN SPLEEN CELL POPULATIONS FOLLOWING REPEATED INJECTIONS OF 1 CMM STE

i. Ig POSITIVE CELLS

Mice were challenged with 10^7, 7xFT, L5178Y cells, or $5x10^8$ HRBC at different intervals during and after daily treatment with 1 cmm of STE given

TABLE XXIX

% Change in Ig+ Spleen Cells

At Day Seven After the First of Four Injections

of STE[a] Given at Daily Intervals.

Volume STE	% Change Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
10 cmm	-4.0 ± 1.8 (2)	-3.9 ± 1.6 (2)	-4.6 ± 3.8 (2)
1 cmm	-2.6 ± 12.8 (8)	-10.5 ± 12.0 (8)	-15.8 ± 13.9 (6)
0.05 cmm	-4.0 (1)	24.7 (1)	
None	0.0 ± 5.4 (142)		

[a]Repeated injection of STE was carried out at three dose levels.

[b]Six hours after challenge with 10^7, 7xFT, L5178Y cells.

[c]Six hours after challenge with $5x10^8$ HRBC.

for four consecutive days. The results obtained show that such repeated treatment abrogated the ability of animals to produce a six hour increase in Ig bearing cells from day one to at least day seven following the initial pretreating injection (Figure 12). On day fourteen mice were able to produce significant increases in Ig bearing spleen cells six hours after challenge with L5178Y cells or HRBC ($p < .005$).

A significant increase in Ig bearing cells was observed on day three, the day when the course of pretreating injection was completed ($p < .001$). Challenge on that day with either L5178Y cells or HRBC produced a significant decrease in Ig bearing spleen cells ($p < .005$).

ii. Θ POSITIVE CELLS

Examination of the Θ-bearing spleen cells of mice pretreated with four injections of 1 cmm of STE showed a significant decrease in Θ-bearing cells ($p < .005$) concomitant with the increase of Ig bearing cells at day three (Table XXX). Challenge by either L5178Y cells or HRBC on that day produced an increase in the Θ-bearing population of spleen cells. No six hour decrease in Θ-bearing spleen cells was observed in response to antigen challenge from day one till at least day seven following the first dose of STE. On day fourteen challenge with either L5178Y cells or HRBC produced a six hour decrease which was significant in the latter case ($p < .005$).

The sera of these mice were also examined for their ability to produce changes in normal spleen cell populations.

XX. EFFECT ON NORMAL SPLEEN CELLS OF SERA TAKEN FROM ANIMALS DURING AND AFTER REPEATED INJECTIONS OF 1 CMM STE

Spleen cells, from normal DBA/2 mice, were incubated in sera taken from

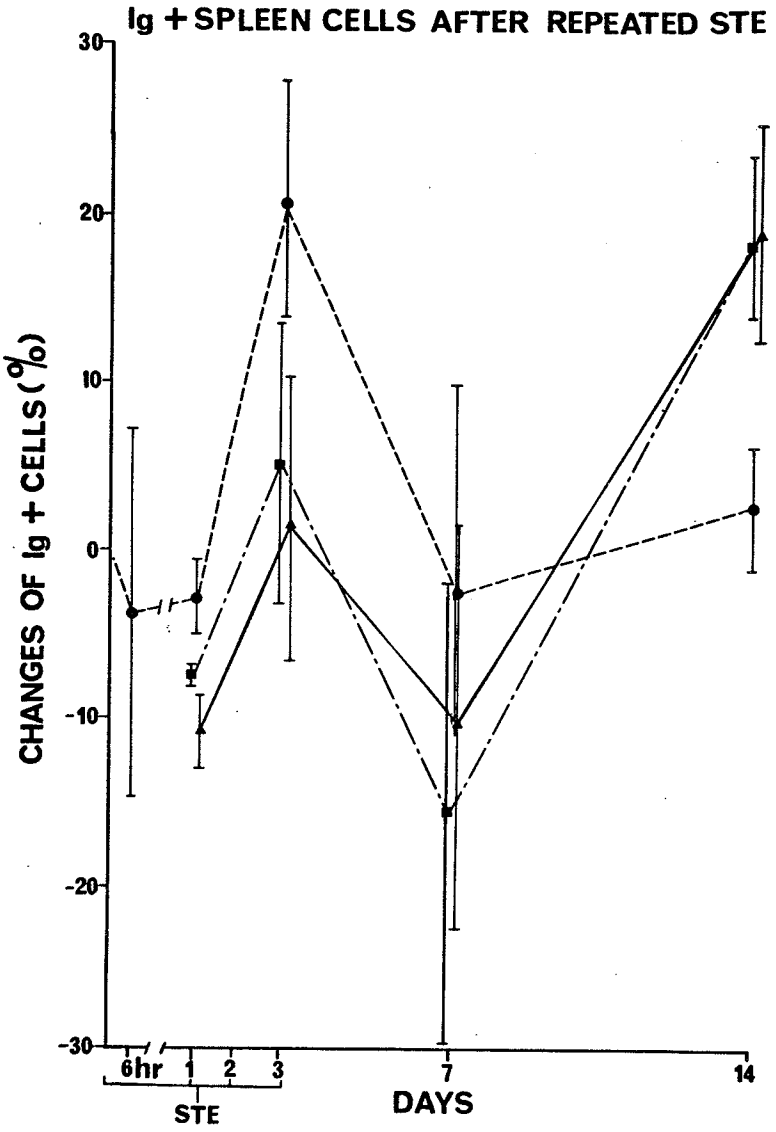

Figure 12: No challenge ●—–●: 6H 10^7,7xFT L5178Y challenge ▲——▲; 6H $5x10^8$ HRBC challenge ■– – –■.

TABLE XXX

% θ Bearing Spleen Cells

After I.P. Administration of 1 cmm STE Given on Four Consecutive Days.

Time After 1st STE Injection	% θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	HRBC Challenge[b]
6 H	25.2 ± 6.9 (7)		
30 H	24.5 ± 0.8 (2)	25.1 ± 3.6 (2)	29.2 ± 3.0 (2)
3 D	18.4 ± 4.3 (4)	25.1 ± 3.2 (4)	27.9 ± 6.0 (6)
7 D	27.8 ± 6.6 (6)	25.3 ± 4.5 (6)	26.8 ± 3.9 (8)
14 D	28.6 ± 5.1 (4)	15.3 ± 8.0 (4)	16.6 ± 4.6 (4)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT, L5178Y cells.

[b]Six hours after challenge with $5x10^8$ HRBC.

mice at different intervals after the first of daily injections of 1 cmm STE given on four consecutive days.

i. Ig POSITIVE CELLS

Such use of sera taken on day three, after the last STE injection produced a significant increase in Ig bearing cells ($p < .001$)(Table XXXI). Sera taken from animals challenged on that day produced a decrease to normal levels. The proportion of Ig positive cells returned to normal by day seven and remained there on day fourteen. No increase resulted from sera taken following antigen challenge till day fourteen when a significant rise was observed in the case of sera from animals challenged with either L5178Y cells or HRBC ($p < .005$ and .001 respectively).

ii. θ POSITIVE CELLS

Concomitant with this, the changes in the θ-bearing cells reflected those in the Ig population (Table XXXII). A decrease was observed after incubation in day three sera ($p < .01$) while sera from animals challenged on that day produced θ-bearing populations in the normal range. All sera taken on day seven produced normal levels of θ-bearing cells. The use of sera taken on day fourteen, resulted in a normal proportion of θ-bearing cells whereas sera from animals challenged with L5178Y cells or HRBC on that day produced somewhat decreased levels.

Thus, neither spleen cells or sera from specifically and non-specifically challenged mice, pretreated with repeated small doses of STE, were able to demonstrate the six hour response from day one to at least day seven after the first injection of STE.

In order to determine whether abrogation of the six hour response was

TABLE XXXI

% Change in Ig+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After IP Administration of 1 cmm of STE Given on Four Consecutive Days.

Time Sera Taken[a]	% Change Ig+ Spleen Cells ± SD		
	No Challenge	L5174Y Challenge[b]	HRBC Challenge[c]
6 H	2.4 ± 7.1 (6)		
30 H	4.4 ± 5.8 (2)	-0.8 ± 4.9 (2)	3.3 ± 6.7 (2)
3 D	16.0 ± 11.4 (4)	-4.3 ± 12.1 (4)	-0.7 ± 18.6 (6)
7 D	-5.9 ± 9.9 (6)	-9.4 ± 12.4 (6)	-9.3 ± 16.2 (8)
14 D	9.0 ± 0.8 (4)	28.9 ± 7.4 (4)	35.3 ± 4.0 (4)
NS	0.0 ± 7.4 (38)		

[a]Time after initial injection of STE when sera were taken.

[b]Sera, from animals treated with STE, taken six hours after challenge with 10^7, 7xFT L5178Y cells.

[c]Sera, from animals treated with STE, taken six hours after challenge with $5x10^8$ HRBC.

TABLE XXXII

% θ+ Spleen Cells:

Effect on Normal Spleen Cells of Sera Taken After IP Administration of 1 cmm STE Given on Four Consecutive Days.

Time Sera Taken[a]	% θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[b]	HRBC Challenge[c]
6 H	30.8 ± 7.1 (6)		
30 H	34.1 ± 1.1 (2)	33.5 ± 4.7 (2)	32.5 ± 0.2 (2)
3 D	20.7 ± 3.2 (4)	23.2 ± 3.4 (4)	25.0 ± 5.1 (6)
7 D	24.4 ± 2.8 (6)	24.9 ± 4.7 (6)	24.3 ± 6.8 (8)
14 D	23.0 ± 4.8 (4)	12.4 ± 5.5 (4)	15.4 ± 7.9 (4)
NS	28.6 ± 5.7 (34)		

[a]Time after initial injection of STE when sera were taken.

[b]Sera, from animals treated with STE, taken six hours after challenge with 10^7, 7xFT, L5178Y cells.

[c]Sera, from animals treated with STE, taken six hours after challenge with $5x10^8$ HRBC.

related to a peculiarity of the tumour cell extract the important points of these experiments were repeated, substituting a heterologous soluble protein, deaggregated Hgg, for STE. The dose given, 50 µg, was suggested by the work of Bonavida and Zighelboim (1973) and will be discussed later.

XXI CHANGES IN SPLEEN CELL POPULATIONS FOLLOWING INJECTION OF 50 µg DEAGGREGATED Hgg

No increase in Ig bearing spleen cells (Table XXIII), nor decrease in Θ-bearing spleen cells (Table XXXIV) was observed six hours after injection of 50 µg deaggregated Hgg. No changes occurred in either population thirty hours after the deaggregated Hgg was given. As well, challenge with Hgg emulsified in FCA or L5178Y cells on day one failed to produce a change in either cell population. On day seven, animals challenged specifically or non-specifically were able to show six hour changes in their Ig bearing and Θ-bearing spleen cell populations typical of the six hour response (Tables XXXIII and XXXIV).

Thus, a single injection of 50 µg deaggregated Hgg transiently abrogated the ability of the animals to produce a six hour response following antigen challenge, similar to a single injection of 1 cmm STE.

XXII CHANGES IN SPLEEN CELL POPULATIONS FOLLOWING REPEATED INJECTIONS OF 50 µg DEAGGREGATED Hgg

The dose of deaggregated Hgg used for repeated injection was suggested by the work of Bonavida and Zighelboim (1973), in which 50 µg of a soluble cell extract, given on five consecutive days, resulted in a depression of both humoural and cell mediated responses.

Injection of 50 µg of deaggregated Hgg, daily, for four consecutive

TABLE XXXIII

% Change in Ig+ Spleen Cells

After Injection of 50 µg Deaggregated Hgg.

Time After 50 µg	% Change in Ig+ Spleen Cells ± SD		
Deaggregated Hgg	No Challenge	L5178Y Challenge[a]	Hgg Challenge[b]
6 H	1.1 ± 9.4 (4)		
30 H	1.6 ± 5.6 (4)	-12.7 ± 11.1 (4)	5.7 ± 7.7 (4)
7 D	-10.5 ± 8.0 (4)	9.8 ± 8.3 (4)	10.1 ± 9.3 (4)
Normal	0.0 ± 5.4 (142)		

[a]Six hours after challenge with 10^7, 7xFT L5178Y cells.

[b]Six hours after challenge with 250 mg Hgg/FCA, 1:1.

TABLE XXXIV

% θ+ Spleen Cells

After Injection of 50 µg Deaggregated Hgg.

Time After 50 µg Deaggregated Hgg	% θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	Hgg Challenge[b]
6 H	30.1 ± 6.4 (4)		
30 H	31.2 ± 10.7 (4)	22.7 ± 2.0 (4)	27.2 ± 2.6 (4)
7 D	24.8 ± 6.4 (4)	20.1 ± 3.3 (4)	17.7 ± 8.7 (4)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT L5178Y cells.

[b]Six hours after challenge with 250 mg Hgg/FCA, 1:1.

days resulted in the animals' inability to produce a six hour response to a challenging dose of antigen, given on the seventh day, after the first pretreating injection. Neither L5178Y cells or Hgg emulsified in FCA produced a six hour increase in Ig bearing spleen cells (Table XXXV) or a significant decrease in θ bearing cells (Table XXXVI).

In contrast, by day fourteen after the first pretreating injection of deaggregated Hgg, animals were able to produce a six hour response to antigen challenge. Administration of 10^7, 7XFT L5178Y cells or 250 mg Hgg/FCA, 1:1, produced a statistically significant increase in Ig bearing cells ($p < .001$ (Table XXXV) and a statistically significant decrease in θ bearing cells ($p < .01$ and .005, respectively) (Table XXXVI).

Thus treatment of mice with a small amount of soluble protein, for four consecutive days, abrogates non-specifically the animals' ability to produce a six hour response to antigen challenge on the first and at least the seventh day after the initiation of treatment with the soluble protein. These results correspond to the results obtained following similar STE treatment and also to the findings in animals bearing tumours growing from a small inoculum of live cells. These three types of treatment, non-specifically, abrogated the animals' ability to produce a six hour response to a challenging dose of antigen given from day one to at least day seven following initiation of the treatment.

It was important to determine whether the loss of the ability to produce the six hour response was related to any change in the animals' ability to produce an immune response. For this purpose, the in vitro growth inhibiting ability of spleen cells from specifically sensitized mice was examined, compared with normal spleen cells.

TABLE XXXV

% Change in Ig+ Spleen Cells

On Days Seven and Fourteen After the First Injection of 50 µg Deaggregated Hgg Given on Four Consecutive Days.

Time After 1st Injection Deaggregated Hgg	% Change in Ig+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	Hgg Challenge[b]
7 D	5.9 ± 2.8 (4)	-1.3 ± 8.8 (4)	1.2 ± 7.0 (4)
14 D	5.7 ± 3.4 (4)	26.7 ± 4.0 (4)	25.4 ± 3.3 (4)
Normal	0.0 ± 5.4 (142)		

[a]Six hours after challenge with 10^7, 7xFT, L5178Y cells.

[b]Six hours after challenge with 250 mg Hgg/FCA, 1:1.

TABLE XXXVI

% θ+ Spleen Cells

On Days Seven and Fourteen After the First Injection of 50 μg Deaggregated Hgg Given on Four Consecutive Days.

Time After 1st Injection Deaggregated Hgg	% Change in θ+ Spleen Cells ± SD		
	No Challenge	L5178Y Challenge[a]	Hgg Challenge[b]
7 D	32.5 ± 2.3 (2)	25.2 ± 2.0 (2)	34.5 ± 8.8 (4)
14 D	30.1 ± 7.0 (4)	18.2 ± 3.3 (4)	12.4 ± 4.6 (4)
Normal	26.8 ± 5.3 (139)		

[a]Six hours after challenge with 10^7, 7xFT, L5178Y cells.

[b]Six hours after challenge with 250 mg Hgg/FCA, 1:1.

XXIII EFFECT OF PRETREATMENT WITH STE OR DEAGGREGATED Hgg ON INDUCTION OF SPECIFIC TUMOUR IMMUNITY, ASSAYED IN VITRO

Radiolabelled deoxyuridine (I^{125}UDR) was employed to determine the ability of tumour cells to take up label for incorporation into new DNA. Using the macrosystem, uptake of label was found to be linear with increasing number of L5178Y cells in culture, and also with the length of the pulse period from four to twenty hours.

It was quickly evident that in order to have statistically significant results, many duplicates of every experiment would be required. For this purpose, subsequent tests were scaled down and performed in a microsystem.

Spleen cells from mice sensitized ten to eighteen days previously with 10^7, 7XFT L5178Y cells were incubated with L5178Y cells. Figure 13 shows the results obtained from twenty-four hour cultures of lymphoid cells and tumour cells combined in L/TC ratios from 12.5/1 to 200/1. The initial culture period was followed by a twenty hour label pulse.

Compared with tumour cells alone, an increase in the uptake of label was observed in cultures having the lowest L/TC ratios. This increase was much greater than that produced by the spleen cells alone, and was considered to result from a "feeder" effect induced on L5178Y cells by the presence of spleen cells. In general, an inverse relationship was observed between uptake of label and increasing numbers of spleen cells in the culture which could be due to crowding. Nevertheless, some growth inhibition was detected in cultures at a L/TC ratio of 100/1. Spleen cells taken from mice fourteen days to eighteen days following specific sensitization showed a tendency to decrease the uptake of radioactive label more than did normal spleen cells. This decrease was signifigant only in the case of spleen cells taken sixteen days after sensitization.

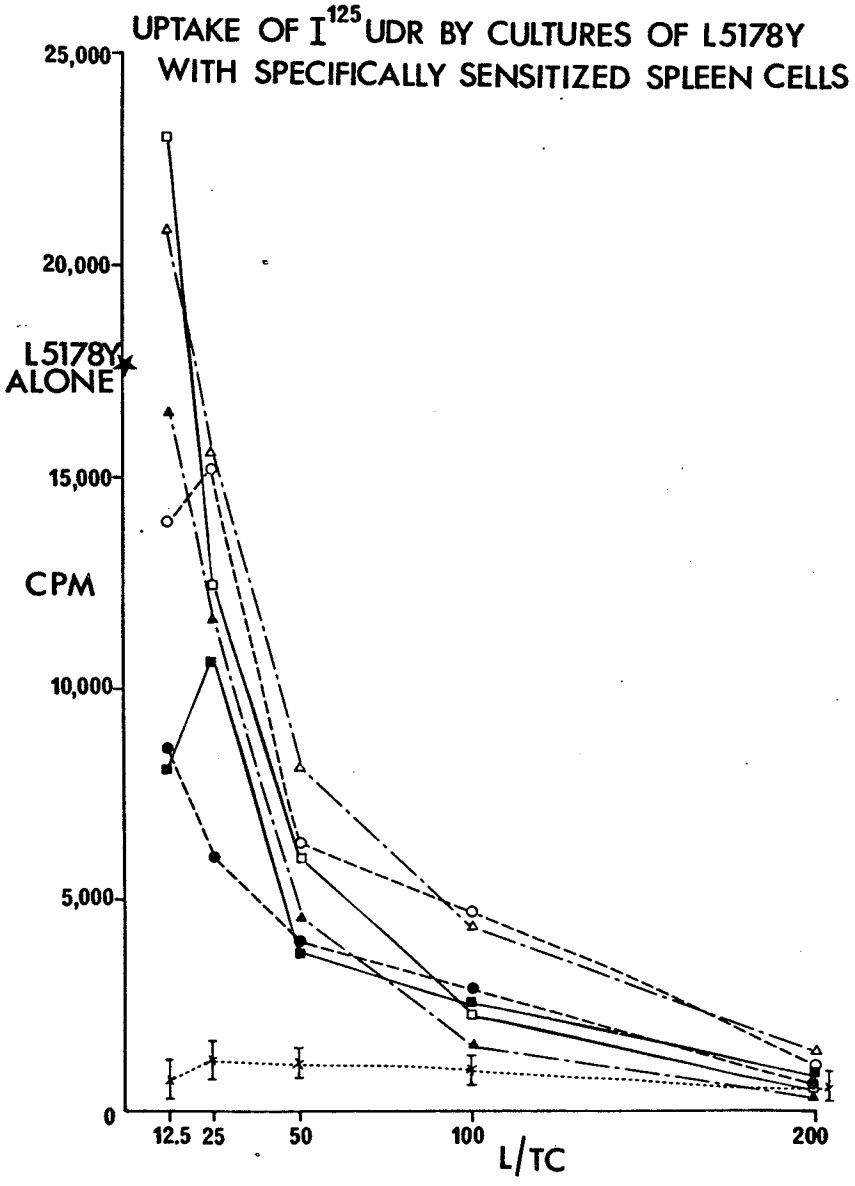

Figure 13: Spleen cells were taken 10-18 days after sensitization with 10^7,7xFT L5178Y cells. L5178Y cells in culture with: normal spleen cells ●——●; 10 day spleen cells ○——○; 12 day spleen cells △——△; 14 day spleen cells □——□; 16 day spleen cells ▲——▲; 18 day spleen cells ■——■. Mean and SD of all cultures containing only spleen cells ✱----✱. L5178Y cells alone ✱

Figure 14 shows the changes in the percent growth inhibition of L5178Y cells with increasing time in culture. Spleen cells sensitized fifteen days previously were used at a L/TC ratio of 100/1. Growth inhibition of L5178Y target cells by the sensitized spleen cells increased from day one to three in comparison to the effect of normal spleen cells. Similarly, the growth inhibition by the specifically sensitized spleen cells increased with culture time in comparison to the effect of spleen cells sensitized fifteen days earlier with 5×10^8 HRBC. Normal spleen cells decreased label uptake in L5178Y cultures more than HRBC sensitized spleen cells did. Thus, the percent growth inhibition by L5178Y-sensitized spleen cells was greater when compared with HRBC sensitized spleen cells than to normal spleen cells. Mice, pretreated with a dose of 1 cmm of STE given on four consecutive days, were "sensitized" with 10^7, 7XFT L5178Y cells on the seventh day after initiation of the pretreatment. Spleen cells from these animals were assayed for their ability to inhibit tumour growth fifteen days later as part of the same experiment already described. Thus Figure 14 also shows that a <u>decrease</u> in growth inhibition occurred in the presence of "sensitized" spleen cells from animals pretreated with STE. This decrease appeared in cultures incubated for two and three days before the twenty hour pulse and increased with increasing culture time. Thus, for further experiments, the cultures set up were incubated in sextuplet, at three different L/TC ratios, for three days before the twenty hour I^{125}UDR pulse.

Six experiments were carried out employing spleen cells from pre-treated and normal mice "sensitized" with L5178Y cells thirteen, fifteen or seventeen days earlier. Normal unsensitized mice and mice sensitized non-

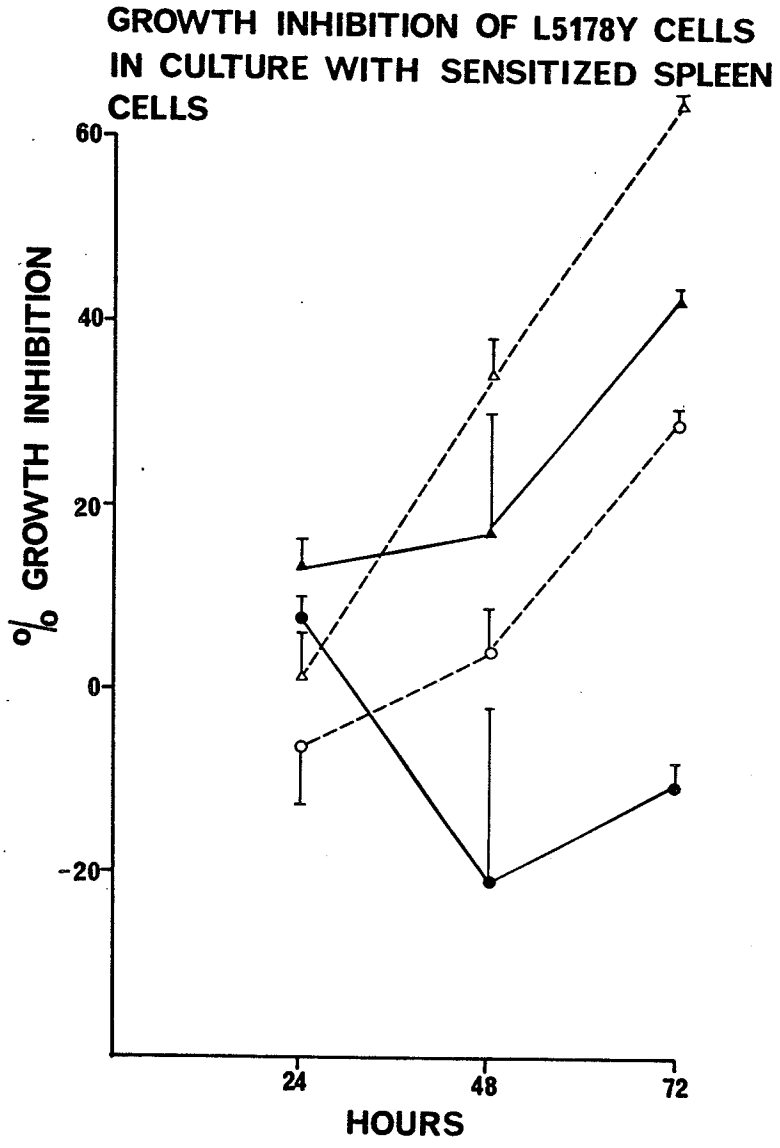

Figure 14: Spleen cells taken 15 days after sensitization with 10^7,7xFT L5178Y cells.Growth inhibition of L5178Y cells by sensitized spleen cells from mice pretreated●——●,and not pretreated○--○ with STE, compared with normal spleen cells. Growth inhibition of L5178Y cells by sensitized spleen cells from mice pretreated▲——▲,and not pretreated △--△ with STE, compared with HRBC sensitized spleen cells.

specifically to HRBC were used as controls. All of the results of these experiments are presented in Tables XXXVII and XXXVIII.

In general, normal spleen cells at an L/TC ratio of 100/1 reduced the uptake of label by tumour cells to such an extent that no further decrease could be detected in the presence of specifically sensitized cells (Table XXXVII). In fact, the percent growth inhibition of tumour cells by specifically sensitized cells was greater overall at an L/TC of 50/1 and was statistically significant at least at the 2.5% level in the first five experiments listed. At this ratio,in every experiment,a decrease in the percent growth inhibition was observed in cultures containing spleen cells from "sensitized" mice pretreated with STE at a dose level of 1 cmm or 10 cmm. At the same ratio, a decrease in growth inhibition was always observed in every culture containing spleen cells from "sensitized" mice pretreated with doses of 50 µg deaggregated Hgg in place of STE. In fact, in most experiments, either pretreatment produced a decrease in percentage growth inhibition.

The inhibition of tumour growth in cultures containing spleen cells from specifically "sensitized" mice was also compared with cultures of tumour cells containing spleen cells from HRBC "sensitized" mice (Table XXXVIII).The presence of HRBC sensitized cells depressed uptake of label less than did normal spleen cells at an L/TC ratio of 100/1. Overall, in a slightly greater percentage of experiments, HRBC sensitized cells depressed uptake of label less than did normal spleen cells. In every experiment carried out at an L/TC ratio of 50/1, a decrease in the percentage growth inhibition was observed in cultures containing spleen cells from sensitized mice pretreated with STE. Similarly, at the same ratio, a

TABLE XXXVII

% Growth Inhibition of L5178Y Cells By Spleen Cells

From Specifically Sensitized Animals Compared with Normal Animals.

Day[a]	Treatment	L/TC	25/1	50/1	100/1
13	L5178Y[b]		25.0	27.3	4.9
	STE + L5178Y[c]		12.3	17.4	0
	Deagg. Hgg + L5178Y[d]		10.1	5.0	0
13	L5178Y		13.9	46.1	0
	STE + L5178Y		34.6	13.3	0
	Deagg. Hgg + L5178Y		30.0	15.4	10.8
15	L5178Y		21.1	12.2	14.9
	STE + L5178Y		11.0	2.9	8.8
	Deagg. Hgg + L5178Y		15.4	11.3	15.4
15	L5178Y		3.8	9.2	8.5
	STE + L5178Y		0	0.9	4.2
	Deagg. Hgg + L5178Y		0	0	0
17	L5178Y		15.4	38.9	0
	STE + L5178Y		0	0	0
	Deagg. Hgg + L5178Y		0	0	0
17	L5178Y		41.6	3.3	0
	STE + L5178Y		12.5	0	0
	Deagg. Hgg + L5178Y		30.3	0	0

[a]Days after sensitization with 10^7, 7xFT, L5178Y cells.

[b]Sensitization with 10^7, 7xFT, L5178Y alone.

[c]Pretreatment with 4 injections of STE followed by sensitization with 10^7, 7xFT, L5178Y on day 7 after first injection.

[d]Pretreatment with 4 injections of deaggregated Hgg following by sensitization with 10^7, 7xFT, L5178Y on day 7 after the first injection.

TABLE XXXVIII

% Growth Inhibition of L5178Y Cells by Spleen Cells From Specifically Sensitized Animals Compared with HRBC Sensitized Animals.

Day[a]	Treatment	L/TC	25/1	50/1	100/1
13	L5178Y[b]		4.4	9.7	24.5
	STE + L5178Y[c]		0	0	15.9
	Deagg Hgg + L5178Y[d]		0	0	0
	L5178Y		16.8	50.5	0
	STE + L5178Y		36.8	20.3	0
	Deagg. Hgg + L5178Y		32.3	22.3	4.2
15	L5178Y		0	45.2	18.2
	STE + L5178Y		0	11.5	12.5
	Deagg. Hgg + L5178Y		0	0	7.4
	L5178Y		16.1	11.8	15.6
	STE + L5178Y		5.4	1.6	9.5
	Deagg. Hgg + L5178Y		10.1	9.8	16.0
17	L5178Y		5.3	14.3	11.2
	STE + L5178Y		0.2	6.5	7.0
	Deagg. Hgg + L5178Y		0	1.8	0
	L5178Y		63.8	84.3	76.2
	STE + L5178Y		34.4	81.6	69.3
	Deagg. Hgg + L5178Y		56.8	77.7	82.2

[a]Days after sensitization with 10^7, 7xFT, L5178Y.

[b]Sensitization with 10^7, 7xFT, L5178Y alone.

[c]Pretreatment with 4 injections of STE followed by sensitization with 10^7, 7xFT, L5178Y on day 7 after first injection.

[d]Pretreatment with 4 injections of "deaggregated Hgg" followed by "sensitization" with 10^7, 7xFT, L5178Y on day 7 after first injection.

decrease in growth inhibition was observed in every culture containing spleen cells from mice pretreated with 50 µg deaggregated Hgg in place of STE. Again, most experiments showed decreased inhibition of tumour growth in cultures containing spleen cells from sensitized mice pretreated with either STE or deaggregated Hgg.

Table XXXIX shows the means and standard deviations of the present growth inhibition for the five experiments in which the growth inhibiting ability of specifically sensitized animals was significantly different from normal animals. The decreases, resulting from pretreatment of sensitized animals with either STE or deaggregated Hgg, were statistically significant using normal or HRBC sensitized animals as controls ($p < .025$).

Thus, repeated exposure of mice to small quantities of soluble tumour cell extract or heterologous protein is correlated with a decreased ability to produce in vitro inhibition of tumour growth following specific sensitization.

TABLE XXXIX

% Growth Inhibition of L5178Y Cells.

Treatment	Normal[a]	% Decrease	HRBC[b]	% Decrease
L5178Y[c]	26.7 ± 16.1		29.9 ± 25.1	
STE + L5178Y[d]	6.9 ± 7.9	(74.8)	5.7 ± 8.6	(80.3)
Deagg. Hgg + L5178Y[e]	6.3 ± 6.8	(71.4)	6.8 ± 9.6	(72.2)

[a]Growth inhibition by spleen cells from specifically sensitized animals compared with normal animals.

[b]Growth inhibition by spleen cells from specifically sensitized animals compared with HRBC sensitized animals.

[c]Sensitization with 10^7, 7xFT, L5178Y cells.

[d]Pretreatment with 4 injections of STE followed by sensitization with 10^7, 7xFT, L6178Y cells on day 7 after the first injection.

[e]Pretreatment with 4 injections of deaggregated Hgg followed by sensitization with 10^7, 7xFT, L5178Y cells on day 7 after the first injection.

DISCUSSION

There is ample evidence in the literature supporting the existence of immune surveillance (Burnet 1970) but its ability to play an effective role in the defence against tumours has been seriously questioned (Prehn 1971). The proponents of this theory have implicated interference with the efferent arm of the immune response in the ability of antigenic tumours to survive and grow (Hellström and Hellström 1969a; Klein 1972). While interference with the immune effector mechanism may explain the inability of the host to eliminate an established tumour, it does not explain the ability of a tumour to survive and proliferate in its earliest stages of development when it should be most susceptible to immune attack.

Investigators have suggested that in certain cases the host is predisposed to tumour growth; it is unable to initiate an immune response against a nascent tumour due to neonatal tolerization (Klein and Klein 1965), or immunosuppression resulting from viremia (Allison 1970b). As well, tumours particularly in their early stages of growth, may not express sufficient immunogenicity to invoke an immune response (Klein 1972).

In contrast little emphasis has been placed on the possible active immune response actually induced by a tumour in its initial stages of growth.

In contrast to a passive, lack of any recognizable immune response, the results of this study indicate that a tumour in its earliest stages of growth actively initiates a state of immunosuppression.

The main features of this thesis will be discussed separately under individual headings.

I. ANTIGENIC QUALITIES OF THE L5178Y LYMPHOMA IN DBA/2 MICE AS EXPRESSED BY THE SIX HOUR RESPONSE

Although the L5178Y lymphoma, demonstrated here to a θ-bearing tumour, is highly compatible with its syngeneic host, DBA/2 (Alexander et al 1966; Goldstein et al 1973), several investigators have previously reported it to be immunogenic in this host strain (Wolf 1969; Alexander et al 1966; Goldenberg and Wilt 1969; Goldstein and Manson 1973).

The six hour response, the increase in Ig bearing spleen cells, has been found to be a generalized phenomenon occurring six hours after administration of a wide variety of antigens including soluble proteins such as BSA, FIB, and KLH given emulsified with FCA and particulate antigens, SRBC and CRBC given without FCA (Paraskevas et al 1972b). It has been demonstrated in Balb/c, DBA/2, NZB and AKR inbred mouse strains. This phenomenon is one of the earliest occurrences following immune stimulation and is considered to correlate with adjuvant activity, whether intrinsic or extrinsic (Orr 1974), such as the promoting effect which FCA exerts on antibody response. Recently the transfer of six hour spleen cells into irradiated mice followed by specific antigen challenge has been shown to have an enhancing effect upon antibody production (PFC), compared with similar transfer and challenge of normal spleen cells (Lee 1975).

A large dose of 10^7, L5178Y leukemia cells, live or repeatedly frozen and thawed, has now been demonstrated to produce an increase in Ig carrying spleen cells in DBA/2 mice, six hours after IP administration. This increase is comparable to that produced by the variety of antigens previously mentioned. Similar administration of $5x10^6$ living DBA/2 peritoneal cells or 10^7 thymocytes

failed to produce a six hour response. The production of the six hour response by a syngeneic tumour was not peculiar to the L5178Y lymphoma in DBA/2 mice, as subsequently a tumour which arose spontaneously in an old AKR mouse, tested after 2-5 passages in vivo, was also found capable of producing the same increase in Ig bearing spleen cells, six hours after administration into AKR mice (Lee 1975).

The increase in rosette-forming Ig bearing spleen cells six hours after administration of tumour cells was not associated with any significant change in total spleen cell count.

Concomitant with the six hour increase in Ig bearing spleen cells, the θ-bearing spleen cell population decreased by approximately the same number, similar to results obtained with antigens previously tested (Lee and Paraskevas 1972).

The incubation of normal spleen cells in serum taken from animals six hours after inoculation of 10^7, L5178Y cells, (6 hour serum), was capable of reproducing the alterations in Ig and θ bearing cell populations observed in the spleens of the serum donors. Thus the increase in Ig bearing spleen cells is considered to be due to uptake of cytophilic Ig, by a subpopulation of spleen cells.

As pretreatment of normal spleen cells with anti-θ serum plus complement produced a cell population which failed to exhibit an increase in Ig bearing cells upon incubation with six hour serum, the cell responsible for taking up cytophilic Ig was demonstrated to be a T cell.

Similar findings have been made for heterologous antigens previously tested in this laboratory. Lee and Paraskevas (1972) have shown that six

hours after antigenic stimulation (POL/FCA), the observed increase in Ig bearing spleen cells is accompanied by a concomitant decrease in the θ-bearing population. Anti-θ serum treated normal spleen cells, exposed to six hour serum, did not show the increase in Ig bearing cells obtained with untreated normal spleen cells incubated in six hour serum. In contrast, anti-Ig treatment of normal spleen cells had no effect on their ability to show an increase in Ig bearing cells, following incubation in six hour serum. Only thymus cells but not bone marrow cells were demonstrated to be capable of exhibiting an increase in Ig bearing cells upon incubation in six hour serum. Furthermore, spleen cells from irradiated mice reconstituted with thymus cells showed a similar to normal increase after antigen challenge in contrast to bone marrow reconstituted mice which did not produce a six hour increase in Ig bearing spleen cells.

The active component of the six hour serum obtained after injection of L5178Y cells was found to reside in the 7S fraction resulting from G-200 separation of the serum. Other investigators have shown that the active fraction of six hour serum (without added 7S Ig or antigen) appears to reside in the 7S or 19S fractions depending upon the molecular size of the antigen used for injection with FCA. Thus, following injection of 4S radiolabelled BSA-I^{125} with FCA the active component was found in the 7S peak or just slightly ahead. This peak also contained BSA and some radiolabel. In contrast, injection of FIB produced a six hour serum having activity in the void volume or 19S peak where FIB is eluted (Orr and Paraskevas 1973).

Incubation of normal spleen cells in six hour BSA/FCA or FIB/FCA serum produced an increase in Ig bearing cells. Washing the cells after incubation

in such six hour sera, followed by treatment with antigen-specific rabbit antiserum resulted in a significant reduction of the increase in rosette formation induced by the six hour serum. This suggested that antigen participates in the complexes involved in the in vitro uptake of cytophilic Ig by T cells. This surface localization of antigen-Ig complexes may involve the Fc receptor or some other mechanism. Similarly Yuan and colleagues (1970) detected antigen complexed to γG immunoglobulin in serum of rabbits 5 hours after stimulation with antigen. Thus, although the hypothesis has not been tested in this case, it is probable that antigen-Ig complexes are also involved in the six hour response induced by L5178Y cells in DBA/2 mice.

It has now been demonstrated that the 4S fraction of six hour serum obtained after injection of 10^7, L5178Y cells, together with normal mouse serum or the 7S fraction of normal mouse serum, when used for incubation with normal spleen cells in the presence of a soluble protein antigen, BSA or FIB, is also capable of producing the same pattern of Ig and θ bearing cells as does six hour serum. This suggests that the increase in Ig bearing spleen cells is due to the uptake by T cells of 7S Ig and antigen in the presence of a serum factor found six hours after administration of antigen. Other investigators in this laboratory have demonstrated that an active 4S factor was regularly found in the six hour serum of mice injected with FCA alone, or a particulate antigen alone (Orr and Paraskevas 1973). It is possible that this may be an observable manifestation of participation of adjuvanticity, extrinsic or intrinsic, in the induction of the immune response. Thus the L5178Y leukemia cells in terms of their ability to induce a six hour response behaves as a soluble protein plus FCA or a particulate antigen.

II. INABILITY OF ANIMALS WITH A DEVELOPING TUMOUR TO PRODUCE A SIX HOUR RESPONSE

In contrast to a large dose of tumour cells, a very small number i.e. 100, live L5178Y cells, produced no statistically significant increase in Ig bearing spleen cells six hours after administration and still killed 95% of mice inoculated. This suggests that although the tumour is antigenic a sufficiently small dose of cells fails to induce the six hour response and as a consequence would alter the components of the immune response which may depend on this phenomenon.

Tumours growing from this small inoculum of cells were started in order to simulate the natural progress of spontaneously arising tumours. The demonstration that mice bearing these tumours were unable to produce a six hour response after a challenging dose of L5178Y cells (10^7), from day one to at least day seven after the inoculum of live cells indicates that the properties of the T cell surface have been altered.

The failure of a challenging dose of heterologous erythrocytes to produce a six hour response for the same length of time indicates that tumour growth in these animals has non-specifically abrogated this phenomenon.

The increase in Ig bearing spleen cells which is observed on day three following initiation of tumour growth is presently not understood.

Normal spleen cells incubated in serum from tumour bearers taken six hours after a challenging dose of antigen failed to show an increase in Ig bearing cells. This indicates that the cytophilic Ig is absent from the sera.

One could speculate that the T cell defect observed in these animals is related to the fact that T cells are not only deprived of taking up cytophilic

Ig which may comprise a signal involved in the induction of some aspect of the immune response but also that cells, whether the same or different, are possibly made incapable of producing the active factors involved.

These results are corroborated by similar observations made on animals bearing tumours growing from an initial inoculum of 1000 live cells, which killed 100% of mice injected. This dose of cells was capable of producing only a partial six hour response and was also found to inhibit the production of a six hour response to a challenging dose of antigen from day one to at least seven days after initiation of tumour growth.

III. MECHANISM OF INDUCTION OF THE T CELL DEFECT

A number of investigators have reported that tumour growth was associated with the presence of tumour antigens in the serum (Ambrose et al 1971; Thomson et al 1973), whether by spontaneous shedding of surface determinants by viable cells (Currie and Alexander 1974; Ben-Sasson et al 1972; Currie and Basham 1972; Thomson et al 1973) or as a result of cell death (Currie and Basham 1972). This suggested that the continual presence of at least initially small amounts of soluble tumour antigen could be responsible for the lack of production of a six hour response.

In order to test this hypothesis a soluble low molecular weight fraction of a papain digest of L5178Y cells, from ascites which also contained red blood cells, was prepared. This soluble extract was shown to be related to surface components of the tumour cell by its ability to block the reaction of tumour specific antiserum and tumour cells. This extract in sufficient quantity was demonstrated to be capable of inducing a six hour response without the benefit of FCA unlike the same quantities of similar preparations from DBA/2 thymocytes with or without syngeneic red blood cells. This surprising evidence suggests that this fraction may bear some unusual adjuvant

properties in contrast with most soluble protein antigens so far tested. From the experience in this laboratory only POL alone could induce a six hour response in the absence of FCA (Paraskevas et al 1972b). Since POL is a highly polymerized product from flagella this is not so surprising. In addition a small molecular weight (circa 30,000 daltons) water soluble glycoprotein known as MAAF, obtained from mycobacteria (Hiu 1972) also induces a six hour response in the presence of soluble proteins (Orr 1975).

Smaller doses of the soluble extract of L5178Y were determined which would not produce a six hour response.

A single injection of a small amount of this extract, below the level which produced a six hour response, was observed to produce only transient abrogation, of the six hour response induced by challenging antigens, lasting for one to three days.

Although a specific, transient unresponsiveness, lasting for one to two days following an immunogenic dose of antigen has been reported in studies of adoptive transfer of primed lymphoid cells with antigen (Sprent and Miller 1973), the defect observed in this study is considered to be different as it is expressed non-specifically and is related to a very small initial dose of soluble antigen.

Repeated daily injection, of the same small dose of soluble tumour cell extract, for four consecutive days was found to be capable of inhibiting the six hour response to a challenging dose of antigen, L5178Y cells or HRBC, from day one to at least day seven, similar to the inhibition observed by a tumour growing from a small inoculum of cells. In fact the peak of Ig bearing spleen cells and concomitant decrease in θ bearing spleen cells occurring

three days after the live tumour cell inoculum was also reproduced by this treatment.

A single injection of 50 µg of deaggregated Hgg, which itself did not produce a six hour response, transiently inhibited the ability of the animal to produce a six hour response to a challenging dose of antigen, Hgg/FCA or L5178Y. Following the protocol used with the tumour cell extract, repeated injection with deaggregated Hgg was found to inhibit the animal's ability to produce a six hour response to antigen challenge at least till day seven after the first injection. This demonstrates that it is not a peculiarity of the tumour cell extract that is related to inhibition of the six hour response but rather a consequence of the type of stimulation resulting from continual exposure to small amounts of soluble antigen.

Animals given an initial inoculum of 100 live tumour cells when tested fourteen days later were able to produce a partial six hour response following challenge with antigen. These animals died, with a large amount of ascites, between days 20-30 after induction of tumour growth. This suggests that the initial inhibition of the six hour response may be abrogated by the presence of larger amounts of soluble and probably particulate antigen occurring in the presence of an established tumour.

In contrast, it may be possible to extend the period of inhibition of the six hour response indefinitely in animals given repeated injections of soluble antigen, by continued administration of soluble antigen.

The results of the experiments involving repeated injections of small amounts of soluble antigen suggest that the continual exposure of the immune system to small amounts of soluble antigen results in a defect on the T cell surface related to participation in the six hour response.

Sera from animals given repeated small doses of soluble antigen similar to sera from tumour bearer animals reproduced the same lack of the six hour response observed in the original animals. This suggests that serum factors involved in the in vitro response are absent. As noted previously, the requirements for reproduction of the six hour response in vitro are 7S Ig, soluble antigen and a 4S serum factor. Only the serum factor must come from stimulated animals. Thus it is likely that the T cell defect lies in the inability of cells to produce the active factor(s) and/or the inability of T cells to localize cytophilic Ig on their surfaces.

Although the defect observed is associated with the T cell population, the subsequent alteration of the pathways of immune processes could be limited or far ranging, effecting the production of a wide range of products of the immune response. The observation that the six hour response involves large scale population changes indicates that antigen stimulation effects a population of cells much larger than the antigen sensitive cells (Paraskevas, et al, 1972b) and thus interference with this response might be expected to have widespread and varying effects on a subsequent immune response. For this reason, the relationship of the T cell defect observed only six hours after exposure to antigen, to the ultimate production of the immune response, was examined by means of an in vitro correlate, a growth inhibition assay, which is possibly one of the more broad based assays, involving a number of products of the immune response (Lamon, et al, 1973a and b; Plata et al, 1974; Plata and Levy, 1974).

IV. IN VITRO IMMUNE CORRELATE OF THE PRODUCTION OF THE T CELL DEFECT

The growth inhibition experiments demonstrated that this assay can detect an antitumour reaction in syngeneic mice which have been given a single injection of frozen and thawed L5178Y cells.

Pretreatment of the animals for 4 consecutive days with daily injection of a small dose of tumour cell extract or deaggregated Hgg, followed by sensitization with frozen and thawed tumour cells, resulted in a significant decrease in in vitro growth inhibition.

The results are reproducible and relatively quantitative over the period of activity (13-17 days). The degree of growth inhibition must be compared under identical conditions as the incorporation of I^{125}UDR depends on the growth characteristics of the tumour cells in the test, the cell concentrations in the culture and the lymphocyte to tumour cell ratio. Thus quantitative data can only be compared within a single experiment.

The large number of effector cells in the system do not seem to interfere with the detection of growth inhibition. The uptake of I^{125}UDR by lymphoid effector cells without tumour target cells is about 10% of the uptake of target and lymphoid cells combined in culture. It might be anticipated that contact with target cells would stimulate cell division in the specifically sensitized effector cells. If this occurs, it must be insigificant, as it would increase the uptake of I^{125}UDR in the test wells and thus result in a decrease in the growth inhibition.

The toxic effects of I^{125}UDR (Le Mevel, et al, 1973; Oldham and Herberman, 1973) have been minimized in this study by labelling the cells at a late stage in the test (Seeger and Owen, 1973) and by the use of a relatively

short labelling time (Oldham and Herberman, 1973).

The advantage of this radioisotope assay of cellular immunity is its objectivity and sensitivity, particularly as low ratios of lymphoid cells to target cells are used. I^{125}UDR is only released into the medium upon cell death and lysis and very little reutilization of I^{125}UDR occurs (Le Mevel, et al, 1973). I^{125}UDR is a gamma emitter and therefore requires little sample preparation before counting.

The apparent cytostatic effect of normal lymphoid cells could be due to overcrowding or the normal mice may be sensitized to cross-reacting antigens (Chia and Festenstin, 1973). Alternatively, the normal unstimulated mice may express some 'natural immunity' against the syngeneic tumour line as mice immunized with heterologous erythrocytes, on the average, exhibited a slightly smaller cytostatic effect upon L5178Y cells than did normal mice. Possibly 'natural immunity' is decreased by non-specific stimulation.

The occasional finding of increased DNA synthesis at low L/TC ratios, and in shorter cultures, suggests that a "feeder effect" (Chia and Festenstein, 1973) may be active in this system, but that it is usually obscured by the depressive effect of increased numbers of lymphoid cells and increased time of culture.

Growth inhibition of tumour target cells, detected here after 44 hours, requires 3-4 days to achieve significance. The observation that cytostasis was maximum after 80-90 hours incubation has been made by other investigators (Chia and Festenstin, 1973; Senik, et al, 1974a). The former investigators have suggested that this time may be required for development of an in vitro secondary response to tumour antigens by lymphoid cells primed in vivo.

The growth inhibition assay has been used in a syngeneic system to demonstrate a specific anti-tumour reaction which can be inhibited by specific sera (Chia and Festenstein, 1973). Other groups have also obtained positive cytostasis in syngeneic systems (Fish, et al, 1974; Finklestein, et al, 1972; Oppenheim, et al, 1970).

The existence of cytostasis of syngeneic, antigenically related MSV tumour cells by lymphoid cells of mice bearing MSV-induced tumours has been demonstrated using a microcytotoxicity assay (Owen and Seeger, 1973; Plata, et al, 1974; Plata and Levy, 1974) and a cytostasis assay (Senik, et al, 1974a).

Currently, there is conflicting evidence concerning the nature of the effector cell, the specificity of the cytostatic reaction, the nature of the effector cell/target cell interactions and the mechanism of tumour cell inhibition involved in cytostasis assays.

Senik and colleagues (1974a) have reported that the same lymphoid cell suspensions taken from spleen or lymph nodes of MSV-tumour-bearing mice were shown to contain several different "immune lymphoid cells" which could be separated in vitro according to the method used to study cell mediated immunity.

Cytotoxic T cells are thought to be the most significant and possibly exclusive mediators of target cell destruction in the chromium release test (CRT) (Cerottini, et al, 1970; Golstein, et al, 1972; Wagner, et al, 1972; Plata, et al, 1973). Although evidence for the participation of these cells in the immune response in syngeneic systems is sparse, as noted previously, the specific cytolysis of tumour target cells employing CRT has been demonstrated in one system, which involves syngeneic, antigenically related

MSV tumours (Plata, et al, 1973; Leclerc, et al, 1973; Herberman, et al, 1973). Cocultivation of syngeneic "cortisone resistant" thymocytes and tumour cells for 6 days has been demonstrated to produce a population of T cells having cytolytic activity measurable by the CRT (Wagner and Röllinghoff, 1973). Consequently, it has been suggested (Perlmann, et al, 1972b; Lamon, et al, 1972a) that cellular immune mechanisms in allograft rejection and in syngeneic tumour regression are not comparable as the effector cells in the former system are essentially thymus-dependent, whereas non-T cells have been revealed to be of major importance in anti-tumour immunity (Lamon, et al, 1972a).

As implied above, the use of other assay systems and, in some cases, different target cells has produced different results. The microcytotoxicity assay has been used to demonstrate both T and non-T effector cell activity against monolayers of MSV tumour cells in the same isologous system employed by Leclerc and colleagues (Lamon, et al, 1973a and b; Plata, et al, 1974; Plata and Levy, 1974). The non-T effector cells are thought possibly to be bone marrow derived (B) cells as they are not eliminated by the carbonyl iron and magnet removal method, which is effective on macrophages.

Macrophages have been shown to be active in the cytostasis assay of cell mediated immunity against tumour cell suspensions in the isologous MSV system of Leclerc (Senik, et al, 1974a and b). Owen and Seeger (1973) have also demonstrated the action of non-T, possibly macrophage effector cells in the growth inhibition of MSV tumours. In a syngeneic system, macrophages have been demonstrated to be capable of inhibiting the growth of lymphoma cells in a 48 hour, in vitro, culture (Evans and Alexander, 1972b). Further studies implicated the activity of a T cell factor for

"activation" of the macrophages for non-specific killing (Evans, et al, 1972).

Thus a number of investigators have demonstrated the susceptibility of tumour target cells to both T and non-T type immune reactions (Lamon, et al, 1972a and b; Senik, et al, 1974b). Although the cytostasis immune reaction has been shown to involve primarily non-T effector cell activity, whether B cell or macrophage, activity of an effector T cell has not been excluded completely (Senik, et al, 1974a; Chia and Festenstein, 1973; Lamon, et al, 1973a and b). Thus the positive cytostatic effect of spleen cells from mice given a single inoculation of frozen and thawed L5178Y cells, upon L5178Y cells in culture could represent effector activity of T cells, B cells or macrophages, or a combination thereof.

The cytostatic reaction observed here is likely specific as spleen cells from animals stimulated non-specifically with heterologous erythrocytes (HRBC) failed to produce a positive reaction against the tumour cells. Other investigators have reported specific cytostasis at low L/TC ratios in an oncornavirus system (Wahren and Metcalf, 1970). Senik and colleagues (1974b) observed that the cytostatic reaction of tumour bearer spleen cells against lymphoma cells in suspension was specific at low L/TC ratios (25/1) with non-specific reaction occurring at higher ratios. Owen and Seegar (1973) also demonstrated non-specific cytostasis caused by macrophages with 500/1 to 3000/1 L/TC ratios. Possibly a specific effect occurs at low L/TC ratios and a non-specific effect appears when a high number of "activated" macrophages are present as suggested by Evans and Alexander (1972a).

Senik and colleagues (1974b) have suggested that the interaction between lymphoid and target cells with a cytostatic effect on the tumour

target cell is probably mediated by a soluble factor as the effector cells can be "armed" or inhibited by the serum of tumour-bearing mice (Senik, et al, 1974b; Chia and Festenstein, 1973). Soluble antigens (Plata and Levy, 1974) and antigen-antibody complexes (Sjögren, et al, 1971; Baldwin, et al, 1973) have been implicated in the inhibition of cytostasis in the microcytotoxicity assay. Antibodies active in this reaction could be carried over into the cultures by sensitized spleen cells or perhaps could be produced in a secondary response which may occur in the in vitro culture (Chia and Festenstein, 1973).

As pretreatment of mice with repeated doses of soluble antigen deaggregated (Hgg), similar to pretreatment with soluble tumour cell extract, was found to depress the in vitro tumour growth inhibiting ability of spleen cells from subsequently sensitized animals,the depression of cytostasis is non-specific. This non-specific inhibition of the specific cytostasis reaction could result from the interference with T and/or non-T effector cells at any point during the process of their production or action. Further, the non-specific inhibition of the cytostatic reaction against tumour cells in vitro is correlated with the production of a T cell defect which is the inability to participate in the six hour response. The defect is observed following repeated injection of animals with small doses of soluble antigen and this treatment simulates the production of the same defect observed originally in mice carrying tumours growing from a small inoculum of live tumour cells.

The observation of the correlation of the same T cell defect with a decrease in immune reactivity has been made by another investigator in this laboratory. Daily injection for 5 consecutive days of a dose of soluble

HRBC hemolysate (which by itself does not produce a six hour response), resulted in the non-specific inhibition of the six hour response to a challenging dose of antigen from day 7 to day 14. This treatment resulted in specific inhibition of both 19S and 7S PFC response to an antigen challenge given on day 7 after the first injection of soluble antigen. In contrast, the same treatment was correlated with the non-specific decrease in the production of the delayed hypersensitivity reaction and cell mediated cytotoxicity, as measured by CRT, in animals stimulated on day 7 after the first injection of soluble antigen. Similarly, the PHA reactivity of spleen cells of mice taken 7 days after the first injection of soluble antigen was also non-specifically decreased, compared with normal controls (Chou, 1975).

Bonavida and Zighelboim (1974) have demonstrated that IP injection of 50 µg of a soluble allo-cell extract, daily for 5 consecutive days, followed by challenge on day 15 after the initial injection, resulted in the production of poor cell mediated cytotoxic responses and a partially suppressed humoural response to tumour cells having the same H-2 specificity as the cell from which the soluble extract was prepared. The specificity of this reaction was not tested. These investigators also found that a single injection of a larger dose of soluble cell extract produced a specific decrease in cell mediated cytotoxicity and complement dependent cytotoxic antibodies and was associated with the production of specific blocking activity in the serum. In contrast, no blocking activity was found in the animals given multiple injections of a smaller dose of cell extract. Thus, the mechanism resulting in decreases in the immune response, following repeated pretreatment, may differ from the specific impairment following a single larger dose of soluble cell extract.

The results presented in this thesis and those of others thus suggest that continual exposure of an animal to small, possibly sub-immunogenic doses of soluble antigen results in the specific interference in the production of the humoural response and the non-specific interference in the production of cell mediated immunity, which is correlated with the appearance of a defect in the T cell surface observed six hours after administration of a challenging dose of antigen. As the T cell defect is detected very early after exposure to antigen, it is considered that the correlated interference with the immune response occurs during the inductive phase. As the same T cell defect observed in animals treated with repeated doses of soluble antigen was demonstrated in mice bearing tumours soon after inoculation of a very small number of live tumour cells, similar interference with the induction of the immune response would be expected. This actively induced T cell defect would thus provide a mechanism for the survival of tumours at a very early stage of development, allowing time for proliferation to an established tumour by which time interference with both inductive and effector mechanisms, as outlined in the Introduction, become important for continued tumour survival.

In recent years, other investigators have suggested that depression of immune reactivity by small inocula of tumour cells is responsible for their survival in contrast with the less successful growth of larger inoculae (Kölsch, et al, 1973; Bonmassar, et al, 1974).

Kölsch and colleagues (1973) also reported that mice inoculated three times at weekly intervals with lethally irradiated syngeneic tumour cells were made more susceptible to a subsequent exposure to a large dose of tumour cells when the pretreatment inoculum was 10^2-10^3 cells, compared with

pretreatment with 10 or 10^4-10^7 irradiated tumour cells. The specificity of this phenomenon was not examined. These investigators interpret this data as evidence for low dose tolerance; antigen in concentrations too low to immunize the host against tumour is able to facilitate the growth of tumour cells. It should be noted that the lowest dose, 10 cells, was not effective in predisposing the host to tumour growth; possibly 10 cells is too few to initiate the facilitative response. Their induction of a facilitative response by repeated small doses of antigen, could be interpreted as active interference with the induction of cell mediated immunity and/or humoural immunity similar to the production of the T cell defect, due to repeated exposure to small amounts of soluble antigen, observed in this study.

Recently, a number of reports in the literature have described the association of suppressive factors with tumour cells. Bonmassar and colleagues (1973, 1974) have demonstrated that a Millipore filtrate obtained from 10^6 frozen and thawed, allogeneic tumour cells, given 3 days before a live tumour cell challenge, predisposed the animals to tumour growth in a non-specific manner. Their soluble antigen, given only once, would be expected to have a suppressive effect on antigen challenge 3 days later, in accord with this study, but probably no later, unless the antigen has the ability to persist in the host. These investigators also report that pretreatment of animals with a single injection of a small number of tumour cells made the animals susceptible to the growth of a subsequent challenge of tumour cells up to 90 days later, regardless of whether the H-2 antigens of challenging and conditioning tumours were alike or different. These authors postulate that an initial

inadequate antigen stimulus coupled with immunosuppressive influences is responsible for the outgrowth of the second tumour cell inoculum. This data corroborates our finding of a state of immunosuppression following inoculation of a small number of tumour cells or repeated injection of small doses of soluble antigen.

Somewhat similar results have been obtained by Nowotny and colleagues (1974) who have demonstrated that treatment with a particulate, membranous preparation from the ascites fluid of tumour-bearers was capable of promoting tumour growth in animals subsequently given a TD50 dose of live tumour cells.

In an allogeneic system, Wong and colleagues (1974) have demonstrated that an ultracentrifuged supernatant from a tumour cell culture, when added to a Marbrook culture containing specifically sensitized spleen cells and antigen in the form of SRBC, resulted in a decrease in the number of direct PFC obtained.

In conclusion, evidence is beginning to appear in the literature which associates immunosuppression with small numbers of tumour cells, tumour cell products and also with repeated doses of small amounts of soluble antigen. This supports the basic hypothesis of this study, which postulates that the initial immune response to a tumour growing from a small inoculum of cells is immunosuppression. This may be mediated by the continuing presence of small quantities of soluble antigen and could last long enough to allow a nascent tumour to become established, thus escaping immune surveillance in the initial stages of growth.

BIBLIOGRAPHY

Abercrombie, M., and Ambrose, E.J. (1962). The surface properties of cancer cells: A Review. Cancer Res. 22: 525-548

Ackerman, G.A. and Knouff, R.A. (1964). Lymphocytopoietic activity in the bursa of Fabricius in "The Thymus in Immunobiology", eds. Good, R.A. and Gabrielsen, A.E, Harper & Row, p. 123-149

Ada, G.L. and Byrt, P. (1969). Specific inactivation of antigen reactive cells with I^{125} labelled antigen. Nature 222: 1291-1292

Alexander, P., Connell, D.I. and Mikulska, Z.B. (1966). Treatment of murine leukemia with spleen cells or sera from allogeneic mice immunized against the tumor. Cancer Res. 26: 1508-1515

Alexander, P., (1968). Immunotherapy of cancer: Experiments with primary tumours and syngeneic tumour grafts. Progr. Exp. Tumor Res. 10: 22-71

Alexander, P., and Evans, R. (1971). Endotoxin and double stranded RNA render macrophages cytotoxic. Nature, New Biol. 232: 76-78

Allison, A.C. (1970a). Tumour development following immunosuppression. Proc. Roy. Soc. Med. 63: 1077-1080

Allison, A.C. (1970b). On the absence of tolerance in virus oncogenesis in "Immunity and tolerance in oncogenesis: 4th Quadrennial Perugia Cancer Conf., L. Severi ed (1969), p. 563

Allison, A.C. (1971). New cell antigens induced by viruses and their biological significance. Proc. Roy. Soc. B. 177: 23-39

Allison, A.C. (1972). Interactions of antibodies and effector cells in immunity against tumours. Ann. Inst. Pasteur, Paris 122: 619-631

Ambrose, K.R., Anderson, N.G., Coggin J.H. (1971). Cytostatic antibody and SV40 tumour immunity in hamsters. Nature 233: 321-324

Andersson, J., Sjoberg, O. and Möller, G. (1972). Mitogens as probes for immunocyte activation and cellular cooperation. Transplant. Rev. 11: 131-177

Aoki, T., Hämmerling, U., DeHarven, E., Boyse, E.A. and Old, L.S. (1969). Antigenic structure of cell surfaces. An immunoferritin study of the occurrence and topography of H-2, θ and TL alloantigens on mouse cells. J. Exp. Med. 130: 979-1001

Aoki, T. and Johnson, P.A. (1972). Suppression of Gross leukemia cell-surface antigen: a kind of antigenic modulation. J. Nat. Cancer Inst. 49: 183-192

Aoki, T., Chieco-Bianchi, L., Plata, E.J., Sendo, F., Hollis, V.W., Jr., Kudo, T. (1974). Host immune response to virus-induced tumours. Progr. Exp. Tumor Res. 19: 23-36

Archer, O.K., Papermaster, B.W. and Good, R.A. (1964). Thymectomy in rabbit and mouse: Consideration of time of lymphoid perlipheralization. in "The Thymus in Immunobiology" eds. Good, R.A. and Gabrielson, A.E. Hoeber Medical Div. Harper and Row Publ. New York

Askonas, B.A. and Rhodes, J.M. (1965). Is antigen associated with macrophage RNA? In "Molecular and Cellular Basis of Antibody Formation" p. 503-514. ed. J. Sterzl. Prague: Czechoslovak Akad. Sci.

Asofsky, R., Cantor, H. and Tigelaar, R.E. (1971). Cell interaction in the graft-versus-host response in "Progress in Immunology" ed. Amos, B.; Academic Press, p. 369-381

Attia, M.A., DeOme, K.B., Weiss, D.W. (1965). Immunology of spontaneous mammary carcinomas in mice. II Resistance to a rapidly and a slowly developing tumour. Cancer Res. 25: 451-457

Axelrad, R. (1965). Antigenic behaviour of lymphoma cell populations in mice as revealed by the spleen colony method. Progr. Exp. Tum. Res. 6: 30-33 (Karger, Basel)

Baker, P.J., Reed, N.D., Stashak, P.W., Amsbaugh, D.F., Prescott, B. (1973). Regulation of the antibody response to type III pneumococcal polysaccharide. I. Nature of regulatory cells. J. Exp. Med. 137: 1431-1441

Baldwin, R.W., Bowen, J.G. and Price, M.R. (1973). Detection of circulating hepatoma D23 antigen and immune complexes in tumour bearer serum. Brit. J. Cancer 28: 16-24

Bankhurst, A.D. and Warner, N.L. (1971). Surface immunoglobulins on mouse lymphoid cells. J. Immunol. 107: 368-373

Bankhurst, A.D., Warner, N.L. and Sprent, J. (1971). Surface immunoglobulins on thymus and thymus-derived lymphoid cells. J. Exp. Med. 134: 1005-1015

Bankhurst, A.D., Rouse, B.T. and Warner, N.L. (1972). Surface immunoglobulins on bursa-derived chicken lymphoid cells. Int. Arch. Allergy 42: 187-195

Bansal, S.C. and Sjögren, H.O. (1971). "Unblocking" serum activity in vitro in the polyoma system may correlate with anti-tumour effects of antiserum in vivo. Nature New Biol. 233: 76-77

Basten, A., Miller, J.F.A.P., Warner, N.L. and Pye J. (1971). Specific inactivation of thymus-derived (T) and non-thymus-derived (B) lymphocytes by I^{125}-labelled antigen. Nature New Biol. 231: 104-106

Basten, A., Miller, J.F.A.P., Sprent, J. and Pye J. (1972a). A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. J. Exp. Med. 135: 610-626

Basten, A., Warner, N.L. and Mandel, T. (1972b). A receptor for antibody on B lymphocytes. II Immunochemical and electron microscopic characteristics. J. Exp. Med. 135: 627-642

Batchelor, J.R. (1967). Assays for cytotoxic and hemagglutinating antibodies against histocompatability antigens in'Handbook of Experimental Immunology! ed. Weir, D.M. Blackwell Scientific Publications. Oxford and Edinburgh, p. 999

Beckwith, J.B. and Perrin, E.V. (1963). In situ neuroblastomas: A contribution to the natural history of neural crest tumours. Amer. J. Path. 43: 1089-1104

Ben-Sasson, Z., Doljanski, F. and Weiss, D.W. (1972). Specific binding of factor(s) from the culture medium of RSV transformed cells to lymphocytes from RSV tumor-bearing chickens.Isr. J. Med. Sci. 8:667

Bergheden, C. and Hellström, K.E. (1966). Studies on allogeneic inhibition. II. Inhibition of mouse tumour cell growth by in vitro contact with cells containing foreign H-2 antigens. Int. J. Cancer 1: 361-369

Bernier, G.H. and Cebra, J.J. (1965). Frequency distribution of α , γ , κ and λ polypeptide chains in human lymphoid tissues. J. Immunol. 95: 246-253

Beinstein, I.D., Wepsic, H.T., Zbar, B. and Rapp, H.J. (1971). Tumor immunity: Impairment in tumor-bearing hosts. J. Nat. Cancer. Inst. 46: 873-880

Bianco, C., Patrick,R. and Nussenzweig, V. (1970). A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. Exp. Med. 132: 702-720

Bianco, C. and Nussenzweig, V. (1971). Theta bearing and complement-receptor lymphocytes are distinct populations of cells. Science 173: 154-156

Biggar, W.D., Lapointe, N., Ishizaka, K., Meuwissen, H., Good, R.A. and Frommel, D. (1970). IgE in ataxia-telangiectasia and family members. Lancet II, 1089

Bonavida, B. and Zighelboim, J. (1974). Modulation of the immune response towards allografts in vivo. I. Selective suppression of the development of cell-mediated immunity by soluble alloantigens. Cell. Immunol. 13: 52-65

Bonmassar, E., Bonmassar, A. Goldin, A. and Cudkowicz, G. (1973). Depression of antilymphoma allograft reactivity by tumour-associated factors. Cancer Res. 33: 1054-1061

Bonmassar, E., Menconi, E., Goldin, A. and Cudkowicz,G. (1974). Escape of small numbers of allogeneic lymphoma cells from immune surveillance. J. Nat. Cancer Inst. 53: 475-479

Bordet, J. (1898). Agglutination and lysis of red blood cells by the serum of animals injected with defibrinated blood. Ann. Inst. Pasteur 12: 688-695

Boyse, E.A. and Old, L.J. (1969). Some aspects of normal and abnormal cell surface genetics. Ann. Rev. Genet. 3: 269-290

Brawn, R.J. (1971). In vitro desensitization of sensitized murine lymphocytes by a serum factor (soluble antigen?) Proc. Nat. Acad. Sci., Wash. 68: 1634-1638

Bretscher, P.A. and Cohn, M. (1968). Minimal model for the mechanism of antibody induction and paralysis by antigen. Nature 220: 444-448

Bretscher, P.A. and Cohn, M. (1970). A theory of self-nonself discrimination. Science 169: 1042-1049

Bretscher, P. (1972). The control of humoral and associative antibody synthesis. Transplant Rev. 11: 217-267

Britton, S. (1972). When allogeneic mouse spleen cells are mixed in vitro the T-cells secrete a product which guides the maturation of B cells. Scand. J. Immunol. 1: 89-98

Burchenal, J.H. (1968). Long term survivors in acute leukemia and Burkitt's tumour. Cancer 21: 595-599

Burkitt, D., Hutt, M.S.R. and Wright, D.H. (1965). The African lymphoma. Preliminary observations in response to therapy. Cancer 18: 399-410

Burkitt, D. (1966). African lymphoma. Observations on the response to vincristine sulphate therapy. Cancer 19: 1131-1137

Burnet, F.M. (1959). "The Clonal Selection Theory of Acquired Immunity" Cambridge University Press

Burnet, F.M. (1963). The evolution of bodily defence. Med. J. Aust. ii: 817-821

Burnet, F.M. (1964). Immunological factors in the process of carcinogenesis. Brit. Med. Bull. 20: 154-158

Burnet, F.M. (1967). Immunological aspects of malignant disease. Lancet I: 1171-1174

Burnet, F.M. (1969). The general character of immune phenomena (p 12) in "Cellular Immunology" Books one and two Melbourne University Press and Cambridge University Press. (ed. Burnet, F.M.)

Burnet, F.M. (1970). The Concept of Immunological Surveillance. Progr. Exp. Tumour Res. 13: 1-27

Byrt, P. and Ada, G.L. (1969). An in vitro reaction between labelled flagellin or hemocyanin and lymphocyte-like cells from normal animals. Immunology 17: 503-516

Cerrottini, J.C., Nordin, A.A, Brunner, K.T. (1970). Specific in vitro cytotoxicity of thymus-derived lymphocytes sensitized to alloantigens. Nature 228: 1308-1309

Chalmers, D.G., Coombs, R.R.A., Gurner, B.W. and Dausset, J. (1959). The mixed antiglobulin reaction in the detection of human iso-antibodies against leukocytes, platelets and Hela cells. Brit. J. Haemat. 5: 225-231

Chan, E.L., Mishell, R.I. and Mitchell, G.F. (1970). Cell interaction in immune response in vitro: requirement for theta-carrying cells. Science 170: 1215-1217

Cheers, C., Breitner, J.C.S., Little, M., Miller, J.F.A.P. (1971). Co-operation between carrier-reactive and hapten-sensitive cells in vitro. Nature New Biol. 232: 248-250

Chia, E., Festenstein, H. (1973). Specific cytostatic effect of lymph node cells from normal and T cell-deficient mice on syngeneic tumor target cells in vitro and its specific abrogation by body fluids from syngeneic tumor-bearing mice. Eur. J. Immunol. 3: 483-487

Chieco-Bianchi, L., Fiore-Donati, L., Tridente, G. and Pennelli, N. (1967). Graffi virus and immunological tolerance in leukaemogenesis. Nature 214: 1227-1228

Chieco-Bianchi, L., Fiore-Donati, L., Collavo, D., and Tridente, G. (1970). Immunological problems in virus-induced leukemia in the mouse in "Immunity and tolerance in oncogenesis": pp 599-612 (Division of Cancer Research, Perugia)

Chieco-Bianchi, L. (1972). The immune status of the host in experimental leukemia in "Immunopathologie und Therapie der Aputen Leukose im Kindesalter" pp. 28-39 (Friedrich Schiller-Universitat Jena)

Chiller, J.M., Habicht, G.S. and Weigle, W.O. (1970). Cellular sites of immunological unresponsiveness. Proc. Nat. Acad. Sci. 65: 551-556

Chiller, J.M. and Weigle, W.O. (1970). Cellular events during in-induction of immunologic unresponsiveness in adult mice. J. Immunol. 106: 1647-1653

Chiller, J.M., Habicht, G.S. and Weigle, W.O. (1971). Kinetic differences in unresponsiveness of thymus and bone marrow cells. Science 171: 813-815

Chiller, J.M., and Weigle, W.O. (1973). Restoration of immunocompetency in tolerant lymphoid cell populations by cellular supplementation. J. Immunol. 110: 1051-1057

Chou, B. (1975) Personal communication

Chuat, J.C., Berman, L., Gunven, P. and Klein, E. (1969). Studies on murine sarcoma virus; antigenic characterization of murine sarcoma virus induced tumour cells. Int. J. Cancer 4: 465-479

Churchill, W.H. Jr., Rapp, H.J., Kronman, B.S. and Borsos, T. (1968). Detection of antigens of a new diethylnitrosamine-induced transplantable hepatoma by delayed hypersensitivity. J. Nat. Cancer Inst. 41: 13-29

Claman, H.N., Chaperon, E.A. and Selner, J.C. (1968). Thymus-marrow immunocompetence. III The requirement for living thymus cells. Proc. Soc. Exp. Biol. Med. 127: 462-466

Claman, H.N. and Chaperon, E.A. (1969). Immunological complementation between thymus and marrow cells - a model for the two-cell theory of immunocompetence. Transplant Rev. 1: 92-113

Clark, S.L., Jr. (1963). The thymus in mice of strain 129/J studied with the electron microscope. Am. J. Anat. 112: 1-33

Cole, R.E. and Maki, S.E. (1971). Differential inactivation of lymphocytes and bone marrow stem cells by heterologous antimouse γ-globulin serum. Nature, New Biol. 230: 244-246

Collins, V.P., Loeffler, R.K. and Tivey, H. (1956). Observations of growth rates of human tumours. Amer. J. Roentgenol. 76: 988-1000

Coombs, R.R.A., Feinstein, A. and Wilson, A.B. (1969). Immunoglobulin determinants on the surface of human lymphocytes. Lancet II: 1157-1160

Cooper, M.D., Peterson, R.D.A., South, M.A. and Good, R.A. (1966). The functions of the thymus system and the bursa system in the chicken. J. Exp. Med. 123: 75-102d

Crone, M., Koch, C. and Simonsen, M. (1972). The elusive T cell receptor. Transplantation Rev. 10: 36-56

Currie, G.A. and Bagshawe, K.D. (1967). The masking of antigens on trophoblast and cancer cells. Lancet I: 708-710

Currie, G.A., and Basham, C. (1972). Serum mediated inhibition of the immunological reactions of the patient to his own tumour: A possible role for circulating antigen. Br. J. Cancer 26: 427-438

Currie, G.A., and Alexander, P. (1974). Spontaneous shedding of TSTA by viable sarcoma cells: Its possible role in facilitating metastatic spread. Br. J. Cancer 29: 72-75

Daguillard, F. and Richter, M. (1970). Cells involved in the immune response. XVI The response of immune rabbit cells to phytohemagglutinin, antigen and goat anti-rabbit immunoglobulin antiserum. J. Exp. Med. 131: 119-131

David, J.R. (1968). Macrophage migration. Fed. Proc. 27: 6-12

Davie, J.M., Paul, W.E., Mage, R.G. and Goldman, M.B. (1971). Membrane-associated immunoglobulin of rabbit peripheral blood lymphocytes: Allelic exclusion at the b locus. Proc. Nat. Acad. Sci. 68: 430-434

Davies A.J.S. (1969). The thymus and the cellular basis of immunity. Transplant Rev. 1: 43-91

De Noronha, F., Post, J.E., Norcross, N.L. and Rickard, C.G.(1972). Induction of group-specific interspecies antibody in a cat by immunization with disrupted feline leukaemia virus. Nature, New Biol. 235: 14-15

Dent, P.B. and Good, R.A. (1965). Absence of antibody production in the bursa of Fabricius. Nature 207: 491-493

Doria, G., Agarossi, G. and Di Pietro, S. (1972). Enhancing activity of thymocyte culture cell-free medium on the in vitro immune response of spleen cells from neonatally thymectomized mice to sheep RBC. J. Immunol. 108: 268-270

Dwyer, J.M., Warner, N.L. and Mackay, I.R. (1972). Specificity and nature of the antigen-combining sites on fetal and mature thymus lymphocytes. J. Immunol. 108: 1439-1446

Dutton, R.W., Falkoff, R., Hirst, J.A., Hoffman, M., Kappler, J.W. Kettman, J.R., Lesley, J.F. and Vann, D. (1971). Is there evidence for a non-antigen specific diffusible chemical mediator from the thymus-derived cell in the initiation of the immune response? in "Progress in Immunology", ed. Amos, B., Academic Press, p. 355-368

Elkins, W.L. (1973). Deficit of specific thymus-dependent lymphocyte in transplantation tolerance in the rat. J. Exp. Med. 137: 1097-1102

Essex, M., Klein, G., Snyder, S.P. and Harrold, J.B. (1971). Correlation between humoral antibody and regression of tumors induced by feline sarcoma virus. Nature 233: 195-196

Evans, R. and Alexander P. (1970). Cooperation of immune lymphoid cells with macrophages in tumour immunity. Nature 228: 620-622

Evans, R. and Alexander P. (1972a). Mechanism of immunologically specific killing of tumour cells by macrophages. Nature 236: 168-170

Evans, R. and Alexander, P. (1972b). Role of macrophages in tumour immunity I. Cooperation between macrophages and lymphoid cells in syngeneic tumour. Immunology 23: 615-626

Evans, R. and Alexander P. (1972c). Role of macrophages in tumour immunity. II Involvement of a macrophage cytophilic factor during syngeneic tumour growth inhibition. Immunology 23: 627-636

Evans, R., Grant, C.K., Cox, H., Steele, K., and Alexander, P. (1972). Thymus-derived lymphocytes produce an immunologically specific macrophage arming factor. J. Exp. Med. 136: 1318-1322

Everson, T.C. (1964). Spontaneous regression of cancer. Ann. N.Y. Acad. Sci. 114: 721-735

Fagraeus, A. (1948). Antibody production in relation to the development of plasma cells. Acta Med. Scand. 130: Suppl. 204, p. 1-122

Fahey, J.L., Barth, W.F. and Law, L.W. (1965). Normal immunoglobulins and antibody response in neonatally thymectomized mice. J. Nat. Cancer Inst. 35: 663-678

Feldman, M., and Palmer, J. (1971). The requirement of macrophages in the secondary immune response to antigens of small and large size in vitro. Immunology 21: 685-699

Feldman, M. and Nossal, G.J.V. (1972). Tolerance, enhancement and the regulation of interactions between T cells, B cells and macrophages. Transplantation Rev. 13: 3-34

Feldman, M. (1972). Cell interaction in the response in vitro. II The requirement for macrophages in lymphoid cell collaboration. J. Exp. Med. 135: 1049-1058

Fenyö, E.M., Klein, E., Klein, G., and Swiech, K. (1968). Selection of an immune-resistant Moloney lymphoma subline with decreased concentration of tumor-specific surface antigens. J. Nat. Cancer Inst. 40: 69-89

Finklestein, J.Z., Byfield, J., Tittle, KL, and Imagawa, DT. (1972). Studies of inhibition of tumor-cell DNA synthesis by immune cells in Gross virus-induced leukemia. Rev. Europ. D'Et. Clin. Biol. 17: 287-292

Fish, F., Yaakubovicz, M. and Witz, I.P. (1974). Incorporation of I^{125}-iododeoxyuridine into target cells as an assay for cell immunity. J. Nat. Cancer Inst. 53: 1743-1747

Fishman, M. (1961). Antibody formation in vitro. J. Exp. Med. 114: 837-856

Fishman, M. and Adler, F.L. (1963). Antibody formation initiated in vitro. II. Antibody synthesis in x-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. J. Exp. Med. 117: 595-602

Foley, E.J. (1953). Antigenic properties of methylcholanthrene-induced tumours in mice of the strain of origin. Cancer Res. 13: 835-837

Ford, C.E., Hamerton, J.L., Barnes, D.W.H. and Lowtit, J.F. (1956). Cytological identification of radiation-chimeras. Nature 177: 452-454

Ford, C.E. and Micklem, H.S. (1963). The thymus and lymph-nodes in radiation chimeras. Lancet I: 359-362

Ford, C.E. (1966). Traffic of lymphoid cells in the body, in "The Thymus, Experimental and Clinical Studies", eds. Wolstenholm, G.E.W. and Porter, R., Churchill, London 64: 131-152

Friberg, S. Jr. (1972). Comparison of an immunoresistant and an immunosusceptible ascites subline from the murine tumour TA3. II. Immunosensitivity and antibody-binding capacity in vitro and immunogenecity in allogeneic mice. J. Nat. Canc. Inst. 48: 1477-1489

Fröland, S.S. and Natvig, J.B. and Berdal, P. (1971). Surface-bound immunoglobulin as a marker of B lymphocytes in man. Nature, New Biol. 234: 251-252

Furth, J. (1953). Conditional and autonomous neoplasms: A review. Cancer Res. 13: 477-492

Gallily, R. and Feldman, M. (1966). The induction of antibody production in X-irradiated animals by macrophages that interacted with antigen. Israel J. Med. Sci. 2: 358-361

Gershon, R.K. and Kondo, K. (1971). Antigenic competition between heterologous erythrocytes. I. Thymic dependency. J. Immunol. 106: 1524-1531

Gilmour, D.G., Theis, G.A. and Thorbecke, G.J. (1970). Transfer of antibody production with cells from bursa of Fabricius. J. Exp. Med. 132: 134-147

Glick, B., Chang, T.S. and Jaap, R.G. (1956). The bursa of Fabricius and antibody production. Poult. Sci. 35: 224-225

Globerson, A., Auerbach, R. (1967). Reactivation in vitro of immunocompetence in irradiated mouse spleen. J. Exp. Med. 126: 223-234a

Golan, D.T. and Borel, Y. (1972). Deletion of hapten-binding cells by highly radioactive I^{125} conjugate. J. Exp. Med. 136: 305-317

Goldenberg, G.J. and Thomas, C.E. (1967). Virus-like particles in murine leukaemia L5178Y. Nature 214: 1339-1341

Goldenberg, G.J. and Wilt, F. (1969). Importance of regional lymph modes in transplantation type immunity to murine leukemia L5178Y. Fed. Proc. 28: 582

Goldstein, L.T. and Manson, L.A. (1973). A tumor specific transplantation antigen present in a murine lymphoma L5178Y. Fed. Proc. 32: 971

Goldstein, L.T., Klinman, N.R. and Manson, L.A. (1973). A microtest radioimmunoassay for noncytotoxic tumor-specific antibody to cell-surface antigens. J. Nat. Cancer Inst. 51: 1713-1715

Goldstein, L.T. and Manson, L.A. (1975). Specificity of the immune response in an L-5178Y-DBA/2 syngeneic mouse system. Transplant. Proc. 7: Supp. 1, 513-515

Golstein, P., Wigzell, H., Blomgren, H. and Svedmyr, E.A.J. (1972). Cells mediating specific in vitro cytotoxicity. II. Probable autonomy of thymus processed lymphocytes (T-cells) for the killing of allogeneic target cells. J. Exp. Med. 135: 890 -906

Good, R.A. and Finstad, J. (1971). Experimental and clinical models of immune deficiency and reconstitution of immunologic capacity in "Cell Interactions and Receptor Antibodies in Immune Responses", eds. Makela, O., Cross, A. and Kosunen, T.V. Acad. Press p 27-40

Good, R.A. (1973). Immunodeficiency in developmental perspective in "The Harvey Lectures 1971-1972". Series 67. Acad. Press New York and London

Grabar, P., and Burtin, P. (1964). Immuno-electrophoretic Analysis Elsevier Publishing Co., p. 4-29

Granger, G.A. and Weiser, R.S. (1964). Homograft target cells: specific destruction in vitro by contact interaction with immune macrophages. Science 145: 1427-1429

Granger, G.A. and Weiser, R.S. (1966). Homograft target cells: contact desctruction in vitro by immune macrophages. Science 151: 97-99

Greenberg, A.H. (1975). Personal communication

Habel, K. (1961). Resistance of polyoma virus immune animals to transplanted polyoma tumours. Proc. Soc. Exp. Biol. (N.Y.) 106: 722-725

Hammond, W.S. (1954). Origin of thymus in the chick embryo. J. Morphol. 95: 501-521

Harada, M., Pearson, G., Pettigrew, H., Redman, L., and Orr, T. (1973). Enhancement of normal lymphocyte cytotoxicity by sera with high antibody titers against H-2 or virus-associated antigens. Cancer Res. 33: 2886-2893

Haran-Ghera, N. (1970). The role of immune impairment in viral leukemogenesis in "Immunity and tolerance in oncogenesis", 4th Quadrennial Perugia Cancer Conf., L. Severi ed. 1969, p. 588

Harris, J.E. and Sinkovics, J.G. (1970). Immunology of Malignant Disease. St. Louis, Mosby.

Hellström, I. and Hellström, K.E. (1966). Recent studies on the mechanisms of the allogeneic inhibition phenomenon. Ann. N.Y. Acad. Sci. 129: 724-733

Hellström, I., Hellström, K.E., Evans, C.A., Heppner, G.H., Pierce, G.E., Yang, J.P.S. (1969). Serum-mediated protection of neoplastic cells from inhibition by lymphocytes immune to their tumor-specific antigens. Proc. Nat. Acad. Sci. U.S. 62: 362-368

Hellström, I., and Hellström, K.E. (1969a). Studies on cellular immunity and its serum-mediated inhibition in Moloney virus-induced mouse sarcomas. Int. J. Cancer 4: 587-600

Hellström, I., Hellström, K.E. and Sjögren, H.O. (1970). Serum-mediated inhibition of cellular immunity to methylcholanthrene-induced murine sarcomas. Cell. Immunol. 1: 18-30

Hellström, K.E. and Hellström, J. (1969b). Cellular immunity against tumour antigens. Advanc. Cancer Res. 12: 167-223

Hellström, K.E. and Hellström, I. (1970). Immunological enhangement as studied by cell culture techniques. Ann. Rev. Microbiol. 24: 373-398

Herberman, R.B., Nunn, M.E., Lavrin, D.H. and Asofsky, R. (1973). Effect of antibody to θ antigen on cell-mediated immunity induced in syngeneic mice by murine sarcoma virus. J. Nat. Cancer Inst. 51: 1509-1512

Herbert, W.J. (1967). Passive hemagglutination, in "Handbook of Experimental Immunology". ed. Weir, D.M., Blackwell Scientific Publications, p. 720-744

Hertz, R., Ross, G.T., and Lipsett, M.B. (1964). Chemotherapy in women with trophoblastic disease: Choriocarcinoma, chorioadenoma destruens and complicated hydatidiform mole. Ann. N.Y. Acad. Sci. 114: 881-885

Herzenberg, L.A., Herzenberg, L.A., Goodlin, R.C., Rivera, E.C. (1967). Immunoglobulin synthesis in mice: suppression by anti-allotype antibody. J. Exp. Med. 126: 701-713

Hibbs, J.B., Lambert, L.H., and Remington, J.S. (1971). Tumour resistance conferred by intracellular protozoa. J. Clin. Invest. 50: 45a

Hibbs, J.B., Lambert, L.H., and Remington, J.S. (1972). Possible role of macrophage mediated nonspecific cytotoxicity in tumour resistance. Nature New Biol. 235: 48-50

Hirsch, M.S., Allison, A.C. and Harvey, J.J. (1969). Immune complexes in mice infected neonatally with Moloney leukaemogenic and murine sarcoma viruses. Nature 223: 739-740

Hiu, I.J. (1972). Water-soluble and lipid-free fraction from BCG with adjuvant and antitumour activity. Nature New Biology 238: 241-242

Howard, J.G., Christie, G.H., Countenay, B.M., Leuchars, E.M. and Davies A.J.S. (1971). Studies on immunological paralysis. VI. Thymic-independence of tolerance and immunity to type III pneumococcal polysaccharide. Cell. Immunol. 2: 614-626

Howard, J.G. and Mitchison, N.A. (1975). Immunological tolerance. Progr. Allergy 18: 43-96

Hudson, L., Sprent, J., Miller, J.F.A.P. and Playfair, J.H.L. (1974) B-cell derived immunoglobulin on activated mouse T lymphocytes. Nature 251: 60-62

Humphrey, J.H., Parrot, D.M.V., and East, J. (1964). Studies on globulin and antibody production in mice thymectomized at birth. Immunology 7: 419-439

Humphrey, J.H. and Keller, H.V. (1970). Some evidence for specific interaction between immunologically competent cells and antigens in "Developmental Aspects of Antibody Formation and Structure", eds. Sterzl, J. and Riha, I. Academic Press. p. 485-516

Humpreys, S.R., Glynn, J.P., Chirigos, M.A., and Golden, A. (1962). Further studies on the homograft response in Balb/c with L1210 leukemia and a resistant subline. J. Nat. Cancer Inst. 28: 1053-1063

Hunter, P., and Kettman, J.R. (1973). Enhancement of the in vitro humoral response of thymus deprived spleen cells by supernatants from activated thymus derived cells. Fed. Proc. 32: 965

Ivanyi, J., Skamene, E. and Kurisu, A. (1970). Stimulation of chicken lymphocytes in vitro by anti-immunoglobulin serum. Folia Biol. (Praha) XVI: 34-46

Jacobson, E.B., L'Age-Stehr, J. and Herzenberg, L.A. (1970). Immunological memory in mice. II. Cell interactions in secondary immune response studied by means of immunoglobulin allotype markers. J. Exp. Med. 131: 1109-1120

Kaliss, N. and Molomut, N. (1952). The effect of prior injections of tissue antiserums on the survival of cancer homografts in mice. Cancer Res. 12: 110-112

Kaliss, N. (1958). Immunological enhancement of tumor homografts in mice. A review. Cancer Res. 18: 992-1003

Katz, D.H., Benacerraf, B. (1972). The regulatory influence of activated T cells on the B cell responses to antigen. Advanc. Immunol. 15: 1-94

Kearney, R., Basten, A. and Nelson, D.S. (1974) in Nelson D.S. Infection, Allograft and Tumour Immunities. Transplantation Reviews 19: 266-254; p. 231

Keller, R. and Jones, V.E. (1971). Role of activated macrophages and antibody in inhibition and enhancement of tumour growth in rats. Lancet II: 847-849

Keller, R., Ogilvie, B.M. and Simpson, E. (1971). Tumour growth in nematode infected animals. Lancet I: 678-680

King, T.P. (1968). Cellulose ion exchange chromatography in "Methods in Immunology and Immunochemistry" eds. Williams, C.A. and Chase, M.W. Academic Press, p. 154-160

Kirby, D.R.S. (1970). The egg and immunology. Proc. Roy. Soc. Med. 63: 59-61

Klein, E., Klein, G. (1964). Antigenic properties of lymphomas induced by the Moloney agent. J. Nat. Cancer Inst. 32: 547-568

Klein, E. and Klein, G. (1965). Antibody response and leukemia development in mice inoculated neonatally with Moloney virus. Cancer Res. 25: 851-854

Klein, E. and Klein G. (1966). Immunological tolerance of neonatally infected mice to Moloney leukaemia virus. Nature 209: 163-165

Klein, E. (1972). Tumour immunology; Escape mechanisms. Ann. Inst. Pasteur 122: 593-602

Klein, G., Sjögren, H.O., Klein, E. and Hellström, K.E. (1960). Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res. 20: 1561-1572

Klein, G. (1966a). in Host defences in Recent Research on Burkitt's tumor: Conference at Kampala, Uganda. Brit. Med. J. 1: 1044

Klein, G. (1966b). Tumor Antigens. Ann. Rev. Microbiol. 20: 223-252

Klein, G., Clifford, P., Klein, E. and Stjernswärd, J. (1966). Search for tumour-specific immune reactions in Burkitt's lymphoma patients by the membrane immunofluorescence reaction. Proc. Nat. Acad. Sci., Wash. 55: 1628-1635

Klein, G., Gars, U., and Harris, H. (1970). Isoantigen expression in hybrid mouse cells. Exp. Cell. Res. 62: 149-160

Knox, W.E. and Pillers, E.M.K. (1958). Time of recurrence or cure of tumours in childhood. Lancet I: 188-191

Kölsch, E., Mengersen, R., Diller, E. (1973). Letter to the editor. Low dose tolerance preventing tumor immunity. Europ. J. Cancer 9: 879-882

Kruger, J. and Gershon, R.K. (1971). Immune response to hemocyanin in normal, thymus-deprived and reconstituted mice. J. Immunol. 106: 1065-1073

Lamon, E.W., Skurzak, H.M., Klein, E. and Wigzell, H. (1972a). In vitro cytotoxicity by a nonthymus-processed lymphocyte population with specificity for a virally determined tumor cell surface antigen. J. Exp. Med. 136: 1072-1079

Lamon, E.W., Skurzak, H.M. and Klein, E. (1972b). The lymphocyte response to a primary viral neoplasm (MSV) through its entire course in Balb/c mice. Int. J. Cancer 10: 581-588

Lamon, E.W., Wigzel, H., Klein, E., Andersson, B. and Skurzak, H.M. (1973a). The lymphocyte response to a primary Moloney sarcoma virus tumour in Balb/c mice: definition of the active subpopulations at different times after infection. J. Exp. Med. 137: 1472-1493

Lamon, E.W., Wigzell, H., Andersson, B. and Klein, E. (1973b). B lymphocytes from animals in which primary MSV tumours have regressed are active in vitro against target cells bearing MLV determined antigens. Nature New Biol. 244: 209-211

Landsteiner, C. (1901). The agglutinating properties of normal human blood. Wien klin. Wochnschr. 14: 1132-1134 in"Three Centuries of Microbiology"(1965). Lechevalier, H.A. and Solotorovsky, McGraw-Hill, New York, p. 247

Lappe, M. and Schalk, J. (1971). Necessity of the spleen for balanced secondary sex ratios following maternal immunization with male antigen. Transplantation 11: 491-495

Law, L.W. and Dawe, C.J. (1960). Influence of total body X-irradiation on tumour induction by parotid tumor agent in adult mice. Proc. Soc. Exp. Biol. (N.Y.) 105: 414-419

Law, L.W., Ting, R.C. and Leckband, E. (1966). The function of the thymus in tumour production by polyoma virus. In "The Thymus", Ciba Foundation Symposium p.214-241 eds.Wolstenholme, G.E. and Porter, R. J. and A. Churchill Ltd., London 1966

Law, L.W. (1968). Immunologic factors in susceptibility and resistance to tumor induction by viruses. In "Thule International Symposia on Cancer and Aging" pp. 111-130, Stockholm.

Lay, W.H. and Nussenzweig, V. (1968). Receptors for complement on leukocytes. J. Exp. Med. 128: 991-1009

Lechevalier, H.A. and Solotorovsky, M. (1965). From Francisco to Pasteur in "Three Centuries of Microbiology", McGraw-Hill, N.Y. p. 9

Leclerc, J.C., Gomard, E., and Levy, J.P. (1972). Cell-mediated reaction against tumours induced by oncornaviruses. I. Kinetics and specificity of the immune response in murine sarcoma virus (MSV)-induced tumors and transplanted lymphomas. Int. J. Cancer 10: 589-601

Leclerc, J.C., Gomard, E., Plata, F., and Levy, J.P. (1973). Cell-mediated immune reaction against tumors induced by oncornoviruses. II. Nature of the effector cells in tumor-cell cytolysis. Int. J. Cancer 11: 426-432

Lee, S-T, Paraskevas, F. and Israels, L.G. (1971). Cell surface associated gamma globulins in lymphocytes. II The pleuripotentiality of mouse spleen lymphocytes. J. Immunol. 107: 1583-1592

Lee, S-T, and Paraskevas, F. (1972). Cell surface-associated gamma-globulins in lymphocytes. IV. Lack of detection of surface γ-globulin on B-cells and acquisition of surface γG globulin by T cells during primary response. J. Immunol. 109:1262-1271

Lee S-T.(1975). Personal communication

Le Mevel, B.P., Oldham, R.K., Wells, S.A., and Herberman, R.B. (1973). An evaluation of I^{125}- iododeoxyuridine as a cellular label for in vitro assays: Kinetics of incorporation and toxicity. J. Nat. Cancer Inst. 51: 1551-1558

Lerner, R.A., Oldstone, M.B.A. and Cooper, N.R. (1971). Cell cycle-dependent immune lysis of Moloney virus-transformed lymphocytes. Presence of viral antigen, accessibility to antibody and complement activation. Proc. Nat. Acad. Sci. (Wash.) 68: 2584-2588

Liacopoulos, P. and Neveu, T. (1964). Non-specific inhibition of the immediate and delayed types of hypersensitivity during immune paralysis of adult guinea pigs. Immunology 7: 26-39

Liacopoulos, P. (1965). Suppression of immunological responses during the induction of immune paralysis with unrelated antigens. Texas Rep. Biol. Med. 23: 63-80

Lindner, O.E.A. (1962). Survival of skin homografts in methylcholanthrene-treated mice and in mice with spontaneous mammary cancers. Cancer Res. 22: 380-383

Malmgren, R.A., Bennison, B.E., and McKinley, T.W. Jr. (1952). Reduced antibody titres in mice treated with carcinogenic and cancer chemotherapeutic agents. Proc. Soc. Exp. Biol. N.Y. 79: 484-488

Malmgren, R.A., Rabson, A.S. and Carney, P.G. (1964). Immunity and viral carcinogenesis. Effect of thymectomy on polyoma virus carcinogenesis in mice. J. Nat. Cancer Inst. 33: 101-104

Manning, D.D. and Jutila, J.W. (1972a). Immunosuppression of mice injected with heterologous anti-immunoglobulin heavy chain antisera. J. Exp. Med. 135: 1316-1333

Manning, D.D. and Jutila, J.W. (1972b). Immunosuppression in mice injected with heterologous anti-immunoglobulin antisera. J. Immunol. 108: 282-285

Marbrook, J. (1967). Primary immune response in cultures of spleen cells. Lancet II: 1279-1281

Marchalonis, J.J., Crone, R.E., and Atwell, J.L. (1972). Isolation and partial characterization of lymphocyte surface immunoglobulins. J. Exp. Med. 135: 956-971

Mason, S. and Warner, N.L. (1970). The immunoglobulin nature of the antigen recognition site on cells mediating transplantation immunity and delayed hypersensitivity. J. Immunol. 104: 762-765

Metchnikoff, E. (1892). Leçons sur la pathologie comparée de l'inflammation. Paris: Masson in "Cellular Immunology" Book 1 and 2 1969. Burnet,FM. Melbourne Univ. Cambridge Univ. Press, p. 15

Micklem, H.S., Ford, C.E., Evans, E.P., and Gray, J. (1966). Interrelationships of myeloid and lymphoid cells: Studies of chromozome-marked cells transfused into lethally irradiated mice. Proc. Roy. Soc. B 165: 78-102

Miller, J.F.A.P., Marshall, A.H.E., and White, R.G. (1962). The immunological significance of the thymus. Adv. Immunol. 2: 111-163

Miller, J.F.A.P. (1963). Immunity and the thymus. Lancet I: 43-45

Miller, J.F.A.P., Grant, G.A. and Roe, F.J.C. (1963). Effect on thymectomy on the induction of skin tumours by 3,4-benzopyrene. Nature 199: 920-922

Miller, J.F.A.P. (1964). The thymus and the development of immunologic responsiveness. Science, N.Y. 144: 1544-1551

Miller, J.F.A.P. and Mitchell, G.F. (1969). Thymus and antigen reactive cells. Transplant. Rev. 1: 3-42

Miller, J.F.A.P. and Sprent, J. (1971a). Cell-to-cell interaction in the immune response VI. Contribution of thymus-derived cells and antibody-forming cell precursors to immunological memory. J. Exp. Med. 134: 66-82

Miller, J.F.A.P. and Sprent, J. (1971b). Thymus-derived cells in mouse thoracic duct lymph. Nature New Biol. 230: 267-270

Mintz, B. and Silvers, W.K. (1967). "Intrinsic" immunological tolerance in allophonic mice. Science 158: 1484-1487

Mishell, R.I. and Dutton, R.W. (1967). Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126: 423-442

Mitchel , G.F. and Miller, J.F.A.P. (1968). Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. J. Exp. Med. 128: 821-837

Mitchel, G.F., Chan, E.L., Nobb, M.S., Weissman, I.L., Mishell, R.I. and Herzenberg, L.A. (1972). Immunological memory in mice. III. Memory to heterologous erythrocytes in both T cell and B cell populations and requirement for T cells in expression of B cell memory. Evidence using immunoglobulin allotype and mouse alloantigen theta markers with congenic mice. J. Exp. Med. 135: 165-184

Mitchison, N.A. (1967). Antigen recognition responsible for the induction in vitro of the secondary response. Cold. Spr. Harb. Symp. Quant. Biol. 32: 431-439

Mitchison, N.A. (1969). Cell populations involved in immune responses, in "Immunological Tolerance"eds. Landy, M. and Braun, W., Acad. Press. p. 113-169

Mitchison, N.A., Rajewsky, K., and Taylor, R.B. (1971). Cooperation of antigenic determinants and of cells in the induction of antibodies in "Developmental Aspects of Antibody Formation and Structure", eds. Sterzl J. and Riha, I. Academic Press p. 547

Möller, E., and Möller, G. (1962). Quantitative studies of the sensitivity of normal and neoplastic mouse cells to the cytotoxic action of isoantibodies. J. Exp. Med. 115: 527-553

Möller, G. and Möller E. (1967). Immune cytotoxicity and immunological enhancement in tissue transplantation (p. 349) in "Antibodies to Biologically Active Molecules". ed. Cinader, B. Pergamon Press

Morrow, R.H., Pike, M.C., and Kisuule, A. (1967). Survival of Burkitt's lymphoma patients in Mulago hospital, Uganda, Brit. Med. J. 4: 323-327

Muramatsu, T., Nathenson, S.G., Boyse, E.A. and Old, L.J. (1973). Some biochemical properties of thymus leukemia antigens solubilized from cell membranes by papain digestion. J. Exp. Med. 137: 1256-1262

Naor, D. and Sulitzeanu, D. (1967). Binding of radioiodinated bovine serum and albumin to mouse spleen cells. Nature 214: 687-688

Nelson, D.S. (1969). Macrophages and Immunity. North-Holland Press, Amsterdam

Neveu, T. (1964). Étude de la compétition des antigens chez le cobaye adult. Ann. Inst. Pasteur 107: 320-339

Ngu, V.A. (1965). The African lymphoma (Burkitt tumour): survivals exceeding two years. Brit. J. Cancer 18: 101-107

Nisonoff, A., Wissler, F.C., Lipman, L.N., and Woernley, D.L. (1960). Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulphide bonds. Arch. Biochem. Biophys. 89: 230-244

Nisonoff, A. and Rivers, M.M. (1961). Recombination of a mixture of univalent antibody fragments of different specificity. Arch. Biochem. Biophys. 93: 460-462

Nossal, G.V.H., and Abbot, A. (1968). Lymphoid cells and antibody formation in "XIth Congr. Internat. Soc. Blood Transf., Sydney, Plenary sessions", 1966, ed. Hollander, L., Basel, New York, Karger, p. 5

Nossal, G.J.V., Cunningham, A., Mitchell, G.F. and Miller, J.F.A.P. (1968). Cell to cell interaction in the immune response. III. Chromozomal marker analysis of single antibody-forming cells in reconstituted irradiated or thymectomized mice. J. Exp. Med. 128: 839-853

Nossal, G.J.V., Warner, N.L., Lewis, H. and Sprent, J. (1972). Quantitative features of a sandwich radioimmunolabeling technique for lymphocyte surface receptors. J. Exp. Med. 135: 405-428

Nowotny, A., Grohsman, J., Abdelnoor, A., Rote, N., Yang, C., and Waltersdorff, R. (1974). Escape of TA3 tumors from allogeneic immune rejection: theory and experiments. Eur. J. Immunol. 4: 73-78

Okumura, K., and Tada, T. (1971). Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. J. Immunol. 106: 1019-1025

Old, L.J., Boyse, E.A., Clark, D.A. and Carswell, E.A. (1962). Antigenic properties of chemically induced tumours. Ann. N.Y. Acad. Sci. 101: 80-106

Old, L.J. and Boyse, E.A. (1965). Antigens of tumors and leukemias induced by viruses. Fed. Proc. 24: 1009-1017

Oldham, R.K., and Herberman, R.B. (1973). Evaluation of cell-mediated cytotoxic reactivity against tumor associated antigens with I^{125}-iododeoxyuridine labeled target cells. J. Immunol. 111 1862-1871

Oldstone, M.B.A. and Dixon, F.J. (1969). Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. J. Exp. Med. 129: 483-505

Oldstone, M.B.A., Aoki, T., and Dixon, F.J. (1972). The antibody response of mice to murine leukemia virus in spontaneous infection: Absence of classical immunological tolerance. Proc. Nat. Acad. Sci., Wash. 69: 134-138

Oppenheim, J.J., Zbar, B. and Rapp, H. (1970). Specific inhibition of tumour cell DNA synthesis in vitro by lymphocytes from peritoneal exudate of immunized syngeneic guinea pigs. Proc. Nat. Acad. Sci. Wash. 66: 1119-1126

Orr, K.B., and Paraskevas, F. (1973). Cell surface associated gamma globulin in lymphocytes. V. Detection of early cytophilic complexes reacting with T-and B-lymphocytes. J. Immunol. 110: 456-464

Orr, K.B. (1974). Induction of soluble factor(s) by Freund's complete adjuvant. A doctoral thesis. University of Manitoba

Orr, K.B. (1975). Personal communication

Ortiz de Landazuri, M., Kedar, E., and Fahey, J.L. (1974). Antibody dependent cellular cytotoxicity to a syngeneic Gross virus induced lymphoma. J. Nat. Cancer Inst. 52: 147-152

Osoba, D. and Miller, J.F.A.P. (1964). The lymphoid tissues and immune responses of neonatally thymectomized mice bearing thymus tissue in millipore diffusion chambers. J. Exp. Med. 119: 177-194h

Owen, J.J.T. and Seeger, R.C. (1973). Immunity to tumours of the murine leukemia-sarcoma virus complex. Brit. J. Cancer 28: Suppl. I: 26-34

Paraskevas, F., Lee, S-T., and Israels, L.G. (1970). Absence of γ--globulin receptors on mouse plasmacytoma cells. Nature 227: 395-396

Paraskevas, F., Lee, S-T., and Israels, L.G. (1971a). Cell surface associated gammaglobulin in lymphocytes. I. Reverse immune cytoadherence: A technique for their detection in mouse and human lymphocytes. J. Immuno. 106: 160-170

Paraskevas, F., Lee, S-T., Orr, K.B. and Israels, L.G. (1971b). Reverse immune cytoadherence. A technique for the detection of surface associated γ-globulins on lymphoid cells. J. Immunol. Methods 1: 1-17

Paraskevas, F., Lee, S-T., Orr, K.B. and Israels, L.G. (1972a). A receptor for Fc on mouse B-lymphocytes. J. Immunol. 108: 1319-1327

Paraskevas, F., Orr, K.B. and Lee, S-T. (1972b). Cell surface-associated gamma globulins in lymphocytes. III. Changes of globulin-carrying lymphocytes during primary response. J. Immunology 109: 1254-1261

Parish, C.R. (1972). The relationship between humoral and cell-mediated immunity. Transplant. Rev. 13: 35-66

Parrott, D.M.V. and deSousa, M.A.B. (1966a). Changes in the thymus-dependent areas of lymph nodes after immunological stimulation. Nature 212: 1316-1317

Parrott, D.M.V., deSousa, M.A.B. and East, J. (1966b). Thymus dependent areas in the lymphoid organs of neonatally thymectomized mice. J. Exp. Med. 123: 191-204g

Pasteur, L. (1880). Compt. Rend. Acad. Sci. 91: 86-94 in "Three Centuries of Microbiology" (1965). Lechevalier, H. and Solotorovsky, M. McGraw-Hill, New York, p. 50

Paul, J. (1965). Preservation, storage and transportation of living tissues and cells. in Cell and Tissue Culture, 3rd ed. E. and S. Livingstone Ltd., Edinburgh and London, p. 289-292

Perkins, W.D., Karnovsky, M.J. and Unanue E.R. (1972). An ultra structural study of lymphocytes with surface-bound immunoglobulin. J. Exp. Med. 135: 267-276

Perlmann, P., O'Toole, C., and Unsgaard, B. (1972a). Lymphocyte-mediated tumor cell destruction in vitro. Some general principles and reactivities in human urinary bladder carcinoma. Nat. Cancer Inst. Monograph 35: 223-229.

Perlmann, P., Perlmann, H., Wigzell, H. (1972b). Lymphocyte mediated cytotoxicity in vitro. Induction and inhibition by humoural antibody and nature of effector cells. Transplant Rev. 13: 19-114

Pernis, B., Forni, L. and Amante, L. (1970). Immunoglobulin spots on the surface of rabbit lymphocytes. J. Exp. Med. 132: 1001-1018

Pernis, B., Miller, J.F.A.P., Forni, L. and Sprent, J. (1974). Immunoglobulin on activated T cells detected by indirect immunofluorescence. Cell. Immunol. 10: 476-482

Peterson, R.D.A., Kelly, W.D. and Good, R.A. (1964). Ataxia-telangiectasia its association with a defective thymus, immunological-deficiency disease and malignancy. Lancet I, 1189-1193

Plata, F., Gomard, E., Leclerc, J.C. and Levy, J.P. (1973). Further evidence for the involvement of thymus-processed lymphocytes in syngeneic tumor cell lysis. J. Immunol. 11: 667-671

Plata, F., Gomard, E., Leclerc, J.C., Levy, J.P. (1974). Comparative in vitro studies on effector cell diversity in the cellular immune response to murine sarcoma virus (MSV)-induced tumours in mice. J. Immunol. 112: 1477-1487

Plata, F. and Levy, J.P. (1974). Blocking of syngeneic effector T cells by soluble tumor antigens. Nature 249: 271-274

Pollack, S., Heppner, G., Brawn, R.J. and Nelson, K. (1972). Specific killing of tumor cells in vitro in the presence of normal lymphoid cells and sera from hosts immune to tumor antigens. Int. J. Cancer 9: 316-323

Pollock, W.F., Hastings, N. and Snyder, W.H. jr. (1960). The Collins "period risk" formula for malignant tumours in children with particular reference to Wilms' tumour and neuroblastoma. Surgery 48: 606-609

Potter, C.W., Hoskins, J.M. and Oxford, J.S. (1969). Immunological relationships of some oncogenic DNA viruses. Arch. Gesamte Virusforsch 27: 73-86

Potter, M., Appella, E. and Geisser, S. (1965). Variations in the heavy polypeptide chain structure of gamma myeloma immunoglobulins from an inbred strain of mice and a hypothesis as to their origin. J. Molec. Biol. 14: 361-372

Prager, M.D., Hollinshead, A.C., Ribble, R.J. and Derr, I. (1973). Induction of immunity to a mouse lymphoma by multiple methods, including vaccination with soluble membrane fractions. J. Nat. Cancer Inst. 51: 1603-1607

Prehn, R.T. and Main, J.M. (1957). Immunity to methylcholanthrene-induced sarcomas. J. Nat. Cancer Inst. 18: 769-778

Prehn, R.T. (1963). Function of depressed immunologic reactivity during carcinogenesis. J. Nat. Cancer Inst. 31: 791-805

Prehn, R.T. (1964). A clonal selection theory of chemical carcinogenesis. J. Nat. Cancer Inst. 32: 1-17

Prehn, R.T.and Slemmer,G.L. (1967). The role of immunity as a homeostatic mechanism during oncogenesis. in "Endogenous Factors Influencing Host-tumor Balance" eds. Wissler, R.W., Day,T.L. and Wood,S.,Jr. University of Chicago Press, Chicago. p. 185-190

Prehn, R.T. (1970). in "Immune Surveillance" Proc. of an Int. Conf. held at Brook Lodge, Augusta, Michigan. eds. Smith, R.T. and Landy, M. Academic Press New York p. 451-462

Prehn, R.T. (1971). Perspectives in oncogenesis: Does immunity stimulate or inhibit neoplasia? J. Retic. Soc. 10: 1-16

Prehn, R.T. and Lappé, M.A. (1971). An immunostimulation theory of tumor development. Transplant. Rev. 7: 26-54

Rabellino. E., Colon.S., Grey, H.M. and Unanue, E.R. (1972). Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. J. Exp. Med. 133: 156-167

Radovich,J. and Talmage,D.W. (1967). Antigenic competition: Cellular or humoral. Science N.Y. 158: 512-514

Raff, M.C. (1969). Theta iso-antigen as a marker of thymus-derived lymphocytes in mice. Nature 224: 378-379

Raff, M.C. (1970a). Two distinct populations of perpheral lymphocytes in mice distinguishable by immunofluorescence. Immunology 19: 637-650

Raff, M.C. (1970b). Role of thymus-derived lymphocytes in the secondary humoural immune response in mice. Nature 226: 1257-1258

Raff, M.C. and Wortis, H.H. (1970). Thymus dependence of Θ-bearing cells in the peripheral lymphoid tissues of mice. Immunology 18: 931-942

Raff, M.C., Sternberg, M. and Taylor, R.B. (1970). Immunoglobulin determinants on the surface of mouse lymphoid cells. Nature 225: 553-554

Raff, M.C. (1971). Surface antigenic markers for distinguishing T and B lymphocytes in mice. Transplant. Rev. 6: 52-80

Reif, A.E. and Allen, J.M.V. (1963). Specificity of isoantisera against leukaemic and thymic lymphocytes. Nature 200: 1332-1333

Reif, A.E. and Allen, J.M.V. (1964). The AKR thymic antigen and its distribution in leukemias and nervous tissue. J. Exp. Med. 120: 413-433

Reif, A.E. and Allen,J.M.V. (1966). Mouse thymic iso-antigens. Nature 209: 521-523

Reithmüller, G.,Rieber, E. and Seeger,I. (1971). Suppression of graft-versus-host reaction by univalent anti-immunoglobulin antibody. Nature New Biol. 230: 248-250

Roseman, J. (1969). X-ray resistant cell required for the induction of in vitro antibody formation. Science 165: 1125-1127

Rouse, B.T., Röllinghoff, M. and Warner,N.L. (1972). Anti-Θ serum suppression of the cellular transfer of tumour-specific immunity to a syngeneic plasma cell tumour. Nature New Biol. 238: 116-117

Sabine, A.B.,Ginder, D.R.,Matumoto, M. and Schlesinger, R.W. (1974). Serological response of Japanese children and old people to Japanese B encephalitis mouse brain vaccine. Proc. Soc. Exp. Biol. N.Y. 65: 135-140

Schlesinger, M. and Yron, I. (1969). Antigenic changes in lymph-node cells after administration of antiserum to thymus cells. Science 164: 1412 1413

Schlesinger, M. and Yron, I. (1970). Serologic determination of a thymus-dependent population of lymph node cells. J. Immunol. 104: 798-804

Sedgwick, R.P. and Boder, E. (1972). Ataxia-telangiectasia in "Handbook of Clinical Neurology" North-Holland Publ., Amsterdam. 14:267-339

Seeger, R.C. and Owen, J.J. (1973). Measurment of tumour immunity in vitro with I^{125}-iododeoxyuridine-labeled target cells. Transplantation 15: 404-408

Segal, S, Cohen, I.R.and Feldman, M. (1972). Thymus-derived lymphocytes: Humoral and cellular reactions distinguished by hydro-cortisone. Science 175: 1126-1128

Sell, S., Rowe, D.S. and Gell, P.G.H. (1965). Studies on rabbit lymphocytes in vitro III. Protein, RNA and DNA synthesis by lymphocyte cultures after stimulation with phytohaemagglutinin, with staphylococcal filtrate, with antiallotype serum and with heterologous antiserum to rabbit whole serum. J. Exp. Med. 122: 823-839

Sendo, F., Aoki, T. and Buafo, C.K. (1974). Inhibition of lymphocyte-mediated cytotoxicity (^{51}Cr.-release) by antisera preabsorbed with various cells. J. Nat. Cancer Inst. 52: 769-775

Senik, A., De Giorgi, L., Gomard, E. and Levy, J.P. (1974a). Cytostasis of lymphoma cells in suspension: Probable non-thymic origin of the cytostatic lymphoid cells in mice bearing MSV-induced tumors. Int. J. Cancer 14: 396-400

Senik, A., De Giorgi, L. and Levy, J.P. (1974b). Cell-mediated anti-tumour immunity in oncornavirus-induced tumours: Specific cytostasis by spleen and lymph-node cells. Int. J. Cancer 14: 386-395

Shearer, G.M. and Cudkowicz, G. (1969). Distinct events in the immune response elicited by transferred marrow and thymus cells. I. Antigen requirements and proliferation of thymic antigen-reactive cells. J. Exp. Med. 130: 1243-1261

Shimpl, A. and Wecker, E. (1972). Replacement of T-cell function by a T-cell product. Nature New Biol. 237: 15-17

Shipley, W.U. (1971). Immune cytolysis in relation to the growth cycle of Chinese hamster cells. Cancer Res. 31: 925-929

Shortman, K., Diener, E., Russel, P.J. and Armstrong, W.D. (1970). The role of nonlymphoid accessory cells in the immune response to different antigens. J. Exp. Med. 131: 461-482

Shortman, K. and Palmer, J. (1971). The requirement for macrophages in the in vitro immune response. Cell. Immunol. 2: 399-410

Simmons, R.L., Rios, A., Ray, P.K. and Lundgren, G. (1971). Effect of neuraminadase on growth of a 3- methylcholanthrene-induced fibrosarcoma in normal and immunosuppressed syngeneic mice. J. Nat. Cancer Inst. 47: 1087-1094

Singhal, S. K. and Wigzell, H. (1969). Antigen specific receptors on the outer surface of 'virgin' immunocytes of normal bone marrow. Fed. Proc. 28: 309

Sjögren, H.O., Hellström,I.and Klein, G. (1961). Transplantation of polyoma virus-induced tumors in mice. Cancer Res. 21: 329-337

Sjögren, H. O.,Hellström,I., Bansal, S.C. and Hellström, K.E. (1971). Suggestive evidence that "blocking antibodies" of tumour-bearing individuals may be antigen-antibody complexes. Proc. Nat. Acad. Sci. 68: 1372-1375

Sjögren, H.O. and Bansal, S.C. (1972). Relationship between blocking and unblocking activity and tumor growth in vivo. Nat. Cancer Inst. Monogr. 35: 231-235

Skurzak, H.M., Klein, E., Takato, O., Yoshida, T.O. and Lamon, E.W. (1972). Synergistic or antagonistic effect of different antibody concentrations on in vitro lymphocyte cytotoxicity in the Molonev sarcoma virus system J. Exp. Med. 135: 997-1002

Slettenmark, B and Klein, E. (1962). Cytotoxic and neutralizing tests with serum and lymph node cells of isologous mice with induced resistance against Gross lymphomas. Cancer Res. 22: 947-954

Sophocles, A.M. and Nadler, S.G. (1971). Immunological aspects of cancer. Surgery, Gynecology and Obstetrics with International Abstracts of Surgery 133: 321-331

Southam, C.M. (1961). Applications of immunology to clinical cancer;past attempts and future possibilities. Cancer Res. 21: 1302-1316

Sprent, J. and Miller, J.F.A.P. (1973). Effect of recent antigen priming and adoptive immune responses. I. Specific unresponsiveness of cells from lymphoid organs of mice primed with heterologous erythrocytes. J. Exp. Med. 138: 143-162

Sternberg, M. (1970). Lack of specific inhibition of graft-versus-host reaction by anti-Fab serum. Experientia 26: 190-191

Stjernswärd, J. (1966a). Effect of noncarcinogenic and carcinogenic hydrocarbons on antibody-forming cells measured at the cellular level in vitro. J. Nat. Cancer Inst. 36: 1189-1195

Stjernswärd, J. (1966b). Age-dependent tumor-host barrier and effect of carcinogen-induced immunodepression on rejection of isografted methylcholanthrene-induced sarcoma cells. J. Nat. Cancer Inst. 37: 505-512

Stjernswärd, J. (1967). Further immunological studies on chemical carcinogenesis. J. Nat. Cancer Inst. 38: 515-526

Stobo, J. D., Paul, W.E. and Henney, C.S. (1973). Functional heterogeneity of murine lymphoid cells. IV. Allogeneic mixed lymphocyte reactivity and cytolytic activity as functions of distinct T cell subsets. J. Immunol. 110: 652-660

Stocker, M. (1964). Regulation of growth and orientation in hamster cells transformed by polyoma virus. Virology 24: 165-174

Sulitzeanu, D. and Naor, D. (1969). The affinity of radioiodinated BSA for lymphoid cells. II. Binding of I^{125}-BSA to lymphoid cells of normal mice. Int. Arch. Allergy 35: 564-578

Takahashi, T., Carswell, E.A. and Thorbecke, G.J. (1970). Surface antigens of immunocompetent cells. I. Effect of Θ and Pc.I allo-antiserum on the ability of spleen cells to transfer immune responses. J. Exp. Med. 132: 1181-1190

Takeda, K., Kikuchi, Y., Yamawaki, S., Ueda, T. and Yoshiki, T.(1968). Treatment of artificial metastases of methylcholanthrene-induced sarcomas by auto-immunization of autochtonous hosts. Cancer Res. 28: 2149-2154

Taylor, R.B. and Iverson, G.M. (1971). Hapten competition and the nature of cell-cooperation in the antibody response. Proc. Roy. Soc. B 176: 393-418

Terino, E.O., Miller, J. and Glenn, W.W.L. (1964). Tolerance induction and skin graft prolongation by competing antigens. Surgery 56: 256-265

Thieffry,St., Arthuis, M., Aicardi, J. and Lyon, G. (1961). L'ataxie-télangiectasie. Rev. Neurol. 105: 390-405

Thomas, L. (1959). Discussion. In "Cellular and Humoural Aspects of the Hypersensitive States". ed. Lawrence, H.S., Hoeber, New York p. 529

Thompson, D.M.P., Eccles, S., and Alexander, P. (1973). Antibodies and soluble tumour-specific antigens in blood and lymph of rats with chemically induced sarocomata. Br. J. of Canc. 28: 6-15

Thorbecke, G.J. and Cohen, M.W. (1964). Immunological competence and responsiveness of the thymus. in'The Thymus" Wistar Inst. Symp. Monograph no. 2, p33. ed. Defendi, V. and Metcalf. Philadelphia

Unanue, E.R. (1970). Thymus dependency of the immune response to hemocyanin: an evaluation of the role of macrophages in thymectomized mice. J. Immunol. 105: 1339-1343

Unanue, E.R., Grey, H.M., Rabellino, E. Campbell, R. and Schmidtke, J. (1971). Immunoglobulins on the surface of lymphocytes. II The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulin. J. Exp. Med. 134: 1188 -1198

Utsumi, S. and Karush, F. (1965). Peptic fragmentation of rabbit γ-G-immunoglobulin. Biochemistry 4: 1766-1779

Vandeputte, M., Denys, P., Leyten, R. and de Somer, P. (1963). The oncogenic activity of the polyoma virus in thymectomized rats. Life Sciences 2: 475-478

van Furth, R., Schuit, H.R.E. and Hijmans, W. (1966). The formation of immunoglobulins by human tissues in vitro III. Spleen, lymph nodes, bone marrow and thymus. Immunology 11: 19-27

Venzke, W.G. (1952). Morphogenesis of the thymus of chicken embryos. Am. J. Vet. Res. 13: 395-406

Vitetta, E.S., Bianco, C., Nussenzweig, V. and Uhr, J.W. (1972). Cell surface immunoglobulin IV. Distribution among thymocytes, bone marrow and their derived populations. J. Exp. Med. 136: 81-93

Wagner, H., Harris, A.W. and Feldman, M (1972). Cell-mediated immune response in vitro: II The role of thymus and thymus-derived lymphocytes. Cell. Immunol. 4: 39-50

Wagner, H. and Rollinghöff, M. (1973). Cell-mediated immunity in vitro against syngeneic mouse plasma tumour cells. Nature New Biol. 241:53-54

Wagner, H., Rollinghöff, M. and Nossal, G.J.V. (1973). T-cell-mediated immune response induced in vitro : A probe for allograft and tumor immunity. Transplant. Rev. 17: 3-36

Wahren, B. and Metcalf, D. (1970). Cytoxicity in vitro of preleukaemic lymphoid cells on syngeneic monolayers of embryo or thymus cells. Clin. Exp. Immunol. 7: 373-386

Walters, C. S. and Wigzell, H. (1970). Demonstration of heavy and light chain antigenic determinants on the cell-bound receptor for antigen. J. Exp. Med. 132: 1233- 1249

Weissman, I.L. (1967). Thymus cell migration. J. Exp. Med. 126: 291-304d

Wepsic, H.T., Bernstein, I.D., Zbar, B., Borsos, T. and Rapp, H.J. (1971). Abrogation of passively transferred tumour immunity in vivo by antigenically related tumour cells. J. Nat. Cancer Inst. 46: 195-202

Wide, L. (1962). An immunological method for the assay of human chorionic gonadotrophin. Acta. Endocrinologica, Suppl. 70, p.20-27

Wigzell H. and Andersson, B. (1969). Cell separation on antigen-coated columns. Elimination of high rate antibody-forming cells and immunological memory cells. J. Exp. Med. 129: 23-36

Wigzell. H. and Mäkelä, O. (1970). Separation of normal and immune lymphoid cells by antigen-coated columns. J. Exp. Med. 132: 110-126

Wolf, A. (1969). The activity of cell-free tumour fractions in inducing immunity across a weak histocompatibility barrier. Transplantation 7: 49-58

Wong, A., Mankovitz, R. and Kennedy, J. C. (1974). Immunosuppressive and immunostimulatory factors produced by malignant cells in vitro. Int. J. Cancer 13: 530-542

Woodruff, M.F.A. (1968). Immunology and cancer. Lancet I: 1299

Wright, A.E. and Douglas, S.R. (1903). An experimental investigation of the role of blood fluids in connection with phagocytosis. Proc. Roy. Soc. 72: 357-370

Wunderlich, J.R., Martin, W.J., Macdonald, J. and Fletcher, F. (1972). Functional capacity of cytotoxic lymphoid cells. Nat. Cancer Inst. Monograph 35: 243-249

Yohn, D.S., Funk, C.A., Kalnins, V.I. and Grace, J.T.Jr. (1965). Sex-related resistance in hamsters to adenovirus-12 oncogenesis. Influence of thymectomy at three weeks of age. J. Nat. Cancer Inst. 35: 617-624

Youn, J.K., Le Francois, D. and Barski, G. (1973). In vitro studies on mechanism of"eclipse"of cell-mediated immunity in mice bearing advanced tumours. J. Nat. Cancer Inst. 50: 921-926

Yuan, L., Garvey, J.S. and Campbell, D.H. (1970). Molecular forms of antigen in the circulation following immunization. Immunochemistry 7: 601-618

Zarling, J.M. and Tevethia, S.S. (1973a). Transplantation immunity to simian virus 40-transformed cells in tumour-bearing mice. I. Development of cellular immunity to simian virus 40 tumour-specific transplantations antigens during tumourigenesis by transplanted cells. J. Nat. Cancer Inst. 50: 137-148

Zarling, J.M. and Tevethia, S.S. (1973b). Transplantation immunity to simian virus 40-transformed cells in tumour-bearing mice. II. Evidence for macrophage participation at the effector level of tumour cell rejection. J. Nat. Cancer Inst. 50:149-157