THE UNIVERSITY OF MANITOBA

IMMUNOLOGICAL MECHANISMS IN PLASMACYTOMA-BEARING MICE

by

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ABSTRACT

The immunobiology of plasmacytomas (MOPC104E, J606, HOPC-1 and S104) was investigated in Balb/C mice. Mice were immunized in various ways such as administration of live or irradiated tumor cells with or without BCG. Upon challenge with a lethal dose of tumor cells, varying degrees of antigenicity was found: MOPC104E and HOPC-1 were comparatively strongly antigenic and J606 and S104 were only weakly antigenic, as judged by the various methods to induce immunity. The effects of whole spleen subpopulations (T-cells, B-cells) and of thymocytes from tumor-bearing and normal animals was also investigated. Lymphoid cells were mixed with a lethal dose of tumor cells and administered s.c. to normal recipients (Winn Assay). Whole spleen cells played, generally, a protective role if taken from tumor-bearing animals, and normal spleen cells had little protective capacity when injected together with tumor cells. Nylon wool columns were used for the separation of whole spleen cells. Fractions containing mostly T-cells or B-cells had varying degrees of effect on tumor induction and growth, depending on the particular tumor investigated. Immunological enhancement could occur with any subpopulation or whole spleen cells. Thymocytes from tumor-bearing animals were found to interfere with tumor inhibition in several experiments. The suppressor action of adherent cells (i.e. removed by the nylon wool column) was also indicated in one experiment. The results demonstrate that various effector and suppressor lymphoid cells co-exist in tumor-bearing hosts, and that the balance between these antagonistic arms of the immune response is extremely important.

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Chapter I

I. INTRODUCTION

A. Foreword

The main purpose, if not the only purpose, of scientific investigation is to increase the quality of life for man on earth. This can be most meaningfully accomplished by increasing longevity and totally obliterating disease, which, aside from man's inherently characteristic selfishness, is his worst enemy. The study of immunology, starting as far back as 200 years ago, has developed and evolved for this explicit purpose beginning with the practice of variolation in India and China in which protection against smallpox was obtained by inoculating live organisms from disease pustules. With disease prevention at its roots, immunology in the past two centuries, and more accurately in the past two decades has advanced in leaps and bounds. Within this time, cancer, the ever debilitating threat to every human alive, came into the cross hairs of modern immunological concepts and theories. The only partially adequate usefulness of chemical and physical therapy added impetus to the concept of immunotherapy, only possible with a fundamentally detailed prospectus of immune reactions to tumors in general and in specific. Although we know much in this respect, we still grope anxiously to crack the anomalies and inconsistencies the literature presents. This essentially defines tumor immunology and its purpose: To prevent or cure cancer through a detailed understanding of the immune mechanism actively underlying reactions to all antigens and through a concise understanding of the pathological process of tumorigenesis. Since these two areas of study are immense, and since knowledge of normal physiological procedures

at the molecular level is comparatively scarce, it seems dubious that the goals of tumor immunology can be achieved in minimal lengths of time. However, man's obsession to achieve the unascertainable prods the immunologist on to gainfully secure some measure of success in terms of further understanding the intensly complex and dynamic discipline of modern immunology. Within this publication is contained a small contribution to this end.

B. Scope of the Present Study

The purpose of this study was to help elucidate underlying cellular mechanisms in host immunity to plasmacytomas, tumors of the plasma cells, which are characteristically responsible for the production of antibodies in normal conditions. The study can be divided into two areas of investigation. (1) lethal tumor cell dose of four plasmacytomas (MOPC104E, J606, HOPC-1, S104) and the antigenic nature of each, and (2) the *in vivo* inhibitions of these plasmacytomas by adoptive transfer of whole spleen cells, and T-cells and B-cells (purified by nylon wool columns) from spleens of tumor-bearing Balb/c mice.

A brief look at the effect of thymocytes through adoptive transfer studies (as well as the cross-reactivity of two plasmacytomas) forms part of the area of investigation.

(a) Lethal Tumor Cell Dose and Antigenicity Studies:

This study was performed as a basis on which to look at cellular mechanisms in immunity to plasmacytomas. The demonstration of the immunogenicity in syngeneic and autochthonous tumors plays an extremely important role in the study of *in vivo* and *in vitro* systems. This becomes difficult sometimes because of poor antigenicity on behalf of plasmacytomas in general. However, Rollinghoff et.al. (15, 39,42,43,49,52) have shown some reactivity in both *in vivo* and *in vitro* procedures. He also discusses the source of antigenicity of these plasmacytomas. However, antigenicity studies are very subjective and antigenicity may indeed change with time (26, 27). Three of the four plasmacytomas investigated (J606, S104, HOPC-1) were not considered by any other groups to this date and therefore, antigenicity had to be ascertained.

(b) In Vivo Destruction of Plasmacytomas:

The basic method used was the Winn Assay (38), which was also extensively used by Rollinghoff et. al. (39). Rollinghoff and other investigators (14, 36,39,40,51) have found reactivity to plasmacytomas using this method as well as in vitro culturing methods. Basically, they have concluded that the T-cell, through the use of anti Thy-1 serum, and antithymocyte serum plays the key role in immunity to the plasmacytomas and that B-cells, through the use of anti-K chain antisera plays no role at all. Also, peritoneal exudate cells are important (47,48). However, other cells have been implicated (50,51). The separation of whole spleen cells into T-cell enriched fractions and B-cell enriched fractions using nylon wool in our investigations leads to some interesting conclusions concerning the actual cell type involved in conferring resistance, if indeed any is conferred with our plasmacytomas. This study indicated the complexity of cell interactions in the hosts and the idiosyncrasies applicable to individual tumors.

Chapter II

II. TUMOR IMMUNOLOGY

A. Introduction

The study of tumor immunology essentially exists to answer one question: Why is a tumor cell, which is by all definitions chemically and biologically foreign, not destroyed by our exquisitely sensitive immunological defense system? Obviously, this is a very broad question to answer and underlying it are manifold complications, which in essence emanate from our relative ignorance of normal biological mechanisms.

The tumor immunologist is really not terribly concerned with the etiology of tumors, although etiologies play a role in the actual immune response potentiated in terms of conferring "antigenicities" to the converted cell, ie. the tumor. In the past four or five decades, concerted efforts to implicate viruses in animal tumors, and even in some human tumors as etiological agents have succeeded. Conclusions indicate that both DNA viruses (such as Papova virus, polyoma virus, Shope papilloma, SV40, Adenovirus & Herpes virus) and RNA viruses (such as fowl leukemia and sarcoma viruses, murine leukemia and sarcoma viruses, Type C virus particles from many animal leukemias and sarcomas, including man, mouse mammary tumor and reoviruses) are able to induce animal and human tumors (74). This viral infection manifests itself structurally as antigenic determinants on the now altered cell, which will be discussed shortly.

Similarly, chemical carcinogens have also been responsible for the formation of new antigenic determinants on the cell surface of a transformed cell. The effect of physical agents such as X-irradiation or ultra violet irradiation on normal cells as a carcinogen undoubtedly also confers some new antigens on the cell.

It is the modern view within the study of carcinogenesis that viral, chemical and physical etiology may not be mutually exclusive and are adamantly underlain by genetic background and environment.

With this brief summary of etiology of tumors, it becomes apparent that tumor immunology really relies on new antigens being formed, ie: neo-antigens on the transformed cell and thereby eliciting an immune response, albeit many times in vain.

B. Tumor Antigens

With the cornerstone of tumor immunology resting with tumor antigens, it is no surprise that this subject has been reviewed extensively many times (74,75,76). The collective term for all the tumor antigens is "Tumor Associated Transplantation Antigens" (TATA) as opposed to Tumor Specific Transplantation Antigens because it was found that tumor antigens can cross-react, even though they are not identical, with normal tissue antigens present during the development of the fetus ie. embryonic life, and that these antigens are not in all cases "specific" transplantation antigens, as formerly thought by using transplantation methods.

Tumor associated transplantation antigens are normally classified according to the etiology of the tumor, ie. chemically induced, virally induced, and other reasons. One consistent observation of TATA from chemically induced neoplasms is the fact that the antigens have a propensity to be individually distinct, in direct opposition to antigens conferred by viruses, which have a propensity to cross-react antigenically, even across species barriers.

1. Virus-Associated Tumor Antigens

a. RNA - virus - induced tumor antigens:

This type of oncogenesis is best exemplified by the avian and

murine leukemia viruses and by the mouse mammary tumor virus. In this type of oncogenesis, the cancerous cells will usually continue to release virus particles and the virus leaves the host cell through a budding process, without causing cell damage.

The murine leukemia viruses possess a group-specific soluble antigen, which is derived from the ribonucleoprotein core of the virus particle. The major representatives of the murine leukemia viruses include Gross, Friend, Moloney, and Rauscher viruses and all share this antigen. Hence, it is referred to as a group specific antigen (77,78). This antigen is released from concentrated virus particles treated with ether or from virus-induced leukemia cells. Soluble antigen extracted from mammary tumor virus particles of mice failed to cross-react with that of the leukemic origin, indicating group, ie. murine leukemia specificity.

However, in contrast to the cross-reactivity of the group-specific antigen of Gross, Friend, Moloney, and Rauscher viruses, the envelope antigens of these viruses differ from one another. This viral envelope antigen, or type-specific antigen, is represented at the membrane of murine leukemic cells, and subunits of the viral envelope are present in the blood and may be adsorbed on cell surfaces. Type-specific antigens are specific for the various strains of oncogenic RNA viruses although some degree of cross-reactivity may be present.

Non-viral surface antigens of RNA induced tumors are also present. Non-virus producing tumors induced by defective viruses (does not produce envelope, although it may induce tumors) have been detected, and transplantation immunity can be induced with them, although no virion antigens were present. The Moloney virus induced hemangiosarcoma XM-1 in mice fails to release virus particles but induces transplantation immunity (74).

The Gross-virus, in particular, in addition to having group and type specific antigens, also has the "Gross Cell Surface Antigen"(GCSA) and also a soluble antigen is present in infected animals which is non-virion in nature, possibly derived from GCSA. G_{IX} is another cell surface antigen induced by Gross virus. It exhibits Mendelian inheritance patterns, but also appears newly synthesized in infected cells. The structural gene coding for G_{IX} is common to all mice, similar to Tla. It is uncertain whether either of the two genes, G_{IX} and Tla, belong to the murine leukemia virus or to the cellular genome.

Not just RNA-induced tumor tissues demonstrate the presence of RNA viral antigens. It has been found that normal cells can as well have RNA virus associated antigens throughout life. Also, methylcholanthrene induced tumors, show expressions of C-type RNA tumor virus genomes in a variety of animals (79).

b. DNA virus induced tumor antigens:

DNA oncogenic viruses are cytocidal in certain cell types, and virus particles are reproduced. Whereas the full genome of the virus is needed for producing de novo infectious virus particles, for transformation only part of the genome is necessary. Generally, the viral genome is integrated into the nucleus of the transformed cell, and as a rule, Papova and Adenovirus induced tumors do not produce virus and hence do not contain virion antigens, while Herpes induced Mareks Disease in chickens and Burkitt's Lymphoma may release virus, and hence possess virion antigens.

DNA - virus transformed cells showing neoantigens appear first within the nucleus. These are the tumor (T) antigens. They are specific for the inducer virus and not for the cell type or species in which the malignant transformation occurs. Adenoviruses cross-react on the basis of their T - antigens.

T - antigens are not identical with the components of the infecting virus but should be regarded as products of the viral genome. Antibodies to T - antigen have also been detected in tumor bearing animals (131).

In addition to intranuclear antigens discussed above, immunologically distinct surface or tumor specific transplantation antigens have also been postulated. They can be detected with membrane immunofluorescence. Presently, it is impossible to determine whether the surface antigen or TSTA are identical, similar or totally distinct. There is some suggestion that they are not identical. SV40 oncogenic cell lines were described which expressed either surface antigen and TSTA or surface antigen only. A direct correlation was found between the absence of T - antigen and TSTA and the absence of viral ^{m}RNA and viral DNA. Some revertants of polyoma virus transformed cells, however, that apparently lose their TSTA still express T - antigen and contain detectable ^{m}RNA (80).

Adenoviruses of the highly carcinogenic group A, weakly oncogenic B groups as well as group C viruses all induce a common TSTA (80).

2. Antigens of Chemically Induced Tumors

As mentioned before, antigens, as a result of chemical carcinogenesis, show an extreme individual antigenic specificity, and therefore, several tumors induced in one host by the same chemical will most likely demonstrate individually distinct neoantigens. These neoantigens are, nevertheless, capable of immunity induction as shown by sensitive bioassay experiments with graded inocula doses. Only occasionally are tumors encountered, eg. urethan-induced pulmonary adenomatosis in mice where no immune response can be demonstrated. The fact that neoantigens of chemically induced tumors display great individual antigenic disparity at least in terms of rejections supports the view that these antigens

are expressions of various gene groups of the host cell and not the result of a commonly occurring infection by viruses. However, in some exceptional cases, cross-reactivity can be detected as in the case of diethylnitrosamine-induced guinea pig hepatomas and methylcholanthrene sarcomas (81,82). Further a high degree of cross-reactivity was found by serological methods between tumors of different etiology, eg. three methylcholanthrene-induced and spontaneous rat sarcomas, or of diverse histological types such as hepatomas and mammary carcinomas (83). Those tumor antigens may be re-expressed embryonic products being identified by their reaction with lymphoid cells or serum antibody from multiparous donors.

3 . Onco-Fetal Antigens (Embryonic Antigens)

Under some conditions not clearly understood mature cells may resume some of the gene activity which was functional during fetal life, and as a consequence of the maturation process, was later repressed. This "derepression" of fetal genes manifests itself with newly synthesized substances normally present in fetal cells, but now found on the normal cell. It has been postulated that, in the process of oncogenesis, fetal gene derepression occurs and genetic coding for new (fetal) antigenic structures begins and visualizes itself as some of the transplantation antigens of tumors. This may occur in either chemically or virally induced tumors, and hence TATA's may be in some cases identic al with fetal antigens. Furthermore, loss of the carcinogen (virus) from the transformed cell, may take place, rendering a state whereby no etiological agent can be assigned with current laboratory methods. The derepression can result in (a) cells with fetal characteristics will outgrow neighbouring cells, (b) will possess fetal antigens recognizable experimentally as TATAs or, (c) will evoke little reaction from the host due to the host's tolerance to

its own fetal antigens. Embryonic antigens of human tumor cells may be listed at present as follows: (a) carcinoembryonic antigen (CEA) of Gold (84), (b) the alpha - 1 fetal globulin antigens of Abelev which are produced by hepatomas (85) and, (c) the widely distributed fetal antigen of Edynak (86).

a. Carcinoembryonic antigen (CEA):

In 1965, Gold and associates found that in human colonic carcinomas, antigens, distinct from those of the normal colonic tissue, as detected by specific antisera produced in rabbits, were present. This antigen could also be found in adenocarcinomas of the digestive system epithelium such as the esophagus, stomach, small intestine, colon, rectum, pancreas, and liver, and was also a normal constituent of fetal digestive tissues in the first six months of gestation, and hence the term "carcinoembryonic antigen". The antigen is detectable by radioimmunoassay and low antibody levels in patients have been observed. CEA is a 6-7S or 10S water soluble glycoprotein with an electrophoretic mobility characteristic of a beta globulin. The fact that the majority of colon or rectal carcinoma patients have elevated CEA blood levels allows CEA to avail itself as a prognostic and diagnostic tool. However, CEA is found in patients with breast, lung and prostatic carcinomas and in patients with non-malignant diseases as well as 50% of healthy normal volunteers (87).

b. Alpha-Feto protein:

The alpha globulin is the first protein to appear in the serum of mammals in early embryonic life and contains trace amount of carbohydrate. It is secreted by cells of the hepatic parenchyma and yolk sac into the blood during embryonic life and hence the term alpha-fetoprotein (AFP). Immunologically, AFP cross-react transgressing species barriers although

electrophoretic mobilities may vary and molecular weights may vary (65,000 - 70,000). In 1971, Abelev (85) found that mouse and rat hepatomas synthesized AFP which was subsequently secreted into the blood. Clinically, similar findings were observed in patients with teratoblastomas of the testis and ovary and hepatocellular carcinoma. Similarly to CEA, AFP is found in the sera of normal individuals, patients with hepatitis or other non-neoplastic hepatic conditions, and also during pregnancy and cirrhosis. Equally misleading is the fact that AFP has not been detected in some cases of hepatoma and teratocarcinomas. Nevertheless, the detection of AFP in serum is a useful diagnostic test when the above mentioned tumors are suspected (88).

c. Gamma Fetal Protein - 2 (Edynak Fetal Protein)

In extracts of many human tumors, such as breast, colon, ovary and stomach carcinomas as well as various sarcomas and leukemia, Edynak and associates (86) have found a fetal antigen with gamma-globulin electrophoretic mobility. Some antibodies have been detected in patients with these cancers, but not in the sera of normal individuals. Therefore, this antigen has been considered to be the product of a malignant or near malignant cell.

d. Other Fetal Antigens (88):

There are a few other fetal antigens associated with malignancy which will be just briefly mentioned here. They include alpha-ferroprotein which is related to ferritin and is produced in fetal and post-natal liver as well as in children afflicted with nephroblastoma, neuroblastoma, hepatoma, teratoma and others. Beta Globulin (88) is another human fetal antigen which occurs frequently in the sera of patients with cancers of nonhepatic origin and cirrhosis and hepatitis. Heterophile fetal antigen (88) is present in the serum of human, bovine, porcine, canine, and feline fetuses.

In a very small number of patients with various cancers, an antibody in their serum was capable of precipitating this antigen from extracts of breast, colon, ovary, kidney, parotid gland, muscle, bone, and nerve tumors.

C. Host Response to Tumor

1. Introduction

It is an accepted fact that most transplantable animal and autochthonous animal tumors can induce an immune response in the host (74,75). However, the immunological responses are complex and diverse, but can be lumped into two general categories - humoral response and cell-mediated response. The evolutionary significance of tumor immunity is not clear at the present time. Burnet proposes that cell-mediated immunity (CMI) has developed in higher organisms for the express purpose of eliminating aberrant cells, whether tumor cells or other transplanted cells. This teleological interpretation, the crux of Burnet's immunological surveillance theory (111), alleges that the immune system is constantly on guard. Another view implies that the major evolutionary forces at work in shaping immunological responses are parasites, bacteria and other microorganisms (90). Higher animals would require such a precise defence mechanism to protect all those surfaces exposed to the environment from invasion by such pathogenic organisms, and indeed, the elegant design of both the humoral and cellular limbs of the immune system deals rather efficiently with these antagonists. Tumor immunity may simply be a "spin-off" from this type of protective immunity. The effector mechanisms in tumor immunity will be discussed under the two conventional divisions: humoral and cellular.

2. Humoral Mechanisms in Tumor Immunity

As a general rule, animal and human tumors do elicit antibody

responses against the aberrant cells (89, 113). Some antibodies in fact, may be non protective to the host and is classified as "enhancing antibody" and will be discussed under another heading further in this text called "Possible Escape Mechanisms of Tumors". However, in the protective sense, the production of antibody can have a variety of physiological effects on the target tumor cells varying from rapid and complete cell destruction to phenotypic changes of the surface of the tumor cell resulting in the aforementioned phenomenon of enhancement (90). Destruction of the target can be mediated by complement dependent cytotoxic antibody, particularly in malignant melanomas, lymphomas and leukemias. However, methylcholanthrene-induced sarcomas, Moloney virus-induced sarcomas and MTV-induced tumors avail themselves more readily to antibody dependent cell mediated cytotoxicity (90).

In Burkitt's lymphoma, the cells of which grow readily in tissue culture and provide an excellent test system for immunofluorescence the main tool for the detection of antibody, complement dependent cytotoxic antibody plays an important role. In about 80% of normal African and American sera, antibodies reacting with these lymphoma cells can be detected, however at a much lower titre than in patients afflicted with the disease. Evidence indicates the immunological reaction is directed towards Epstein-Barr Virus (EBV). A similar antibody is detected in high titre in Africans with nasopharyngeal carcinoma. Also, antibodies to EBV was associated in American college students with the development of infectious mononucleosis (90). Their antibodies have also been found in patients with Hodgkins disease and acute leukemia. The exact role of EBV is unclear although it seems obvious it has some function in the diseases mentioned above. It has been hypothesized that EBV infection may be the primary infective agent, the gross manifestations in the form of clinical conditions

above being the ultimate affliction really shaped by the environment or other local modifying factors (90). Therefore, mononucleosis is the result in a healthy individual, whereas in the disease-ridden regions of Africa, a malignant lymphoid cell proliferation known as Burkitt's lymphoma ensues (90).

Malignant melanoma also elicits complement-dependent antibody mediated reactions. Anti-melanoma antibodies were found in the sera of 67% of melanoma patients and only 20% in normal sera. When spontaneous regression of melanoma was the case, all those patients showed high titres of antibody, which can be considered a general rule. But, on the other hand, only 26% of patients with advanced metastatic melanoma showed cytotoxic antibody activity (89, 90).

Sarcomas are notorious for defying complement-antibody directed action of the host defense mechanism and are generally affected by antibody dependent cell mediated cytotoxicity, (ADCC). In this mechanism, non sensitized "lymphoid cells" in the presence of heat inactivated sera from mice bearing the respective neoplasm or whose tumor had been removed are the cytolytic agents (91). This antibody was consequently called "lymphocyte-dependent antibody" (LDA) because cytotoxicity was thought to have been imparted by lymphoid-like cells. However, subsequent studies have indicated the fact that the cells responsible for cytotoxicity in ADCC are more likely of the monocyte/macrophage series. The attractive feature of ADCC is the economic use of the antibody produced to trigger this type of activity, with as few as 100 molecules of IgG required to lyse a chicken red cell. Also, the non-specific requirements for effector cells is expedient. However, in some sarcomas such as osteogenic sarcoma, serum antibodies can be detected by immunofluorescence, and in fact shows localization on cultures from human sarcomas

of any histological origin. Also, these antibodies were found in a high percentage of family members. These antibodies can be shown to participate in fixing complement with lysis imminent (92).

Other less dramatic mechanisms whereby antibody can help the host overcome the effect of aberrant cells are possibly also active. When specific antibody reacts with the surface of cells, the amoeboid motility of the cells is impaired. It has been shown that syngeneic immune serum will inhibit the *in vitro* movement of lymphoma cells. With this in mind, it is conceivable that this may be an active mechanism to prevent or delay metastases (93).

When less than optimal amounts of antibody bind to the tumor cell, enough complement may not be subsequently bound to result in cytolysis. In fact, membrane damage may be quite limited as a result of this lack of complement and also as a result of repair mechanisms at work. However, with minimum complement binding, it has been observed that cell division may be inhibited (90).

A discussion of humoral mediators of anti-tumor mechanisms would not even be elementarily complete if no mention was made of soluble chemical mediators. Lymphocytes, largely T-lymphocytes, are capable of synthesizing and releasing a large number of pharmacologically active macro molecules in response to immunological stimulation. Collectively, they are known as *lymphokines* and their effects are non-specific although their production is precipitated by specific lymphocyte reactions (132). Some of these activities are: (a) the inhibition of movement of macrophages and polymorphs caused by mediator known as MIF (macrophage inhibitory factor); (b) skin reactive factor which causes vascular dilatation and increased capillary permeability;

(c) lymphotoxin which is allegedly a humoral cytotoxic factor capable of lysing target cells, or inhibiting their growth; (d) chemotactic factors which attract macrophages and polymorphonuclear leukocytes; (e) blastogenic factor which causes lymphocytes to synthesize DNA and divide (132). Many other activities have been described, and whether they are nuances of the same is not known presently. The enterprising nature of these collectively potent humoral modulators of anti-tumor activity, needless to say, has a profound influence on this immunological detente.

3. Cellular Mechanisms in Tumor Immunity

Many histological studies had referred to the cellular infiltration seen around tumors, but it was not until the work of Murphy in 1926 that the lymphocyte was incriminated as a possible mediator of immunological reactions to grafted tumor cells (94). When the similarity became apparent between allogeneic graft resistance and tumor immunity, in that they could both be adoptively transferred by cells, only then did it become clear that the lymphocyte occupied a central role in tumor immunity. The most frustrating component of studying cellular mechanisms is undoubtedly the ignorance surrounding the cell-type responsible for tumor immunity. In adoptive transfer experiments, using the spleen generally, it is impossible to know which cell types are actually responsible since the spleen contains a variety of types. Morphological criteria as a means of distinction is inappropriate and rarely co-relate with data derived from studies of cell function. Some of the properties which have been used to define cells involved in cellular immune reactions against tumors are phagocytosis, adherence to glass or synthetic polymers, presence of special antigenic determinants,

presence of immunoglobulin or immunoglobulin receptors on the cell surface, resistance to irradiation or metabolic inhibitors, and of course, morphological features. This preoccupation of assigning specific roles to specific cells has led to some conclusions, but unfortunately has left most investigators in a false sense of security in terms of a fundamental understanding of cellular co-operation, a phenomenon destined to further confusion.

Basically, there are two types of lymphocytes: thymus-derived lymphocyte, or T-cell, and the bone marrow-derived lymphocyte, or B-cell. The T-cell is characterized by the presence, in the mouse, of the Thy-1 (formerly theta) antigen and a lack of or sparse amount of surface immunoglobulin. In modern immunology, T-cells can be further divided into sub-groups on the basis of differences in antigenicity. The antigenicity differences are defined by the different Ly antigens (Ly 1,2,3 and others) first discovered by Boyse and co-workers (95). The various biological manifestations of T-cells have been linked to the particular Ly phenotype, which has led investigators to believe that Ly 1^+ cells are allegedly "helper T-cells" and Ly $2,3^+$ cells are allegedly "cytotoxic T-cells". The majority of immature T-cells are Ly $1,2,3^+$. Nuances surrounding these recent revelations indicate skepticism and confusion and it is, at most, extremely difficult to categorically draw conclusions in terms of a pragmatic assignment of cellular occupations. The recent discovery of "suppressor" cells which allegedly play some role in ablating or modulating the immune response to antigenic challenge further complicates this extremely volatile area currently so vogue (96). The co-operation of all the above cell types amongst each other is further complicated by the discovery of

certain genetic products such as Ir gene products and other histo-compatibility products as a requirement for maximum co-operative efficiency to antigenic challenge (97). Furthermore, the macrophage/monocyte cell type has come under intense scrutiny and is somehow apparently intimately involved as a "specific and non-specific coll-aborator" in effecting a maximal immune response (98,99,100).

The T-cell and B-cell and macrophage have been implicated as the responsible cell type for tumor destruction "independently". In other words, each of these cell types can, by itself, kill tumor cells to which they are specifically sensitized. Needless to say, it is difficult to make this brash statement candidly, due to technical restraints inherent in any separation technique employed to isolate T, B or macrophage cell types. Similarly, the presence or absence of soluble mediators, such as Ir gene product for example, on the surface of a cell type may or may not influence the subsequent reaction with the isolated cell type, and it is difficult to prove the presence or absence of this product on the surface. Therefore, what may seem to be the result of exposure of the tumor cell to only one cell type, may in fact not be the case. However, cognative of this drawback, Lamon et al, and O'Toole et al, have used anti-immunoglobulin antiserum to deplete the cell population of B-cells and concluded that B-cells are important cytotoxic effectors in tumor immunity. They also found that anti-Thy 1 failed to ablate cytotoxic effects demonstrated, which further corroborates his contentions (101,102).

To implicate T-cells as the cell type involved, many authors have succeeded. Cerrottini and his colleages have shown that a specifically cytotoxic activity found in lymphocytes from alloimmune

mice can be abolished by anti-Thy 1 serum (103). Grant, Evans and Alexander have confirmed this finding but have also emphasized that this population of cytotoxic T-cells is only one transient facet of the response. Other mechanisms may be operating concurrently (104). In studies of Moloney sarcoma virus induced tumors, tumor specific cytotoxic cells appeared to be T-cells and no evidence of B-cell cytotoxicity was detected (105). In our laboratory, we have also confirmed that T-cells play a major role in cytotoxic responses using a rabbit antiserum specific for guinea pig thymus-derived lymphocytes. We have found that the cytotoxic reaction against methylcholanthrene-induced tumor cells (MC-D) by cytotoxic lymphoblasts was inhibited by pretreatment of the lymphoid cell populations with the anti T-cell antiserum (138). In all studies of mouse plasmacytomas, T-cells were unquestionably involved (15). Also, thymectomy effects the carcinogenic effect of benzpyrene and methylcholanthrene and greatly increases susceptibility to oncogenesis due to adenovirus (107,109). This is powerful evidence to suggest an influential role of T-cells in tumorigenicity.

Macrophages as the cytolytic cell type have also been unilaterally implicated. Evans and Alexander have examined monolayers of macrophages removed from mice immunized with lymphoma cells. It was shown that these macrophages were capable of specifically inhibiting the <u>in vitro</u> replication of the lymphoma (106). In syngeneic systems, they found growth inhibition, but lysis by macrophage was reserved for allogeneic systems. An "arming factor" was shown by the same authors to be released as a soluble mediator which essentially "informs" the macrophage of the aggressive invader cell. Upon contact of the armed macrophage with the specific target, they become hyperactive, vacuolated and physiologically "angry".

The short mention of T, B and macrophage cell types above as being responsible for tumor immunity from the cellular point of view should indicate the extreme complexity and status quo in this aspect of cellular immunology today. In concrete terms, we can only really categorically emphasize that cellular mechanisms as a protective contingent in tumor immunity is very real. Countless numbers of studies in many different systems confirm and reconfirm this contention. Some have already been mentioned above. Other studies use delayed hypersensitivity skin reactions as a means to assess cellular involvement, since this reaction is classically cell-mediated. This is performed by intradermal injection of tumor extract or whole tumor cells in animals that are immune to syngeneic tumors. In mice, delayed hypersensitivity was shown easily (109,110). In the human, delayed-type reactions were noted in patients with melanomas, Burkitts lymphoma, colon carcinoma, breast cancer and various other sarcomas, and carcinomas (88). A detailed study of mouse plasmacytomas concluded that immunity could be adoptively transferred with the spleens of tumor bearing mice (39).

Other possible effector cells could include the mast cell with its release of various soluble mediators in reaginic antibody reactions. There is also a close association of mast cell degranulation in epithelial pyknosis (88). Other cells in the polymorphonuclear lineage may be involved, perhaps acting as armed or activated cells co-operating with humoral mechanisms. The well documented association of eosinophilia with tumors such as Hodgkins disease lends courtesy to this thought (90). A recent paper published by Glade and associates provides a good overview of the role of cellular immunity in neoplasia (141).

D. Possible Escape Mechanisms of Tumors

The undisputed antigenicity of tumors, a priori, presents an

enigma, since malignant disease does occur, and indeed occurs at an uncomfortably high frequency. Immunological surveillance is, therefore, prostituted to the aberrant cell benefactors. Somehow, the reconnaissance arm of the immune mechanism is despicably undermined, resulting in its vigilance being ominously compromised. A recently published paper concerning the factors interfering with immunological rejection of tumors is written by Baldwin and Robins (140). Some of the possible underlying mechanisms will now be briefly discussed.

1. Immunological Unresponsiveness

Burnet's theory of immunological surveillance implies that the development of a tumor must represent the failure of the host's responses: an immunological failure on the part of the host (111). The increased incidence of tumors in immunodeficient patients is often used as evidence in scaffolding the surveillance hypothesis. The allografting of malignant cells in renal transplant recipients, while on immunosuppressive therapy, is successful only until therapy is removed (90). This also further corobborates Burnet's theory of surveillance and the fact that immunological unresponsiveness, in this case induced unresponsiveness, is a possible escape route for tumors. Unresponsiveness can fundamentally be exercised through specific immunological tolerance, or non-specific immunodeficiency.

a. Specific Immunological Tolerance:

Tumor viruses which are vertically transmitted, such as AKR mouse leukemias induced by the Gross virus impart a specific immunological tolerance to the virus and to the tumor cells. These animals cannot be immunized against Gross leukemia cells. It is quite possible that these animals are actually mounting an immune response which is

ineffective and is perhaps overwhelmed by viral antigen. Hence, functionally, they are rendered tolerant. In this disease associated with the vertical transmission of an oncogenic virus, the state of non-reactivity preceeds the development of the disease (90). In human tumors, there is no apparent analogue. Human neoplasms indicate no evidence to suggest specific tolerance and reactive lymphoid cells are readily detectable in the peripheral blood. Histological studies of tumors and their regional lymph nodes, hypersensitivity studies and anti-tumor antibody studies of cancer patients all indicate that patients are not tolerant (90). Experimental animal tumors induced by chemical carcinogens and by some horizontally-transmitted DNA viruses grow progressively in the host without any evidence of specific tolerance occuring. The existence of concomitant immunity and post-excision resistance to challenge in these models suggests that specific immunological tolerance plays no important role in the immunological escape of tumor cells.

b. Non-Specific Immunodeficiency:

The relationship between immune deficiency states with high incidence of malignant disease has led investigators to test the hypothesis that the escape of tumors may be a consequence of the general lack of immunocompetence. In fact, patients with general hyporeactivity have more rapidly growing tumors and following surgical treatment, have a poorer prognosis (133). This type of a study makes a cause or effect assignment difficult. In general, patients with early tumors do not have any detectable immunological deficit and it is only after extensive tumor growth that such defects become evident. The implication here is that defects in immune reactivity are a consequence of tumor growth and are, in fact, not involved in the initial escape of the tumor. However,

in the reticulo-endothelial system, which is composed of macrophages and monocytes within an extensive network throughout the body, immunological deficiency is detectable early in the disease, manifested by an inability to mount delayed cutaneous hypersensitivity reactions to a variety of antigens and an inability with sensitized lymphoid cells to convert to positive skin reactivity after adoptive transfer (90). In general, however, current assay methods have failed to detect substantial immunological defects in cancer patients.

Animal studies have implicated immunodeficiency as a route for tumor cell escape. If age can be used as a tool indicative of immunodeficiency, ie. very young and progressively older immune systems are generally less effective than "mature" systems, then much evidence is in favor of correlating cancer and immunodeficiency. Aged Swiss mice are less capable of rejecting skin homografts and tumor homo and heterografts than young adult mice. In addition, spontaneous tumor incidence increased with age. Similarly SV40 oncogenesis in hamsters was directly related to the age of the animal: 95% of animals inoculated within 3 days (ie. very young immune system) of birth developed tumors after 90 - 150 days and the incidence of tumors fell to 20-25% in animals infected at 1 or 2 weeks of age and to 0% in animals infected 3 weeks after birth (88). Immunodeficiency can be artificially contrived using antilymphocyte serum (ALS), and results also generally indicate the correlation between immunodeficiency and tumor growth. It has been found that ALS injection into mice promotes the occurence of spontaneous neoplasms, facilitates lung adenoma induction by urethan and enhances viral carcinogenesis (88). Chemical immunosuppression also leads to similar results. Further, chemicals considered carcinogenic such as benzpyrene, demethylbenzanthracene and

methylcholanthrene in general reduce the number of total plaque-forming cells per spleen to sheep red blood cell antigens and there seems to be an apparent correlation between carcinogenicity of a compound and immunosuppressive capability (112).

2. "Sneaking Through"

In the early stages of tumor growth, a few somewhat isolated tumor cells may present too little antigen to sensitize the host. By the time the immunological response has become established, the tumor cells may have replicated several times and the consequent cell mass may be beyond the capabilities of the immune response. The hypothesis only provides a mechanism of escape at the very early stages and cannot explain why the immune system cannot effectively deal with a well established tumor. The establishment of this theory came as a result of experiments with mice using graded tumor cell doses. Transplanted leukemias grew and regressed when inocula of 0.1 ml. containing 1% leukemic spleen cells were used. With further dilution of the inocula, the proportion of tumor takes terminating in death was increased markedly. Therefore as the dose of tumor cells became lower, the degree of difficulty of the tumor to establish itself became less (88).

3. Phenotypic Modification of Tumor Cell Antigens

During the development of a tumor, there could be a progressive loss of antigenic determinants from the cell surface brought about by immuno-selection, analogous to the development of drug-resistant bacteria. However, other mechanisms underlying antigenic change in neoplasims can also be at work. The two most common interpretations are briefly mentioned below.

a. Antigenic Modulation:

The phenomenon of antigenic modulation, as described by Boyse and Old in 1969 (134), refers to the fact that some cell types, when exposed to a persistant immune response in vivo against a single antigenic determinant, lose that particular determinant. This is a reversible phenotypic change in that the antigen may reappear after in vitro culturing, and hence the appropriate term "modulation" has been assigned. It was originally detected in thymus-leukemia (TL) antigens in mice and hasn't been adequately demonstrated in human tumors. We do not know whether the antigen is shed from the cell surface (see below) or is internalized. Needless to say, modulation can be an extremely potent escape mechanism for tumors but more work must be carried out which ultimately will herald the truth.

b. Shedding of Antigens:

The glycoprotein coating of all nucleated mammalian cells known as the glycocalyx is constantly being shed and resynthesized. It is possible that tumor cells shed their surface antigenic structures faster and more extensively than normal cells. One illustration of the shedding of the glycocalyx is in the work of Gold and Freedman concerning CEA in colon carcinoma patients. They maintain that CEA is a component of the tumor cell glycocalyx which is shed in large quantities into the serum, and can readily be detected there (84).

Recent studies from this laboratory have also suggested that antigenic modulation may be a very powerful method to escape immunological attack (136). Tumor cells coated with rabbit anti-tumor antiserum and incubated with various fluids (e.g. serum from tumor bearing animals, serum from tumor immune animals, ascites) before the

addition of complement resulted in resistance induction to complement mediated lysis. One reason suggested for the resistance to lysis upon complement addition is that the tumor antigens are stripped from the cell surface through cross-linking by immune complexes which were detected in the ascitic fluid and sera of tumor bearing animals.

The protective capacity of shedding became immediately obvious because, if prodigious shedding is the rule, the immune system is left without an effective attack mechanism. Furthermore, the shed antigens could serve as an antigenic "smokescreen" virtually tying up the immune system artillery, which would make it easy for the tumor to cellularly expand and metastasize.

4. Immunologically "Privileged" Sanctuaries

There are regions in the body hidden from the surveillance and vigilance of the host's immune system which may lend themselves to tumor initiation. First set graft rejections are prolonged if allografts are placed in these sites. Several sites are known including the hamster cheekpouch, the brain, the anterior chamber of the eye and the testes. Burkitt's lymphoma, for example, frequently involves the central nervous system, an immunological sanctuary (88). The relative rarity of tumors in these sites however, prevents one to strongly advocate this potential mechanism.

5. Humoral Interference Factors

There is some evidence to indicate that specific serum factors may exist in tumor bearing hosts which could be potentially lethal in patients in whom the tumor grows. Several distinct hypothetical serum factors have been incriminated in this inhibitory activity and will be discussed under separate headings.

a. Immunological Enhancement and Blocking Antibody:

Snell and colleagues have reviewed the subject of tumor resistance following the injection of inactivated tumor cells and have drawn attention to a considerable number of reports in which the results were exactly opposite to the expected, ie. tumor growth was promoted by prior immunization (113). This phenomenon was shown to be transferred with serum and is referred to as "enhancement". Although immunological enhancement can be readily demonstrated with allogeneic tumor grafts, it is more difficult with syngeneic tumor grafts, however possible (114).

The mechanism of enhancement is still poorly understood but was originally seen as a form of efficient blockade in which antibody, for some obscure reason without any cytotoxic activity, coated the tumor cells and protected them from further attack by the effector arms of cell mediated immunity. Experimental data also implicate evidence for both central and afferent effects of antibody. Central inhibition advocates contend that the tumor's antigens reach the lymphoid centres of the host, but as a result of high titres of serum antibodies or antigen-antibody complexes, these centres are rendered incapable of cellular response. Afferent inhibition advocates believe that the antigenic material released by the tumor is prevented from adequately sensitizing the host by virtue of prior union with and pre-emption by antibodies (115).

The Hellström's consolidated much enthusiasm with the aid of *in vitro* growth inhibition or tumor cell destruction assays. It was demonstrated that most human and animal tumor bearing hosts have detectable cellular immunity against the tumor. This cell-mediated immunity, however, could be abrogated consistently with serum factors of hosts with growing tumors. These factors were not detectable in sera of animals

which spontaneously caused regression of a tumor or of animals which were specifically immunized. This blocking effect was found to be specific (116, 120). An "unblocking factor" was found in normal sera, and when this serum was added to blocking serum, the enhancing effect was abrogated (119). Later the active ingredient of serum imparting this enhancement was found to be antibody of the IgG type. However, subsequent studies by Baldwin, attempting to correlate blocking of the tumor destruction with the presence of antibody, was unsuccessful since the sera of tumor-bearing rats containing no detectable antibody was capable of blocking cytotoxic lymphoid cells whereas serum taken after tumor excision with detectable anti-TSTA antibody was devoid of such blocking activity (117).

The more than subtle conclusion is that the blocking effect of serum components cannot categorically lay with a specialized antibody.

b. Circulating Antigen, Immune Complexes, Blocking and Inhibition:

Our own laboratory and many others have shown evidence which indicates that together with or independent of blocking antibody, free circulating soluble tumor antigens or tumor-antigen-antibody complexes can play a role through efferent inhibition of the effector arm of the immune response (136). The addition of autologous patients' serum to washed lymphocytes will abolish cytotoxic activity. The inhibiting material is found in larger concentrations in patients with more advanced disease and the factor(s) seems to be immunologically specific. Molecular weight studies indicate the inhibitory activity substance is of low molecular weight (ca. 30,000) but in patients with less advanced disease such activity has been detected in the IgG region as well, suggesting immune complexes as also being suspect (136). Baldwin and associates have shown that tumor cell membrane components isolated from human colon carcinoma by papain treatment are capable of inhibiting cytotoxic activity

of the lymphocytes from patients with colonic tumors. The specificity of this inhibition further suggested that tumor antigen was responsible (118).

Other investigators have shown that lymph nodes from SV40-bearing mice contain specifically cytotoxic cells, detectable only after extensive washing. The "inhibitor" was apparently reversibly bound to the membrane of the effector cell and it was postulated that the circulating antigen shed from the surface of tumor cells was responsible for the inhibition of cellular immune mechanisms. In fact, the addition of tumor antigens by injection to animals bearing syngeneic tumors have resulted in the expected increase in tumor growth (90).

At different stages of tumor progression, it is conceivable that both immune complexes and free antigen make vital contributions to the escape of the tumor, as shown by experiments above. The presence of free antigen and complexes can also be used advantageously to detect tumors, diagnose them and monitor effects of treatment. Radioimmunoassays have proven valuable in this respect because of the extreme sensitivity inherent in this method.

E. Immunotherapy and Immunodiagnosis - An Overview

Before the advent of immunotherapy, approximately 10 years ago, surgery and irradiation were the main tools used in treating cancer patients. Also, chemotherapy played a role in controlling the tumor growth. However, the current inadequacy of these treatments reflects their lack of selectivity, ie. the absence of specific target sites on the malignant cell and the consequent inability to distinguish between normal and malignant. It is within this context that the immunological approach to cancer treatment provides such an attractive concept. An immunological attack would be directed only at the cells expressing tumor specific antigenicity, the cornerstone of modern tumor immunology. In simple terms, the fundamental

goals of immunotherapy are clear: We must do one of three things in the face of the failing response of the patient - either amplify the overall effectiveness of the immune response, sensitize the tumor cells to immunological attack or remove any factors interfering with the potentially cytotoxic confrontation between these responses and the tumor cells.

Until we know which are the important effector mechanisms, it is difficult to rationally approach the development of a lucrative tool for immunotherapy. Controversy as to whether antibody, T-cells, B-cells, macrophages or any subset therein, either unilaterally or collectively, effect the ultimate response to a tumor, forms the pivotal point surrounding intelligent investigation.

Some of the potential methods of immunotherapy of human tumors are shown in the table below:

Table II - 1 Some of the potential methods of immunotherapy of human tumours

	Specific	Non-specific
Active	Sterilized tumour cell vaccines. Antigen extracts. Helper determinants. Heterogenization	BCG Zymosan antireticular serum. Corynebacteria. Levamisole
Passive	Anti-tumour antisera. 'Deblocking' sera	Normal serum components. Properdin. Complement
Adoptive	Sensitized allogeneic or xenogeneic lymphoid cells	Non-sensitized allogeneic lymphoid cells. Graft-versus-tumour

Miscellaneous	Comments
Local immunotherapy—DNCB (Klein) BCG (Morton). Vaccinia (Hunter-Craig *et al.*)	Probably inflammation-therapy. Delayed hypersensitivity reactions are non-specifically tumoricidal
Adoptive transfer with products of sensitized lymphocytes e.g. transfer factor (Levin *et al.*) Immune RNA (Alexander *et al.*)	Require more extensive validation in experimental models
In vitro 'arming' of effector cells e.g. immune complexes used to arm monocytes–macrophages. Also specific *in vitro* stimulation of lymphocytes then reinfusion	Requires characterization of effector cells and co-operating humoral factors
Oncolysis, Coley's toxins. Interferon inducers. Viruses	Besides a direct tumoricidal effect, there is an additional boost to immunological response

Taken from Currie, G.A.
in "Cancer and the Immune Response" (90)

Specific passive and active immunotherapy has been quite discouraging in all but isolated cases (121, 122). Some success has been reported using non-specific active immunotherapy. Many substances are known to induce non-specific stimulation of the activity of the reticulo-endothelial system (RES), mostly derived from yeasts and mycobacteria. The immune response to a variety of antigens can be boosted in a non-specific fashion by such agents. Bacillus Calmette-Guérin (BCG), an attenuated live tubercle bacilli was shown to reduce heterologous infections in mice. Also, BCG accelerates allogeneic skin rejection. Furthermore, it was found that intact and fractionated tubercle bacilli would protect mice against syngeneic tumors (123, 124). Among the excitement, other non-specific RES stimulants were sought out and include zymosan, shark liver lipid, bamboo leaves, wheatstraw, edible mushrooms, and bacteria such as Proteus vulgaris and C. parvum (90). BCG in particular has stepped into the limelight as a potential tool for non-specific stimulation of the RES and some success has been observed. Much work is necessary and caution is exercised before it can be routinely used in humans in order to avoid the theoretical danger of immunological enhancement.

Adoptive immunotherapy is a relatively recent development and is based on the demonstration of the central role of this cell type in transplantation immunity, through the use of delayed hypersensitivity reactions. However, in humans little work has been performed using adoptive transfer experiments, and what has been done is not encouraging (125). The main contention facing adoptive transfers in humans is histocompatibility disparity. Animal experiments, such as in the case of mouse plasmacytomas, have been quite encouraging, and genetically identical animals add greatly to the proper evaluation of results, a point which may ultimately

lead to the abandonment of this approach for human cancers.

It has been experimentally borne out that the most efficient treatment of tumors is a combination of chemical, physical, surgical and immunological approaches (126). The immunological aim of treatment, although very weak relatively speaking, in terms of removing or killing a tumor compared with the surgical removal or X-irradiation of the tumor, is very important. It may serve as a final momentum to favor a tumor-free state for the patient. It would be the immunological sensitivity and vigilance which would specifically rid the host of a small number of tumor cells which would be missed through the use of other forms of treatment, and thereby preventing and obviating an incipient tumor infection.

Immunoprophylaxis is an interesting possibility and an obvious extension of immunotherapy, especially now since we realize that the immune system plays a central role in controlling cancers. If the immune system can be kept alert or non-specifically activated to increase its surveillance ability, it would be possible to either prevent or postpone cancer in man. In mice, BCG has been shown to prevent some syngeneic neoplasms, as cited already, by non-specifically activating the RES. Vaccines seem to be a realistic method of proplylaxis in light of the current knowledge. Neoantigen extracts of tumor cells, particularly if they are extensively cross-reactive with other neoantigens are attractive in the production of a vaccine. The viral etiology theory also lends itself to the implication that a vaccine containing attenuated virus particles, or antigens thereof, may be successful in preventing cancers with known or suspected viral etiology such as some leukemias. It becomes evident that, even through a brief overlook of immunotherapy,

good reasons exist for the excitement in this field, but much has yet to be uncovered.

The relationship between immunotherapy and immunodiagnosis, at closer look, is more cognate than at first apparent. It is not uncommon in the diagnosis of cancer that a clinically observable manifestation of the disease comes late in the development of the cancer, and by the time it is noticed, it may be too late for any meaningful treatment. Such is the case with lung cancer, which, next to heart disease, is the number one killer of males. On the other hand, other cancers, such as cervical cancer, can be detected early and treated generally very successfully. This cancer, if caught in situ through the use of a simple biopsy known as a "Papanicolau smear", therefore presents relatively little danger.

The increase of knowledge in the field of tumor biology and immunology have lent some important tools to the diagnostician which can make the diagnostic procedure much more easy and accurate, but more importantly, much sooner. This has obvious implications for some cancers not clinically evident until its later developments. Immunodiagnosis may make it possible to diagnose any incipient cancer, and hence allow treatment to begin earlier and thereby increasing the chance for recovery.

The detection of tumor products, which in the incipient disease, will be in low concentrations, provides the most convenient method of diagnosis in the early stages, and specific antisera to these products through the use of extremely sensitive radioimmunoassays, could make the detection relatively easy.

Alkaline phosphate isoenzymes of fetal origin are sometimes found in patients with ulcerative colitis and hepatic cirrhosis (127).

It can be of value in monitoring the tumor burden in response to treatment. Hormones produced in excess by some tumors also provide a tool to monitor tumor burden. Bronchial tumors are known to produce many hormones. Similarly, choriocarcinoma cells, of the placental trophoblast, produce large amounts of chorionic gonadotropin, which can actually be used in monitoring the course of disease, through radioimmunoassay quantitation (128).

Carcinoembryonic antigen (CEA) of Gold (84) was found to be released by colonic carcinoma and not by normal colon. It is also associated with Crone's disease, ulcerative colitis, polyposis coli, bronchogenic carcinoma and in tumors of the urinary bladder. CEA is used as a monitor to assess tumor progression and regression in response to therapy through the sensitive radioimmunoassay.

Other fetal protein antigens may also be used in immunodiagnosis. The previously mentioned alpha-fetoprotein of Abelev is one such antigen and has been associated with hepatomas in mice and man. Detection of alpha-fetalprotein antigens in hepatoma patients ranges from 40% to 70% of all cases, and therefore indicates a potential value as a diagnostic tool (85).

The direction which immunodiagnosis research is going now is to seek out new materials produced only by the tumor as an index of the degree of susceptibility, possibly, or as a means to monitor the tumor responses to drug or other therapeutic treatments in patients. CEA and alpha-fetoprotein as well as chorionic gonadotropin are encouraging examples of materials useful in diagnosis, and the "universal cancer test" may be rapidly becoming a reality.

Besides the search for macromolecules as a diagnostic substance,

we should also consider the host responses to tumors as a diagnostic tool. As discussed in previous sections and in future sections, the immune response, although ineffective in some cases, is indeed a real phenomenon in a host's struggle against tumors. Therefore, anti-tumor responses can conveniently be used as a handle to infer the status of the incipient or established neoplasm. Circulating anti-tumor antibodies have been demonstrated in many tumors, in both animals and humans. In Burkitt's lymphoma and in some sarcomas, some clinical correlation between antibody level and disease status is evident.

In our laboratory, we have developed a microradioimmunoassay to detect extremely small amounts of antibodies directed to tumor-associated antigens (130). Assays like our radioimmunoassay allow a means of monitoring tumor progress or diagnosing tumor conditions. Cell mediated immunity also plays a functional role and its reactivity to the tumor can be assessed to determine the status of the malignant growth. Quantitation of serum blocking and inhibiting factors can be used as a method to correlate the progression or regression of a tumor. In fact, Hellström has associated the disappearance of blocking factor with a clinical cure (135). However, methods of quantitation are extremely cumbersome and not feasible on a large scale, and do not show consistently co-relating results. Radioimmunoassays may however make things easier if antisera against these factors can be developed.

The contribution of immunology to the diagnostic aspects of clinical oncology will then be twofold. Firstly, for the detection of tumor products, it will contribute to methodology. The fundamental approach here is to develop an antiserum to the macromolecule and thereby employ a highly sensitive radioimmunoassay. The second contribution of immunology

to the diagnostic aspects of clinical oncology will be to increase the ability to examine the reactions of the host to his own tumor. However, both contributions are totally contingent upon further understanding of the tumor-host relationship from the immunological point of view, and if great strides are gained in a general sense, then it must follow that great strides will be gained in the immunodiagnostic potentiation of immunology. Indeed, the study of tumor immunology essentially culminates in immunoprophylaxis, immunotherapy and immunodiagnosis, all of which assist in increasing the quality of life for man on earth.

Chapter III

III. IMMUNOLOGY OF PLASMACYTOMAS

A. Induction and Modulating the Induction of Plasmacytomas

The induction of plasmacytomas in strain Balb/C mice can be accomplished with relative ease by a variety of methods. Potter and Boyce in 1962 used (incomplete Freund's Adjuvant) IFA by itself, IFA with heat-killed staphylococcus, mineral oil (Bayol F), corn oil and Locke's solution in various volumes with various numbers of injections (1). The following table taken from Potter and Boyse outline their results.

Development of Plasma-Cell Neoplasms in BALB Mice

Table III - 1

Experiment group	Material injected	Volume injected (ml.)	No. of injections	Months of age when injected	No. of mice	Plasma cell neoplasms	Other neoplasms Reticular*	Other neoplasms Non-reticular†	Dead from other causes	Unaccounted deaths
1 *A*	IF + S	0·3	1	2	32	3	1	27	0	1
B	IF + S	0·3	1	2	31	9	7	3	10	2
2 *A*	IF + S	0·5	1	2	18	4	7	0	4	3
B	IF + S	0·5	2	2, 2½	19	8	2	2	6	1
3 *A*	IF + S	0·4	1	2	32	1	2	1	21	7
B	IF + S	0·4	1	2	39	2	9	6	14	8
C	IF + S	0·4	1	2	28	2	7	5	8	6
4 *A*	IF + S	0·4	1	2	39	1	11	5	13	6
B	IF	0·4	1	2	32	0	2	5	11	13
C	M.O.	0·4	1	2	40	8	8	5	6	5.
5 *A*	M.O.	0·5	1	2	32	2	2	0	10	12
B	M.O.	0·5	3	2, 4, 6	32	22	0	0	5	2
C	C.O.	0·5	1	2	40	0	0	2	8	6
D	Locke's	0·5	1	2	40	0	2	1	9	6

* Reticular neoplasms include lymphocytic, histiocytic and the Hodgkin-like lesion. † Most common neoplasm was pulmonary adenoma.

Taken from
Potter, M. et. al. Nature 193: 1086 (1962)

It can be seen from the table, the most effective method of inducing plasmacytomas was to use mineral oil in a 0.5 ml volume, injected intraperitoneally (i.p.) 3 times at 2 month intervals. However, all except corn oil injections and Locke's solution injections resulted in various degrees of tumor induction.

Plasmacytomas can also be induced by the intraperitoneal introduction of solid plastic foreign bodies such as lucite discs or even lucite borings (144). The plastic material causes a marked thickening of the peritoneal connective tissues as well as a fibrous proliferation on the peritoneal surfaces. Within weeks of implantation, hyperplastic plasma cells were found.

Recent studies (2,3) have indicated that the induction of plasmacytomas can be made easier if other antigens are injected at the same time. According to the studies of Bober et. al. (2), at 2, 4, and 6 months of age, 0.5 ml of Primol D (mineral oil) was injected i.p. Within 24 hours of the first oil injection, groups of mice were given 1 of 4 antigens in a volume of 0.1 ml. The appropriate dose was then given one time per week thereafter for the course of the experiment. Mice given no oil injection were given antigen injections on the same schedule. The antigens used were (a) E. coli endotoxin, (b) S. typhimurium endotoxin, (c) BSA and (d) SRBC. The results of the work indicated that five of eleven animals treated with 5.0 ng. E. coli endotoxin developed recognizable plasmacytomas throughout the mesentery between six and seven months of age. The two of fifteen mice treated with 5 ng. BSA which developed ascites did not show plasmacytomas upon autopsy. The following table shows the relationship of dose of antigen together with mineral oil. Again, 5 ng. E. coli endotoxin was most effective as well as 5 ng. S. typhimurium endotoxin given together with mineral oil.

Chronic Antigenic Treatment and Induction of PCT in MO-treated BALB/c Mice

Table III - 2

Treatment	No. of mice with ascites/total no. at 8 mos.	No. of mice with ascites/total no. at 12 mos.	% gross tumor incidence at autopsy	Microscopic diagnosis
MO (Primol D)	0/30	3/27	11.0%	7/27
0.1 ng *E. coli* endotoxin	1/30	11/26	42.2% ($p < 0.025$)[a]	20/26 ($p < 0.005$)
1 ng *E. coli* endotoxin	1/30	7/26	26.8%	14/26
5 ng *E. coli* endotoxin	1/30	13/29	44.8% ($p < 0.025$)	13/29
5 μg *E. coli* endotoxin	0/30	8/28	28.4%	13/28
0.1 ng *S. typhimurium* endotoxin	0/27[b]	1/27	3.7%	11/27
5 ng *S. typhimurium* endotoxin	1/30	14/25	55.0% ($p < 0.005$)	20/25 ($p < 0.005$)
5 μg *S. typhimurium* endotoxin	1/30	2/29	6.9%	4/24
0.1 ng BSA	0/30	4/30	13.6%	12/30
1 ng BSA	0/21[b]	1/21	4.6%	9/21
5 μg BSA	0/24[b]	4/23	17.4%	11/23

[a] p values were determined by χ^2 test with Yates, correction between Primol D control and each treated group.
[b] Mice died prior to 3rd oil injection.

Taken from
Bober, L.A. et. al., Cancer Research 36: 1947 (1976)

However, despite the increase of plasmacytoma incidence by injecting endotoxin (5 ng) together with mineral oil, small amounts of E. coli endotoxin (1 ng to 50 ug) can significantly protect normal female Balb/C mice against challenge with low doses of syngeneic MOPC315 (3). However, beyond 5×10^2 cells as challenge, no protection was afforded in the i.p. treated group. These experiments indicated that: (1) Significant protection was most evident when mice were pretreated with endotoxin (11 days and 5 days) before inoculation with 50-100 tumor cells i.p. (as opposed to other injection schedules such as post-tumor cell injection inoculation with endotoxin) twice a week for the entire experiment. Tumors induced with 10^4 tumor cells i.p. give similar effects. (2) Higher challenges of tumor cells obliterated the endotoxin antitumor effect. (3) Endotoxin omission as a pretreatment resulted in a loss of the preventative effect against i.p. induced tumors, but not with s.c.-induced tumors.

It is difficult to come to grips with this apparent paradox where, in one case, endotoxin helps to induce plasmacytomas when injected with mineral oil and in the other, pretreatment of the animal, and even lack of pretreatment with endotoxin when using s.c. route of injection, causes the animal to be able to reject small doses of tumor inocula. This fine balance between protection and enhancement of tumor growth using the same agent but being administered in different ways is indeed a most puzzling phenomenon.

Besides endotoxin, Amphotericin B (AmB) can also afford protection, if pretreatment consisted of a low dose of the drug (4). If a high dose of AmB was used as a pretreatment, the protective capacity was abrogated and, in fact, enhancement of tumor growth and incidence resulted. The results are summarized in the following table.

Incidence of MOPC-315-C Subcutaneous Tumors

Table III - 3

Experiment No.	Number of drug injections	Control (%) [a]	Incidence (%) of tumors in mice given		Tumor incidence ratio in [c]	
			20 μg AmB [a, b]	500 μg AmB [a, b]	20-μg group	500-μg group
1	1	90 (10)	70 (10)	ND	0.40	—
2	1	40 (5)	0 (10)	60 (10)	0	3.0
3	3	60 (10)	30 (10)	ND	0.50	—
4	3	20 (10)	0 (10)	90 (10)	0	1.0
5	3	40 (10)	10 (10)	80 (10)	0	1.5
6	5	40 (20)	10 (20)	70 (20)	0.25	1.8
7	5	10 (20)	0 (10)	90 (10)	6	4.0
8	5	20 (20)	10 (20)	70 (20)	0	1.5
9	5	90 (21)	55 (9)	ND	0.60	—
10	5	88 (27)	70 (10)	100 (5)	0.52	2.27
11	5	40 (10)	10 (10)	70 (10)	0.35	1.7
12	5	5 (20)	ND	90 (20)	—	1.0

[a] Numbers *in parentheses* indicate number of mice in each group.
[b] ND = not done.
[c] Ratio of tumor incidence in the AmB group to the control when the latter group had developed 50% of their final tumor incidence.

Taken from

Valerote, F., et.al. J.National Cancer Institute 56(3):557 (1976)

As can be seen from the table, a 20 μg pretreatment afforded protection with respect to control group and a 500 μg pretreatment resulted in enhanced tumor incidence, as expressed by the ratio of incidence of tumor in experimental group to incidence of tumor in the control group, when the control groups had developed 50% of their final tumor incidence. Although the mechanism of the action of AmB is unknown, as are the actions of other adjuvants, it has been useful as a chemotherapeutic agent by increasing the uptake of other chemotherapeutic agents eg. 1.3 bis (2-chloroethyl)-1-nitrosourea, as well as acting as an adjuvant in its own right (5).

Another method of increasing tumor incidence, compared to treatment with just mineral oil, is to consistently treat the animals with antithymocyteserum (ATS) (6). Not only is the incidence increased with this schedule, but also growth rate of the plasmacytoma was increased. The experiments of Mandel are shown in the table below.

Effect of Therapy on Plasmacytoma Induction

Table III-4

Therapy	Total No. Mice in Each Group	Mice with Plasmacytomas	Mice without Tumors	Deaths from Other Causes	Therapy	Total No. Mice in Each Group	Mice with Plasmacytomas	Mice without Tumors	Deaths from Other Causes
Bayol	65	32	27	6	Chronic Cytoxan	20	0	0	20
Bayol	50	25	21	4					
Saline	20	0	19	1	Modified Cytoxan, Bayol	25	3	17	6
Acute ATS, Bayol	30	18	6	6	Modified Cytoxan, Bayol	30	4	16	6
Acute ATS, Bayol	25	15	6	4	Modified Cytoxan	20	0	14	6
Acute ATS	20	0	16	4					
Chronic ATS, Bayol	60	38	10	12	Imuran, Bayol	25	7	14	4
Chronic ATS, Bayol	25	16	4	5	Imuran, Bayol	25	5	17	3
Chronic ATS	20	0	16	4	Imuran	20	0	17	3
Chronic NRS, Bayol	20	11	9	0	Irradiation, Bayol	25	15	8	2
Chronic NRS	20	0	20	0	Irradiation	25	0	24	1
Chronic Cytoxan, Bayol	25	0	2	23	Thymectomy, Bayol	20	12	8	0
					Thymectomy	20	1	19	1

[a] Groups of mice were treated by different protocols which included three 0.5-ml intraperitoneal (i.p.) Bayol injections 1 month apart. In the acute ATS groups, four i.p. doses were administered on alternate days with Bayol started on the day of the last ATS injection. The chronically treated ATS, Cytoxan and Imuran groups received four weekly and then six bimonthly doses; the initial Bayol injection was given the day after the first suppressive treatment. Each dose size was for ATS 0.5 ml, Cytoxan 200 mg/kg mouse body weight, and Imuran 50 mg/kg mouse. Modification of the Cytoxan regimen resulted in two weekly injections followed by a single dose before each Bayol inoculation. Irradiation was of the total body and consisted of 250 R the day before each Bayol dose. Thymectomy was performed at 10 days of age. Control groups received each of the therapeutic regimens but without Bayol.

The total number of mice in each group, and the numbers that developed tumors, remained free of tumor, and died from other causes are listed. The length of animal follow-up was up to 15 months.

Taken from

Mandel, M. et. al. J. Immun 109: 360 (1972)

The conclusions from the table indicate that ATS treatment, concomitant with Bayol, results in the increased incidence of tumors of plasma cells (mostly secreting IgA). Suppression of host reactivity with other methods such as Imuran and Cytoxan and adult thymectomy did not significantly increase the rate of tumor induction when injected with Bayol, and resulted in no tumor incidences when Bayol was omitted. Therefore, the increased plasmacytoma incidence results from selective destruction of lymphoid cells by the ATS with little direct effect on plasmacytoma elements. To confirm this conclusion, a study performed by the same authors revealed that, when tumor inocula were followed by an ATS inoculum on the same day, death as a result of accelerated

tumor growth resulted twice as early as in the ATS-untreated group (7). Also, the number of spleen cells inoculated for reconstitutional studies and the time after ATS treatment where the spleen cells were given played an important role. The results are clearly shown in the table below.

Survival of BALB/C Mice Treated with MPC 37 Cells, ATS and Syngeneic Spleen Cells

Table III - 5

Animal Treatment			Animal Survivals	Mean Survival and Standard Deviation
Tumor inoculum (cells)	ATS	Spleen cells		
	ml		*days*	*days*
5×10^4	None	None	48, 52, 52, 52, 52, 55, 55, 55, 59, 59	53.9 ± 3.4 $p < 0.001$
5×10^4	0.5	None	20, 24, 24, 24, 27, 27, 27, 27, 31, 31, 31	26.6 ± 3.5
5×10^4	0.5	7.5×10^7 (day 0)	20, 24, 24, 27, 27, 27, 27, 31, 31, 34	27.2 ± 4.0 $p > 0.1$
5×10^4	0.5	2.5×10^7 (day 2)	27, 27, 27, 31, 34, 34, 34, 34, 38, 38	32.4 ± 4.2 $p < 0.001$
5×10^4	0.5	7.5×10^7 (day 2)	34, 34, 38, 41, 41, 45, 48, 48, 48, 52	42.9 ± 6.3 $p < 0.001$
5×10^4	0.5	7.5×10^7 (day 7)	24, 24, 27, 27, 27, 31, 31, 31, 31, 34, 34	29.2 ± 3.6 $p = 0.05$

Taken from

Mandel, M. et.al. J. Immun. 103: 1288 (1969)

Similarly, the time of ATS injection was crucial, being most effective in reducing mean survival time one day before or after tumor injection, or on the same day. These results indicate that the accelerated tumor growth rate found in mice treated with ATS seems to be the result of the immunosuppressive effects of this antiserum, lowering the ability of the host to respond effectively to this inocuous challenge. We shall consider the immune response to plasmacytomas via humoral/cellular mechanisms in total at some length in section D of this chapter.

The development of plasma cell tumors is also effected by several endocrine factors, including glucocorticoids and adrenocorticotropic hormone which inhibit tumorigenesis and the early inflammatory reaction which occurs after i.p. mineral oil injection. Testosterone stimulates oil-induced tumor formation, while progesterone retards it. Contrarily, follicle stimulating hormone and thyroid-stimulating hormone suppresses

tumorigenesis by a mechanism involving mast cell proliferation. The effect of growth hormone on the development of plasmacytomas has also been studied and the conclusion indicates that daily administration of bovine growth hormone stimulated an inflammatory reaction in the peritoneal cavity and accelerated plasma cell tumor development where bovine prolactin and bovine serum albumin had no effect (41, 54).

It seems evident from the discussion above that plasmacytomas can be effectively induced in Balb/C mice with relative ease and success. Just mineral oil alone seems to be adequate for reasonable success. However, we have seen that many parameters are involved in the tumor induction process, some seemingly paradoxical, all of which are unexplained. It seems, however, in all cases, that the immune system is somehow integrally involved, with bacterial endotoxins and amphotericin B acting non-specifically as adjuvants with the actual mechanism unknown, and with ATS acting directly as an immunosuppressant, crippling the necessary arm of attack to the point where defence to an oncogenic challenge is impossible. Hormones have also been implicated and show correlation with the immune system. Mechanisms of tumor escape have already been discussed in the proceeding section as have the mechanism of host defence to tumors and in the following chapters further evidence will be presented to illustrate the fine balance between the normal and abnormal status.

B. Association of Viruses with Plasmacytomas

Plasmacytomas, as in any other cancer, has a cause, generally referred to as an etiological agent(s). Also, as in the case of other cancers, it becomes extremely difficult to pinpoint these etiological agents of plasmacytomas. Although, as shown above, mineral oil can cause plasmacytomas, mineral oil alone may be unable to cause plasmacytomas. In other words, some other factor(s) may be necessary to accumulatively contribute, and only when all "contributing factors" are present, either with time, or at any one instance, may a tumor cell arise. Carcinogenic chemicals, and irradiation as well as genetic susceptability form the cornerstone of the study of carcinogenesis. Ultimately, genetic background becomes extremely important since everything biological (within our body) is the result of gene expression,

and this includes the ability to mount an immune response to a particular antigen such as viruses for example, as well as cell susceptability to carcinogenic chemicals and irradiation, it may be to some extent academic to label an agent carcinogenic, since it may not cause neoplasia in some individuals and only those susceptible, for whatever reason, develop a tumor when exposed to the agent.

However, in the past, viruses have been found to play a very important causative role in animal cancers, and in some cancers of man. Plasmacytomas fall in this category. Howatson and McCulloch in 1958 reported a virus-like body in a transplantable mouse plasmacytoma, designated at X5563 (8). They found that in a "variable but sometimes high proportion of the tumor cells, a large, dense, osmiophilic inclusion can be seen occupying a central position within the cell, a position normally associated with the Golgi apparatus". High magnification micrographs showed that the central portion of the body is of uniform but variable density and that the limiting membrane is double-layered within a diameter of ca. 65 mμ. Individual particles were found to be indistinguishable from the intracytoplasmic form of the virus-like bodies described by Bernhard et. al. in mammary tumors of mice (9).

In a more recent study concerning the association of viruses and plasmacytomas, a more complex conclusion was the result. In PC - 1^{+} strain Balb/C mice, where PC-1 is a surface alloantigen, primary myelomas which were PC-1^{+} produced many type-C viruses, classified into 3 subpopulations: (1) murine myeloma-associated viruses (MuMAV) carrying a type-specific virus envelope antigen, (xVEA), distinct from known typical murine leukemia virus (MuLV). (2) MuLV (Gross). (3) other uncharacterized type-C viruses.

In PC-1^{-} strains, e.g. C57/b16 where normal plasma cells carried no PC antigen, myeloma cells from some mice became PC^{+} and released complete type-C viruses, either $xVEA^{+}$MuMAV alone or all 3 populations. But, also, some mice developed PC-myelomas with no viruses or only uncharacterized viruses. These results as concluded by these authors indicate that either PC-1 antigen was perhaps induced by $xVEA^{+}$ MuMAV and that $xVEA^{+}$MuMAV and/or the uncharacterized type-C viruses may have had an important role in the development of some myelomas (10).

In another study dealing with syngeneic antibody production with plasmacytomas and naturally occuring antibodies to plasmacytomas, viral association and etiology was also implicated (11). Induced antibodies to a plasma cell tumor antigen by immunization with viable or inactivated tumors was found to be cytotoxic to all the plasma cell tumors tested. However, antibody of the same specificity was found in the sera of normal Balb/C mice and other strains of mice. This is shown in the tables below.

Cytotoxic Activity of Sera of Normal Mice of Various Strains Against Plasma Cell Tumors (MPC 113)

Table III-6

Strain	Age	No. of mice tested	% of mice positive	Mean titer
	months		%	
B6	2	11	82	3.6
"	4	6	83	7.6
"	10	5	80	4.5
DBA	3	5	80	6.5
C3H/HeN	3	5	80	4.7
AKR	3	5	60	2.8
A/He	1	4	0	0
"	4	5	100	4.9
SJL/J	3	5	80	6.7
NZB	3	5	80	7.6
BALB	2	40	85	5.7
"	4	30	90	7.4

Cytotoxic Activity of Normal BALB Serum* Against Variety of Target Cells

Table III-7

Target cell	Cytotoxic titer
BALB plasma cell tumors	
MPC-110	6
MPC-113	8
MPC-80	4
MPC-86	3
MPC-105	2
RPC-20	8
MOPC-28A	8
MOPC-70A	6
MOPC-104E	6
TEPC-18	2
BFPC-3511	4
BALB reticulum cell sarcomas	
RCS-A	8
MC-1934	4
MC-2103	2
BALB leukemias, induced by leukemia virus	
LSTRA	0
MCDV-12	0
BALB normal cells	
Spleen cells	0
Lymph node cells	0
C3H plasma cell tumors	
X5563	4
C1	4
XC1	0

* Pool of serum from 20 BALB mice, 3 months of age.

Tables taken from:

Herberman, R.B. et.al
J.Exp.Med. 136:94
(1972)

This natural antibody reacted with an antigen with characteristics indistinguishable from the alloantigen PC-1 and with the viral envelope antigen xVEA, both these antigens being present on all plasmacytomas tested. Normal Balb/C mice had highest titres at 3-4 months of age, and were lower in 9-12 month old mice. Germ-free mice had a lower reactivity but soon increased their titres when placed in conventional environments (11). One reason for the presence of the PC-1 alloantigen could be that a virus is in a latent form in some normal tissues of Balb/C (liver, kidney, spleen, brain, lymph nodes) and other PC-1^{+} strains (10,11). The antibody, either induced with a plasmacytoma or naturally occurring, can simply be an immunological manifestation to this virally produced PC-1 alloantigen (11), which is found in all Balb/C plasmacytomas and as mentioned above, in some normal tissues as well. It is known that the distribution of the antigen reactive with the immune and natural antibody was identical with that reported for the PC-1 antigen and presumably, the two antigens are the same (12). These studies present more evidence for the viral association in the induction of plasmacytomas, in view of the specificity of the induced and naturally occurring antibodies for xVEA and PC-1.

Although, the aforementioned discussion is not an extensive or in any way complete review of the subject of virus-association with plasmacytomas, it does however provide some indication of the general information currently available. Therefore it can, safely be concluded that virus particles are intimately associated with plasmacytomas.

C. Antigens in Plasmacytomas and Surface Markers

1. Tumor Associated Transplantation Antigens (TATA)

Neoplastic Transformation is associated with a marked modification of cellular antigens, not akin to the normal counterpart. These antigens are called tumor-specific transplantation antigens, or TSTA for short. Perhaps, more correctly, they should be referred to as tumor-associated transplantation antigens (TATA) because some of these new antigens are not specific. The discovery of these new antigens comes from transplantation studies in syngeneic hosts of various animal tumors, falling in three categories (a)carcinogen-induced, (b) virus-induced and (c) spontaneous. In this sense, TATA/TSTA are simply general terms used to describe, a wide variety of new antigens, the origin being unknown.

Studies have been conducted by several laboratories in the past in an effort to establish whether plasmacytomas, as other tumors, do indeed possess new antigens, (14, 15, 36,37).

The work of Williams and Krueger (14), helped pave the road for further investigation. They immunized Balb/C mice with irradiated myeloma cells, then challenged with viable tumor cells and found in each case that the percentage of successful tumor grafts was significantly lower in immunized animals relative to controls. This indicated that murine myelomas have TATA and, further, that the TATAs were fairly weak since only after several immunizations was immunity established, and then easily abrogated with high tumor cell challenges.

This significant reduction in tumor incidences upon challenge with viable tumor cells and the abrogation of this effect with increasing tumor cell numbers in the challenge dose is shown in the table below.

Tumor Immunity of Immunized Mice to Isografts of Homologous Myelomas

Table III-8

Experiment	Immunization*	Challenge cell dose	Tumor incidence (%)	Mean time of tumor appearance (days)
I	None	2×10^6	5/5 (100)	5
	Normal spleen	$3\text{-}4\times10^6$	9/9 (100)	5
		1.0×10^6	7/7 (100)	8
		$1\text{-}1.5\times10^5$	8/8 (100)	13
	MOPC-104E	$3\text{-}4\times10^6$	4/11(53)	8
		1×10^6	1/11(9)	>60
		$1\text{-}1.5\times10^5$	0/11(0)	0
II	None	2×10^6	5/5 (100)	2
	Normal spleen	2×10^6	4/4 (100)	2
		1×10^6	8/8 (100)	3
		3×10^5	3/3 (100)	8
	ADJ-PC5	2×10^6	4/4 (100)	2.5
		1×10^6	6/13(46)	5
		3×10^5	3/6 (50)	11

* Expt. I, mice immunized by 6 weekly injections of irradiated normal spleen or tumor cells; expt. II, 3 weekly injections given.

Taken from

Williams, W. et. al. J. National Cancer Institution 49: 1613 (1972)

The immunological cross-reactivity of the immune mice is shown by the results of Winn Assays (38) in the table below:

Immunologic Specificity of Myeloma TATA Demonstrated by the Winn Test with Immune Spleen Cells From Mice Immunized with Irradiated MOPC-104E Cells*

Table III-9

Challenge tumor	Tumor cells incubated with:	Challenge cell dose	Tumor incidence (%)	Mean time of appearance (days)
MOPC-104E	Normal spleen cells	10^6	7/7(100)	5
		10^5	7/7(100)	9
		10^4	7/7(100)	15
	Immune spleen cells	10^6	0/7(0)	—
		10^5	0/7(0)	—
		10^4	0/7(0)	—
MOPC-173D	Normal spleen cells	10^6	4/4(100)	8
		10^5	4/4(100)	10
		10^4	4/4(100)	16
	Immune spleen cells	10^6	4/4(100)	17
		10^5	3/5(40)	16
		10^4	0/4(0)	—
MOPC-21	Normal spleen cells	10^6	4/4(100)	4
		10^5	4/4(100)	7
		10^4	4/4(100)	12
	Immune spleen cells	10^6	4/4(100)	5
		10^5	2/4(50)	11
		10^4	0/4(0)	—
ADJ-PC5	Normal spleen cells	10^6	4/4(100)	6
		10^5	4/4(100)	9
		10^4	4/4(100)	14
	Immune spleen cells	10^6	4/4(100)	6
		10^5	1/5(20)	11
		10^4	0/4(0)	—
FLOPC-1	Normal spleen cells	10^6	4/4(100)	23
		10^5	4/4(100)	28
		10^4	4/4(100)	32
	Immune spleen cells	10^6	4/4(100)	25
		10^5	3/4(75)	31
		10^4	0/4(0)	—
MCA-1085	Normal spleen cells	10^6	4/4(100)	5
		10^5	4/4(100)	7
		10^4	4/4(100)	10
	Immune spleen cells	10^6	4/4(100)	5
		10^5	4/4(100)	7
		10^4	4/4(100)	10

*5 days before tumor challenge each animal was irradiated with 450R of X-rays.

Taken from

Williams, W. et.al. J. National Cancer Institution 49: 1613 (1972)

These results indicate besides the fact that cell mediated processes are responsible, that by immunization with X-irradiated MOPC104E plasmacytomas, suppression of tumor incidence was possible in four other plasmacytomas tested, all of which were induced with mineral oil, mineral oil-adjuvant or plastic embeddation, and no suppression possible with MCA-1085, a chemically induced plasmacytoma, which therefore possesses no similar cell surface antigens.

These results were corroborated later by McCoy et.al. (36) as well as establishing the requirement for T-cells to manifest this cell-mediated phenomena in vitro with the Winn Assay. This is shown in the table below.

Neutralization of ADJ-PC5 Plasmacytoma In Vitro by ADJ-PC5 Immune Spleen Cells and Abrogation of the Neutralization with Anti-Theta Serum

Table III-10

Treatment of spleen cells with	Ratio of viable spleen cells to tumor cells (1 × 10)	Growth of tumor in recipients
Tumor cell control	—	6/8 (22) [1]
Normal spleen cells + HBSS	300/1	6/8 (21)
ADJ-PC5 immune spleen cells + HBSS	300/1	2/8 (18)
ADJ-PC5 immune spleen cells pretreated with normal AKR mouse serum and guinea-pig complement	300/1	0/8
ADJ-PC5 immune spleen cells pretreated with AKR anti C3H thymocyte sera (anti-theta) + guinea-pig complement	300/1	6/8 (21)
ADJ-PC5 immune spleen cells pretreated with AKR anti C3H thymocyte sera (anti-theta) absorbed twice with an equal volume of C3H brain cells + guinea-pig complement	300/1	0/8

[1] Number of mice developing palpable tumors per number of mice inoculated (mean latency period to tumor detection). Last day of observation = 33.

Taken from

McCoy, J.L. et.al. Int. J. Canc. 14: 264 (1974)

Also, studies by Kolb et.al. (37) established the presence of TATA by a hemagglutination assay as well as anti-TATA activity in the serum by a similar method. By coupling soluble tumor extract, previously shown to indeed be tumor extract by inhibiting the migration of immune syngeneic spleen cells, to SRBC, the anti-TAA activity could be shown by agglutination of the treated SRBC, and TAA could be shown by the inhibition of this phenomenon.

However, studies in other laboratories (15) have not so convincingly demonstrated what Williams in particular has shown in the cross-reactivity studies. Following is a table of all the tumors used in Rollinghoff's experiments.

Tumors Investigated

Table III-11

Tumor line	Strain of origin	Tumor type*	Immunoglobulin synthesis†	Passage generation‡	Mode of tumor induction‖
HPC 6	NZB	PCT	IgA	TC	m.o.
HPC 10	NZB	PCT	IgA	19	m.o.
HPC 18	NZB	PCT	IgA	10	m.o.
HPC 19	NZB	PCT	IgA	14	m.o.
HPC 20	NZB	PCT	IgA	15	m.o.
HPC 32	(NZB × BALB/c)F_1	PCT	IgG1	8	m.o.
HPC 33	(NZB × NZC)F_1	PCT	IgA	7	m.o.
HPC 39	(NZB × NZC)F_1	PCT	IgG1	8	m.o.
HPC 64	(NZB × BALB/c)F_1	PCT	K chain	8	m.o.
HPC 73	(NZB × BALB/c)F_1	PCT	IgA	5	m.o.
HPC 78	BALB/c	PCT	IgG1	9	m.o.
HPC 103	BALB/c	PCT	IgG1	18	m.o.
HPC 108	BALB/c ↔ C57BL§	PCT	IgA	TC	m.o.
HPC 116	(NZB × BALB/c)F_1	PCT	IgA	7	m.o.
HPC 120	(NZB × BALB/c)F_1	PCT	Nil	10	m.o.
HPC 122	(C57BL × BALB/c)F_2	PCT	Not done	3	m.o.
MOPC 104E	BALB/c	PCT	IgM	>20	m.o.
MOPC 315	BALB/c-2	PCT	IgA	TC	m.o.
MOPC 460	BALB/c	PCT	IgA	>20	m.o.
WEHI 3	BALB/c	Myelomonocytic leukemia	Nil	>20	m.o.
WEHI 7	BALB/c	Thymoma	Nil	17	IRR
WEHI 11	BALB/c	Fibrosarcoma	Nil	>20	spont.
WEHI 22	BALB/c	Thymoma	IgM	TC	IRR
WEHI 73	C57BL	Thymoma	Nil	5	IRR
WEHI 91	(NZB × C57BL)F_1	RCS*	Nil	17	m.o.
WEHI 140	(NZB × C57BL)F_1	RCS	Nil	5	spont.
WEHI 145	BALB/c	Thymoma	Nil	TC	IRR
WEHI 150	(NZB × C57BL)F_2	RCS	Nil	6	m.o.
WEHI 156	(NZB × C57BL)F_2	RCS	Nil	4	m.o.
EL 4	C57BL	Thymoma	Nil	TC	MC

*Reticulum cell sarcoma type A, RCS.

†Synthesis of immunoglobulin as determined by radioimmunoelectrophoresis of cell cultures in ^{14}C-labeled amino acids.

‡Tissue culture line of tumor, TC; numbers indicate serial transplant generation of tumor when first used in these experiments.

‖Mineral-oil injections intraperitoneally, m.o.; fractionated (4×150 rad) whole-body irradiation, IRR; spontaneous tumors, spont.; methylcholanthrene-induced, MC.

§Allophenic mouse of BALB/c and C57BL type.

Taken from

Rollinghoff, M. et.al. J. National Cancer Institution 50: 159 (1973)

In all of Rollinghoff's studies, the immunity was induced by surgically removing a tumor approximately 10 days after its appearance. This method in their hands was more effective than X-irradiated tumor cell immunization, killed cells and CFA, or iodoacetate-treated cells,

as shown in the table below.

Immunization, with Different Methods, Against HPC 6 in (NZBxC57bl)F_1 Mice

Table III-12

Number of injections	Number of cells/ injection	Route of injection and treatment of HPC 6 cells*	Tumor growth†	
			Test mice	Controls
1	10^6	im followed by removal of tumor	0/36	36/36
1	10^6	sc followed by tumor regression	0/12	12/12
1	10^8	ip killed cells + CFA	8/8	8/8
2	10^8	ip killed cells + CFA	6/6	8/8
3	10^8	ip killed cells + CFA	1/7	8/8
1	5×10^6	sc irradiated cells (2000 R)	4/4	4/4
2	5×10^6	sc irradiated cells (5000 R)	4/4	4/4
3	5×10^6	sc irradiated cells (5000 R)	5/5	5/5
3	5×10^6	sc irradiated cells (2000 R)	4/4	4/4
1	10^6	sc iodoacetate-treated cells	6/6	4/4
3	10^6	sc iodoacetate-treated cells	6/6	4/4
6	10^6	sc iodoacetate-treated cells	4/4	4/4
3	10^8	sc iodoacetate-treated cells	6/6	4/4
1	10^6	sc iodoacetamide-treated cells	4/4	4/4
3	10^6	sc iodoacetamide-treated cells	4/4	4/4
3	10^8	sc iodoacetamide-treated cells	4/4	4/4

*im: intramuscular; ip: intraperitoneal; sc: subcutaneous.
†Immunized mice and control groups were challenged with 10^6 viable HPC 6 cells injected subcutaneously. The incidence of tumor growth 14 days later is shown.

Taken from

Rollinghoff,M. et.al. J. National Cancer Institution 50:159 (1973)

Using this method of immunity induction, it was found that only 1 of 5 plasmacytomas(HOPC 108) tested resulted in any demonstration of immunity (but NZB-derived plasmacytomas were highly effective) as shown in the table below.

In Vivo Immunity to Several Mouse PCT

Table III-13

Immunizing and challenge tumor*	Recipient strain	Tumor growth†	
		Test mice	Controls
HPC 6	(NZB × C57BL)F_1	0/4	4/4
HPC 18	(NZB × C57BL)F_1	0/4	4/4
HPC 19	(NZB × C57BL)F_1	0/4	4/4
HPC 64	(NZB × BALB/c)F_1	0/4	4/4
HPC 73	(NZB × BALB/c)F_1	4/4	4/4
HPC 116	(NZB × BALB/c)F_1	2/5	4/5
HPC 108	BALB/c	0/16	16/16
HPC 78	BALB/c	8/8	8/8
HPC 103	BALB/c	4/4	4/4
MOPC 104E	BALB/c	4/4	4/4
MOPC 315	BALB/c	4/4	4/4

*Mice were immunized by im injection with $1\text{-}3 \times 10^5$ tumor cells; 10-15 days after tumor removal, animals were challenged with the same tumor by sc injection of $1\text{-}3 \times 10^6$ tumor cells.
†Incidence of tumor growth 14-21 days later.

Taken from

Rollinghoff M et al J National Cancer Institution 50:159 (1973)

No cross-protection was afforded by preimmunization with HPC 108, as shown in the table below (except to HPC 108 and HOPC 116).

Cross-Protection of Mice Immunized with HPC 108

Table III-14

Challenge tumor line	Mouse strain immunized with HPC 108 cells*	Tumor growth†	
		Test mice	Controls
HPC 108	BALB/c	0/16	15/16
HPC 108	(BALB/c × NZB)F_1	2/6	8/8
HPC 6	(BALB/c × NZB)F_1	8/8	8/8
HPC 64	(BABL/c × NZB)F_1	4/4	4/4
HPC 116	(BALB/c × NZB)F_1	0/4	4/4
HPC 78	BALB/c	4/4	4/4
HPC 103	BALB/c	4/4	4/4
HPC 122	BALB/c	3/4	4/4
MOPC 104E	BALB/c	4/4	4/4
MOPC 315	BALB/c	4/4	4/4
MOPC 460	BALB/c	4/4	4/4
WEHI 3	BALB/c	4/4	4/4
WEHI 7	BALB/c	4/4	4/4
WEHI 11	BALB/c	3/4	4/4
WEHI 22	BALB/c	4/4	4/4
WEHI 86	BALB/c	4/4	4/4
WEHI 145	BALB/c	4/4	4/4

*Mice of the indicated strain were immunized with 3×10^5 viable HPC 108 cells. Ten to twelve days after tumor removal, the mice were challenged subcutaneously with other tumors.
†Incidence of tumor growth 14-21 days later.

Taken from

Rollinghoff, M. et.al. J. National Cancer Institution 50: 159 (1973)

However, protection was afforded with preimmunization with HPC 6 by all challenge NZB-hybrid or NZB tumor challenges and by HOPC 108 as indicated by the table below.

Cross-Protection of Mice Immunized with HPC 6

Table III-15

Challenge tumor line	Mouse strain immunized with HPC 6 cells*	Tumor growth†	
		Test mice	Controls
HPC 10	(NZB × C57BL)F_1	0/4	4/4
HPC 18	(NZB × C57BL)F_1	0/5	4/4
HPC 19	(NZB × C57BL)F_1	0/10	10/10
HPC 20	(NZB × C57BL)F_1	0/4	4/4
HPC 32	(NZB × BALB/c)F_1	0/4	4/4
HPC 33	(NZB × NZC)F_1	0/4	3/4
HPC 39	(NZB × NZC)F_1	2/5	4/4
HPC 73	(NZB × BALB/c)F_1	0/4	4/4
HPC 78	(NZB × BALB/c)F_1	4/4	4/4
HPC 103	(NZB × BALB/c)F_1	4/4	4/4
HPC 108	(NZB × BALB/c)F_1	0/8	8/8
HPC 116	(NZB × BALB/c)F_1	0/4	4/4
HPC 120	(NZB × BALB/c)F_1	0/4	4/4
HPC 122	(NZB × BALB/c)F_1	3/4	4/4
MOPC 315	(NZB × BALB/c)F_1	3/4	4/4
MOPC 460	(NZB × BALB/c)F_1	4/4	4/4
WEHI 73	(NZB × C57BL)F_1	3/4	3/4
WEHI 91	(NZB × C57BL)F_1	8/8	8/8
WEHI 140	(NZB × C57BL)F_1	4/4	5/5
WEHI 150	(NZB × C57BL)F_1	4/4	4/4
WEHI 156	(NZB × C57BL)F_1	4/4	3/4

*Mice of indicated strain were immunized with 10^5 HPC 6 viable cells. Ten days after the tumor-bearing limb was removed, the animals were challenged subcutaneously with $1\text{-}3 \times 10^5$ cells from other tumors.
†Incidence of detectable tumor growth 14-23 days later.

Taken from

Rollinghoff, M. et.al. J. National Cancer Institution 50: 159 (1973)

However, with adoptive transfer experiments using normal spleens and spleens from immunized mice injected together with the challenging tumor cells, into sublethally irradiated recipients, (Winn Assay) (38), very little cross-protection could be demonstrated.

Adoptive Transfer of Immunity to HPC 6 and Cross-Reactivity*

Table III-16

Type and No. of spleen cells		Type and No. of tumor cells		Ratio of spleen cells to tumor cells	Tumor growth†
Normal (NZB × C57BL)F_1	15×10^6	HPC 6	3×10^5	50:1	4/4
	15×10^6	HPC 20	3×10^5	50:1	4/4
	15×10^6	WEHI 91	3×10^5	50:1	4/4
	15×10^6	WEHI 140	3×10^5	50:1	4/4
Normal (NZB × BALB/c)F_1	15×10^6	HPC 6	3×10^5	50:1	4/4
	15×10^6	HPC 108	3×10^5	50:1	4/4
	15×10^6	WEHI 22	3×10^5	50:1	4/4
HPC 6 immune (NZB × C57BL)F_1	15×10^6	HPC 6	3×10^5	50:1	0/4
	15×10^6	HPC 20	3×10^5	50:1	0/4
	15×10^6	WEHI 91	3×10^5	50:1	4/4
	15×10^6	WEHI 140	3×10^5	50:1	4/4
HPC 6 immune (NZB × BALB/c)F_1	15×10^6	HPC 6	3×10^5	50:1	0/8
	15×10^6	HPC 108	3×10^5	50:1	8/8
	15×10^6	WEHI 22	3×10^5	50:1	4/4

*Mice of the indicated strain were immunized by im injection of 10^6 viable HPC 6 tumor cells. Spleens were removed 10-12 days after tumor removal. Mixtures of spleen and tumor cells were prepared at various ratios in vitro and the mixture was injected subcutaneously into sublethally irradiated (450 R) mice syngeneic to the transferred spleen cells.
†Incidence of tumor growth 14-21 days later.

Taken from

Rollinghoff, M. et.al., J. National Cancer Institution 50: 159 (1973)

These results indicate spleen cells from HPC 6 immunized mice protected only against HPC 6 challenge and HPC 20 challenge.

One can conclude on the basis of the immunization studies that the NZB plasmacytomas share a common antigen. Also, from a concurrent experiment, HPC 108, a Balb/C originated plasmacytoma, did not grow when the animal was immunized with HPC 64, a NZB-Balb/C hybrid plasmacytoma. Therefore, HPC 108 possibly shares an antigen with NZB inbred and hybrid plasmacytomas and some Balb/C plasmacytomas.

The nature of these antigens is completely unknown, but speculation based on viral studies, indicate that these antigens may be a manifestation of the virus which infects it (9,10,11,12).

2. Viral Antigens

Strengthening this hypothesis, other studies using immunofluorescent microscopy have indicated that IgG is produced as a response to plasmacytomas and is found attached to both the cytoplasm and nucleus. Rabbits immunized with A and G type particles produced IgG antibodies which, with fluorescent studies, very similarly followed

the pattern of staining the cells of the tumor (16). These studies indicate the nature of the surface antigens of plasmacytomas as well as strengthening the viral etiology theory surrounding plasmacytomas. Indeed, the association of virus particles with some plasmacytomas is well known (19). Other studies with heterologous antiserum to MOPC104E have localized the labelled antiserum in the tumor itself, as well as in the liver, spleen and brain (73).

Mouse myeloma cells also have the differentiation antigen called PC-1 found on normal plasma cells, liver, kidney, brain and lymph nodes but not on lymphocytes (12). However, some authors have already attempted to correlate the PC-1 antigen with viral infection of the cell (11,12). Plasmacytoma cells do not have the antigens normally associated with lymphocytes such TL, thy-1, Ly 1 or Ly 2 (17), but in addition, some plasmacytomas have been found to have the Gross leukemia cell surface antigen and Gross viral envelope antigen (GVEA) (17).

The G_{IX} cell surface alloantigen associated with MuLV has been located on one myeloma (18), and is compatible with the current theory that most myelomas produce MuLV-like virus particles (16,19). The xVEA of Aoki and Takahashi (17) on MOPC-70A is an antigen not common to Gross leukemia virions and GVEA. A classification of type-C viral envelope antigens is shown in the table below.

Classification of Type-C Viral Envelope Antigens

Table III-17

	Designation	Antiserum used for demonstration	Description
①	gsVEA:		
	Group-specific viral envelope antigen.	Serum of autoimmune aged NZB mice.	A common antigen of MuLV* and MuMAV. †
	Subgroup-specific cross-reacting MuLV envelope antigen.	Rat or rabbit anti-MuLV serum.	A common antigen of MuLV (GFMR‡) but not of MuMAV.
②	tsVEA:		
	Type-specific MuLV envelope antigen.	Mouse anti-MuLV serum-----	Individual antigen of MuLV.
③	xVEA:		
	MuMAV envelope antigen§-------	PC1-typing serum (C57BL/6 × DBA/2)F_1 anti-BALB/c myeloma MOPC-70A and the serum from normal BALB/c mice.	BALB/c myeloma MOPC-70A-associated MuMAV specific antigen.

*MuLV = murine leukemia virus.
†MuMAV = murine myeloma-associated virus.
‡GFMR = Gross, Friend, Moloney, and Rauscher.
§xVEA is a tsVEA.

Taken from

Aoki, T. et.al., J. National Cancer Institution 51: 1609 (1973)

These studies indicate the general complexity of the study of virus-related antigens on the surface of plasmacytomas. To draw a line between viral antigens as distinct from TSTA/TATA is impossible since, indeed, the TSTA or TATA may essentially be the viral antigen, or any other surface antigen, which is manifesting itself by inducing immunity under certain conditions.

One cannot conclude even a relatively brief discussion of antigens on plasmacytomas without mentioning oncofetal antigens (OFA), since fetal antigens have been extensively studied in human and experimental tumor systems (28,29). Whether an immune response (either humoral or cell mediated) actually is elicited by OFA remains the subject of some debate. In some systems it is possible (30) and in many it is not (31,32). But it is certain that OFA exists on the surface of at least some tumors and in some cases elicits an immune response (28,32).

In a recent study by Chisholm et.al., (33), they compared the degree of in vitro and in vivo sensitization with OFA after challenging with the tumor.Although they found it possible to induce an in vitro T-cell immune response to several different tumor types after immunizing with syngeneic Balb/C fetal liver cells in vitro (34), the in vivo sensitization by OFA was very poor in contrast to the in vitro response. Their conclusion, then, was that OFA does not act as a potent tumor transplantation antigen in the plasmacytomas which they studied. However, whether this is the general rule remains to be established.

3. Surface Markers

Cell surface markers on plasmacytomas have also been investigated. PC-1 with its viral implications has already been mentioned and is present on most plasma cells, but not on all in appropriate strains of mice (11,12). There is a lack of TL, thy-1, Ly 1 and Ly 2 antigens on the surface of plasmacytoma cells, as previously mentioned (17).

In a study by Ramasay et.al. (20), of the three plasmacytomas which he looked at, only one possessed surface immunoglobulin as detected by antiglobulin technique, although by immunoflorescence the other two showed faint traces of surface immunoglobulin. All three tumor lines lacked Fc and C3 receptors. This, however, may simply reflect the properties of normal plasma cells, for their purpose is exclusively for the production of antibody. The results are tabled below.

Properties of the Plasmacytomas

Table III-18

Tumour	Fc receptor	C3 receptor	Immunoglobulin mixed antiglobulin	Immunoglobulin fluorescence
MOPC-104E	–	–	–	±
ADJPC5	–	–	–	±
MOPC-21	–	–	+	+
Non-secretor mutants of MOPC-21	* n.d.	n.d.	–	n.d.

* N.d. = not done.

Non-secretor mutants U7 X22 and X21 were tested for immunoglobulin determinants.

100 per cent of MOPC-21 cells were positive by immunofluorescence while up to 90 per cent reacted in a mixed antiglobulin reaction when cells were examined from culture. A faint patchy fluorescence was observed on all MOPC-104E and ADJPC5 cells *ex vivo* using an anti MOPC-104E myeloma protein and anti-Fab antiserum respectively.

Taken from

Ramasay, R. et.al., Immunology 26: 563 (1974)

However, other studies have conclusively shown that surface immunoglobulin (SIg) does in fact exist on the surface of plasmacytomas (21,22) and in fact some SIg has been isolated from three plasmacytomas (23). Princler et.al. (24) has looked at 22 different plasmacytomas and tested them against fourteen different class and type specific antisera and has concluded that SIg on plasmacytomas is quite likely and attributes negative findings to the variety of techniques used and to the variation of their sensitivities.

Another receptor discovered on MOPC 104E plasmacytoma cells, which secrete IgM with natural affinity for α1,3 dextran linkage from L. mesenteroides, was that discovered by Hiramoto et.al. (25). It was found that MOPC 104E cells can bind dextran-coated SRBC and CRBC forming typical rosettes. In addition, some of the cells reacted with autologous erythrocytes and that this receptor does not cross-react with determinants on the bacterial dextran. This non-cross reactivity was established because soluble dextran, antimouse IgM and antimouse IgG, (and not antimouse IgG_2) blocked rosette formation by dextran-coated CRBC, but autologous erythrocyte rosettes were not blocked by anti-IgM, or dextran and only by antimouse IgG_{2a}. These results indicated that the MOPC 104E carries an IgG_{2a}-like receptor for autologous erythrocytes. Obviously, another conclusion is that more than one class of immunoglobulin may be produced by the same cell, if indeed, the IgG (1or2) was not passively

acquired. Evidence for this idea is further corroborated by the work of Morse et.al. (35). Morse and co-workers found that out of 778 plasmacytomas, IgA was produced by 338 tumors, IgG (1,2a,2b,or 3) by 163 tumors, IgM by 7 tumors. Free κ or λ chains were produced by 14 tumors and no detectable paraprotein was produced by 202 tumors. Two or more paraproteins were detected in the primary ascites of 54 plasmacytomas. Evidence has indicated that when this is the case, the multiple paraproteins are produced by simultaneously occurring, independent plasma cell tumors. However, it was determined that two tumors (SAMM 368 and TEPC 609) produced two paraproteins for over 30 generations in transplantation, implying that single clones of plasma cells have the capacity to produce more than one class of immunoglobulin.

It is well known that surface markers we detect or markers we do not detect may be altered upon transplantation or masked through successive transplantations (26,27).

Ohno et.al., (27) demonstrated that with plasmacytoma 58-8 of Balb/C origin, upon exposure to rabbit anti 58-8 and mouse anti H-2^d, these plasmacytoma cells were killed if the cells had been transplanted for seven or eight generations. However, with the twelfth generation, anti 58-8 susceptibility dropped from 72% to 50% and with anti H-2^d, from 35% to 0%. With the thirteenth transplantation generation, reactivity decreased to 0% with both antisera. However, if these high generation transplanted plasmacytomas were exposed to pronase, a proteolytic enzyme, susceptibility to both the anti 58-8 and the anti H-2^d was regained. Protein synthesis inhibitors such as actinomycin D and puromycin inhibited the loss of susceptibility, by suppressing the "masking" of the antigenic determinants with protein-like material.

This study along with others indicate the difficulties in arriving at consistent results because of mechanisms in cell biology we do not understand. In the case cited above, antigenicity is clearly present at one point in time on the tumor surface, but as transplantation passages or generations increase, this antigenicity may clearly change or be totally lost. This presents a rather obvious problem in studying transplantation biology, both in tumor and normal systems.

The antigens and possibly the surface markers discussed above are a key issue in understanding the incipient immune response in plasmacytoma-bearing mice directed toward rejecting the tumor. Although, generally,

the immune response is relatively weak, it does in fact exist, as we have seen from the discussion above of tumor antigens. In the following section, we shall discuss in detail the cellular and humoral responses of plasmacytoma-bearing mice.

D. Cellular and Humoral Anti-Tumor Responses of Mice Bearing Plasmacytomas

It is impossible to divorce the subject of tumor antigens from the subject of cellular and humoral immune responses, since the proof underlying the former is found in large by the observations in the latter. With this in mind, I shall attempt not to repeat all of the data already outlined in the previous section. The data already outlined however confers upon the reader the fact that an immune response to plasmacytomas does exist, and the purpose of this section is to further this interest.

The work of Rollinghoff et.al. (39), concerning activation of T-lymphocytes is very nicely covered in his review article in 1974. *In vivo* studies have indicated that resistance to a second dose of tumor cells as a challenge was present. Similarly, tumor growth followed by surgical removal of the tumor as an immunization method and challenged nine days later resulted in resistance to tumor induction. As well, Winn assays indicated that this phenomenon was transferable to normal mice by spleen cells (15,39). Similar results were recorded by McCoy et.al. (36), Poupon et.al. (40), Williams (14), and Boyer et.al. (51).

In vitro studies utilizing a Marbrook culture-like system have indicated that killer cells can be activated *in vitro* and can inhibit the growth of tumor cells against which the animals have been immunized (42). Specificity of immunized spleen cells to the tumor cells in question was also shown (43). The table below explains this result.

Specificity of in vivo HPC-108 Tumor Growth Inhibition by Syngeneic in vitro Activated Lymphocytes

Table III-19

Pretreatment of BALB/c[a] spleen cells	Number and type of tumor cell mixed with activated lymphoid cells	Ratio of lymphoid cells to tumor cells	Tumor growth[b] in recipient mice	% *in vitro* ^{51}Cr lysis ± S.E.
HPC-108 activated	3×10^5 HPC-108	20:1	0/4	53.4 ± 2.8
HPC-108 activated	1×10^5 WEHI-164	60:1	4/4	14.3 ± 1.3
HPC-108 activated	1×10^5 EL-4	20:1	4/4	15.4 ± 3.1
C57BL activated	3×10^5 HPC-108	20:1	3/4	13.4 ± 2.3
C57BL activated	1×10^5 EL-4	20:1	0/4	92.6 ± 2.6
Not activated	3×10^5 HPC-108	20:1	4/4	12.7 ± 2.1
Not activated	1×10^5 WEHI-164	60:1	4/4	12.4 ± 1.9
Not activated	1×10^5 EL-4	20:1	4/4	14.9 ± 2.7

a) BALB/c spleen cells were activated by *in vitro* cocultivation with irradiated HPC 108 cells or mitomycin C-treated C57BL spleen cells, then mixed with the indicated tumor cells and injected subcutaneously into irradiated CBA mice.

b) Incidence of tumor growth 14–21 days later.

Taken from

Rollinghoff, M. et.al., Eur. J. Immun. 3: 471 (1973)

In the experiments of Rollinghoff (15), indicated, however, was that the sera of immunized mice had very little effect on the induction of immunity. However, Lespinats (44) using multiple injections of small numbers of iodoacetate treated plasmacytomas, detected anti-plasmacytoma antibodies by fluorescence. They fell, however, to very low titres after approximately six weeks. Other authors have also recorded the detection of anti-plasmacytoma antibodies such as Kolb et.al. (37), Yamada (45), Poupon et.al., (40,46) and Krueger et.al. (16).

The work of Aoki and Herberman (11) also found natural antibodies in the sera of immunized syngeneic mice reactive to 1. viral envelope antigen xVEA present on plasma cell tumors and, 2. a cell surface antigen indistinguishable from the plasma cell differentiation antigen PC-1, characterized by Takahashi et.al. (12).

In the cell mediated immunity experiments, the lymphocyte was not the only cell which seemed to be involved in immunity induction, as indicated in Rollinghoff's studies. Peritoneal exudate cells (PEC) also seemed to confer some resistance to subsequent tumor challenge. The table below compares the protective efficiency of splenic lymphocytes, thoracic duct lymphocytes, and PEC from mice immunized

with HPC 6 tumor cells.

Comparison of Protective Efficiency of Splenic Lymphocytes, Thoracic Duct Cells and Peritoneal Exudate Cells from Mice Immunized with HPC 6 Tumor Cells[a)]

Table III-20

Ratio immune cells to tumor cells	HPC6 tumor growth[b)] Spleen cells	TDL	PEC	adh. PEC
10 : 1	0/6	0/3	0/6	0/4
1 : 1	0/6	0/3	0/6	0/4
1 : 5	4/6	1/3	2/6	1/4
1 : 20	6/6	3/3	5/6	3/4
1 : 100	6/6	3/3	6/6	4/4

a) (NZB x C57BL)F_1 mice were immunized by i.m. injection of 10^6 viable HPC6 tumor cells. Indicated cells: splenic lymphocytes, thoracic duct lymphocytes and peritoneal exudate cells were obtained 10 – 12 days after surgical tumor removal. A sample of peritoneal exudate cells attached to glass surface are referred to as adh. PEC. Mixtures of immune cells and HPC6 cells were prepared at various ratios *in vitro* and the mixture injected subcutaneously into sublethally irradiated (450 rad) (NZB x C57BL)F_1 mice. Each recipient mouse received 2 x 10^5 viable HPC6 cells.

b) Incidence of tumor growth 14 – 21 days later

Taken from

Rouse, B. et.al. Eur. J. Immun. 3: 218 (1973)

Other studies corroborate this conclusion (45,50). The use of macrophages in the macrophage migration inhibition test further provides evidence for a cellular component in plasmacytoma immunity (40).

To determine whether T-cells alone, B-cells alone or both T and B cells were necessary to confer immunity, a series of experiments were performed. In using specific anti B-cell and anti T-cell reagents such as anti-κ chain and anti-thy-1 antisera, respectively, it was shown that only T-cells can transfer immunity to a syngeneic host (48). Similar conclusions were reached by Rouse et.al. (47) using anti-theta serum and also in vitro studies of the secondary cytotoxic immune response have revealed the necessity of T-cells for this indication through the use of anti-theta serum (49). Inability of nude mice to elicit any detectable immunity to plasmacytomas further corroborates T-cells as the cell-type involved (47).

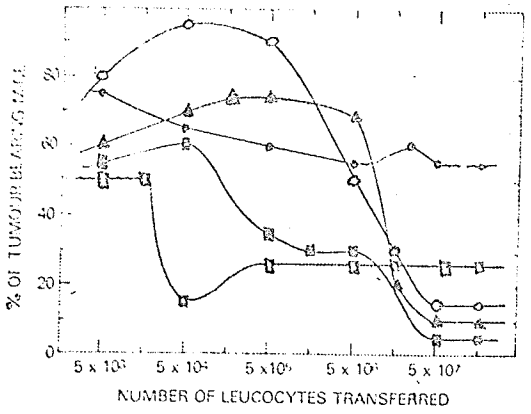
The studies of Mandel and deCosse (6,7) mentioned in an earlier section also provide strong evidence for the ability of a cell-mediated

component (T-cell) to induce a state of immunity through their studies using anti-thymocyte sera.

Since the T-cell is strongly implicated in the tumor immunity elicited (although not exclusively since B-cells because of antibody detection, and macrophages as indicated earlier also play a role), Rollinghoff et.al. (52), proceeded to look for a T-cell receptor for TATA, based on evidence by Cone et.al. (53) that T-cells have IgM-like immunoglobulin on their surface and that these IgM-like molecules are released upon immunization and bind to the stimulating antigen. In fact, Rollinghoff et.al. (52), indeed, found binding of cortisone-resistant thymocyte IgM-like immunoglobulin to the immunizing tumor, presumably to the TATA of the plasmacytoma.

A recent study by Giovarelli (50) has indicated very nicely the fact that different numbers and different cell types of leukocytes from normal adult mice injected into newborns influences in different ways the growth of a transplantable syngeneic chemically induced plasmacytoma (460) in the form of either inhibition or enhancement of tumor growth. The graph below shows this summarily.

Figure III-1



Percentage of tumour incidence in groups of newborn mice pre-inoculated with increasing numbers of leucocytes from adult donors. Groups of 20, 8 12-h-old (newborn) mice were inoculated i.p. with thymus (△), bone marrow (□), spleen cells (○), neutrophils (▽), or macrophages (◇) obtained from adult mice. Twenty-four h after the injection, all groups were challenged s.c. with 3×10^4 MOPC-460 cells.

Hatched area: percentage of tumours ± s.e. mean in control groups pre-inoculated with progressive doses of freeze-thawed leucocytes and in groups not pre-inoculated at all.

The points in the dotted area indicate significant values (P 0·05 to < 0·001).

Taken from

Giovarelli, M. et.al., Brit. J. Canc. 34: 233 (1976)

The results are self-explanatory and indicate that more than just T-cells are involved within the cell-mediated machinery of immunity and other authors do agree (51).

Another recent study has indicated that, not only immunized lymphoid cells, but also normal lymphoid cells could be stimulated in vitro by means of a one-way mixed lymphocyte-tumor interaction assay (MLTI) (51). The activity observed could not be blocked or enhanced by plasmacytoma myeloma protein products indicating that MLTI activity was directed against non-idiotypic cell surface determinants. No cross-reactivity was observed in this system, which is contrary to earlier findings (36,39). As with other studies, anti-theta serum abrogated both the response of normal and immunized lymphoid cell reactivities to the mitomycin-treated tumor cell stimulator. The abrogation, however, was not complete, which means that other cell types are involved. This is not surprising since other studies have implied antibody in the sera of immunized and normal mice (44,45,46) as well as non T-cells conferring resistance (50) as mentioned earlier.

In a very recent study, some interesting results were obtained by Freedman et.al. (56). By using subcutaneous (s.c.) immunization with 1 mg of isolated M component of various plasmacytomas, specific immunity with respect to subsequent s.c. or i.p. challenge resulted, if relatively low tumor cell numbers (10^4) were used as a challenge. The results are clearly shown in the following two tables.

Resistance of Immunized and Nonimmunized Mice to sc MOPC-11 Challenge(s)

Table III-21

and

Specificity of M-component Immunization

Table III-22

Immune status	Number of MOPC-11 tumor cells	Number of mice in each group	Number of sc doses given every 14 days	Significance	Time, in days, until tumor nodule = 2 cm [a]
Immune	10^7	6	1		12, 15, 15, 16, 18, 13 (av, 14.8)
Immune	10^6	8	1	$P<0.001$	18, 24, 31, 30, 45, 49, 49, 54 (av, 37)
Nonimmune	10^6	8	1		15, 16, 17, 16, 16, 22, 30, 30 (av, 20)
Immune	10^5	8	1	$P<0.001$	28, 30, 43, 29, 45, 49, 56, 64 (av, 41)
Nonimmune	10^5	8	1		18, 18, 18, 15, 19, 19, 21, 31 (av, 20)
Immune	5×10^4	2	1		No growth, no growth
		2	2		19, 31
Immune	5×10^4	3	3		36, no growth, no growth
Nonimmune	5×10^4	5	1		16, 16, 16, 22, no growth
Immune	10^4	13	3	$P<0.006$	50, 55, 57, 54, no growth [b]
		5	4		65, no growth, no growth, no growth
Nonimmune	10^4	10	3		26, 29, 31, 37, 40, 21, 24, 24, 28, 40 [c] (av, 30)

[a] No growth refers to no visible tumor after 60 days' observation.
[b] Eight animals developed tumors that never reached 2 cm; 5 of the 8 showed regression in size of tumors beginning at 45 days; all 8 were still alive with tumor nodules (2–10 mm) after 120 days.
[c] All 10 nonimmune animals were dead by 55 days after inoculation.

Specificity of M-component immunization

Immunogen	Tumor challenge	Dose	Number of mice with tumor (2 cm)/No. tested	Time,[a] in days
None (nonimmune)	MOPC-70A	1×10^4	3/3	20, 21, 30
MOPC-11 M component-CFA+IFA	MOPC-70A	1×10^4	3/3	18, 19, 28
None (nonimmune)	MOPC-11	1×10^5	3/3	17, 17, 17 [b]
Normal saline-CFA+IFA	MOPC-11	1×10^5	3/3	15, 17, 18
MOPC-11 M component-CFA+IFA	MOPC-11	1×10^5	3/3	28, 30, 43 [b]

[a] Time until tumor diameter reached 2 cm.
[b] $P<0.025$

Taken from

Freedman, P.M. et.al., J. National Cancer Institution 56: 735 (1976)

However, a visible in vitro demonstration using ^{51}Chromium assay with spleen cells from immunized and normal animals together with ^{51}Cr - tumor

cells failed to show any killing of the tumor cells. In vivo methods, using the Winn assay (38), so much utilized by others, failed also to demonstrate resistance of tumor take over normal spleen cells. Using sublethally irradiated recipients (450R) such as used in experiments by Rollinghoff et.al. (15) to successfully indicate the presence of tumor immunity, failed also to show any significant protection from tumor growth by these authors. Similarly disturbing results were achieved with peritoneal exudate cells from immune mice where again, no immunity was conferred. In this experiment, all previously obtained data concerning the ability of plasmacytomas to induce immunity seemed to have been defied. However, preimmunization with specific M component (myeloma tumor cell product) confirmed previous studies that immunity induced by this method is possible (48,56). However, the lack of cross-protection is not disturbing since the immunization is against only the antibody produced by that particular plasmacytoma, most likely against the idiotypic determinants of the Fab portion, and these antigenic determinants would be expected to be peculiar only to that particular myeloma protein. Cross-protection demonstrated by others (15,36) is easily understood since the immunizing agent was the entire tumor cell, therefore exposing a multitude of antigens to the immune system with some antigens being similar , as discussed earlier. A similar explanation for the negative results in vitro and in vivo methods indicating no tumor immunity can be applied, as these authors have (56). Therefore, the substantial conclusion to be made from this study is not that all other data accumulated in the past has been defied, but rather that immunization by M-component alone is not significant enough to manifest itself in vitro through a ^{51}Cr- release assay, or in vivo in Winn assay transfer experiments. However, clearly, immunity has developed and is demonstrable through low dose tumor challenge with the result that no tumor appears. Other studies have indicated that immunity is not against idiotypic cell surface determinants, as demonstrated by the inability of plasmacytoma protein products to block or enhance the in vitro stimulation of lymphoid cells in a lymphocyte-tumor interaction assay (51). However, the results of Freedman et.al. (56) above, clearly show that idiotypic determinants are important, but may not be too easily demonstrable. Another study using the plasmacytoma TEPC-15 bore out Freedman's conclusions, since

in this study, a transient antibody response to the combining region of the myeloma protein produced by the tumor was observed. In fact, the antibody production lent a protective effect upon viable tumor challenge and could even be transferred by the serum of the immunized animal (74).

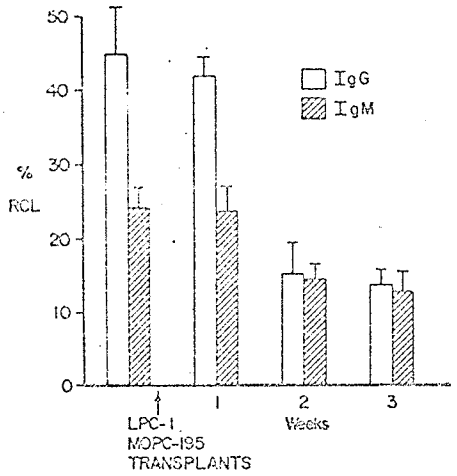
This now completes the accumulative evidence in favor of humoral and cellular responses of the immune system. It must be clear to the reader that only the predjudiced or the naive would deny the intimate association of the immune system with the presence (or absence) of plasmacytomas. The evidence presented is complete to the date of this writing, and all authors cited have demonstrated some immune reactivity in the presence of a plasmacytoma. However, the degree of reactivity, of course, varies widely and can, if nothing else, be attributed to the different methods used and the inherent sensitivity of each. In a very brief summary, it can be said that the exact cell type responsible for immunity is unknown, although evidence from various sources indicate that many cells are involved. This is in keeping with modern immunological theories. However, the blatant need for T-cells is apparent and it is possible that other cells simply help in manifesting an immune response which can be ablated by destroying the T-cells by selective agents. However, the undeniable presence of serum antibody in immune (and normal) mice also implicate B-cells. Macrophages have also been implicated as being able to transfer immunity. One author even shows that neutrophils can be important. The complexity of cellular interactions by these results becomes very evident. The pilgrimage to the understanding of these interactions constitute the ultimate goal in immunology.

E. Effect of PC-RNA on Normal Lymphocytes

One effort to elucidate cell interactions during the precipitation of an immune response has been extensively carried out by Bhoopalam and Yakulis and associates over the past five years. Their initial interest was captured in 1972 when they found that through the use of anti-idiotypic antisera (72), and hemagglutination following implantation with plasmacytomas, the percentage of the normal receptor-carrying lymphocytes (RCL) was decreased. As the tumors progressed in growth, the proportion of lymphocytes having receptors with the structural properties of the specific plasmacytoma globulin gradually increased, shown by anti-

idiotypic antisera to the plasmacytoma globulin. These changes in the type of immunoglobulin receptor on normal lymphocytes were found to be dependant on the tumor presence and were not found to be caused by non-specific absorption of the immunoglobulin to the normal lymphocytes as demonstrated through studies physically separating tumor from lymphocytes via millipore chambers or conversion of the cell to a plasmacytoma cell, as judged by acceptable criteria. The results are clearly shown in the histograms shown below excerpted from their studies.

Figure III-2



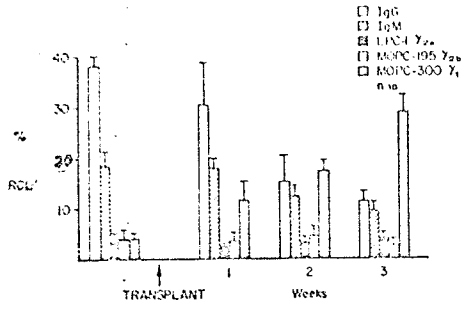
Diminution of IgG and IgM receptor-carrying lymphocytes (RCL) of BALB/c mice after tumor transplant.

Taken from

Yakulis, V., et.al., Blood 39: 453 (1972)

The specificity of this increase in PC-lg is shown very nicely in the following figure.

Figure III-3



Receptor-carrying lymphocytes of BALB/c mice during MOPC-300 development. Note absence of reaction with antiserum to LPC-1 γ_{2a} and MOPC-195 γ_{2b}. Ig of MOPC-300 belongs to the γ_1 class of mouse immunoglobulins.

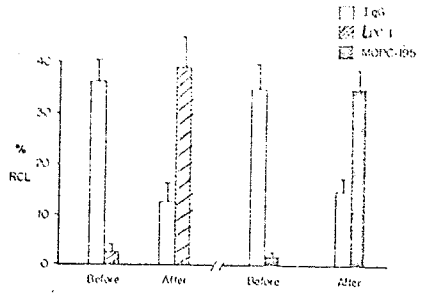
Taken from

Yakulis, V., et.al., Blood 39: 453 (1972)

The significance of this phenomenon is not clear, but it may be one reason why patients with myelomas usually have impaired antibody production following any type of antigenic stimulation (64,65).

Shortly after this discovery, it was found that RNA extracted from plasmacytomas was capable in every way to duplicate results in vivo and in vitro obtained with whole plasmacytoma implantation. In other words, surface immunoglobulin characteristics of normal lymphocytes were changed to the characteristics of the specific plasmacytoma globulin. This phenomenon was found to be RNA dose dependent and transient to some extent (57). The results indicating this "conversion" is shown below.

Figure III-4



Receptor-carrying lymphocytes in vitro after addition of LPC-1 or MOPC-195 plasmacytoma RNA to normal BALB/c lymphocytes.

Taken from

Bhoopalam, N., et.al., Blood 39: 465 (1972)

In a further study, in an effort to rationalize this phenomenon in terms of a biological implication, Yakulis et.al., found that, not only does PC-RNA alter characteristics of the surface immunoglobulin on normal lymphocytes, but that this PC-RNA also impairs the normal antibody response to sheep red blood cells as measured by agglutination titres and plaque forming cells (59). However, the secondary response, if PC-RNA was injected prior, was uneffected. Similarly normal RNA or RNAase-treated PC-RNA had no effect.

These results further corroberate the contention that the impairment

of normal immune responses to antigenic challenges in plasmacytoma-bearing mice and patients with myeloma is related to the conversion of surface immunoglobulin of normal B-cells into idiotypic ones with immunochemical characteristics of the immunologically restricted corresponding plasmacytoma.

Further studies have indicated that contaminating DNA and protein as a result of the RNA preparation method of hot phenol extraction do not play a role in this "cell conversion" phenomenon. Fractionation with RNAase and sucrose density centrifugation indicated that activity was found only in the 12S to 23S fractions, anything less being inactive, and that intact RNA was best (63). Further studies of Katzmann et.al. (62) have shown that the RNA fraction had activity in both the 14-18S region and in the 40-50S region, this high sedimentation velocity value probably due to an aggregation of RNA molecules, possibly with viral RNA, as reported by others (66). Also, these RNAs were found in the plasma of plasmacytoma-bearing mice, with equal cell-converting ability and having the same electrophoretic mobility as the tumor cell isolated fraction. When preparing the subcellular fraction from tumor cells, it was noted that in the active fraction before subjected to sucrose gradient centrifugation, intracisternal type-A particles as well as polysomes and mitochondrial and cellular membranes were identified by electron microscopy (62).

These findings suggest that the RNA in question produced by plasmacytoma cells is released into the serum and incorporated into the normal lymphocytes to yield the specific immunoglobulin on their surface shortly after. This immunoglobulin then impairs normal immunological recognition upon antigen challenges. The persistence of this effect over the length of experimental observation of six to eight weeks suggests the intracellular stabilization of the RNA, its replication and prolonged existence manifested by converted immunoglobulin.

Bhoopalam et.al. (60) have confirmed these hypothesis in later studies. They have found that when spleen cells from mice killed one and twenty four hours after injection of PC-RNA were cultured, the amount of new surface immunoglobulin on the lymphocytes increased with culture time. It, however, depended on when the spleens were taken from the animals because at seventy two hours, maximal conversion had already

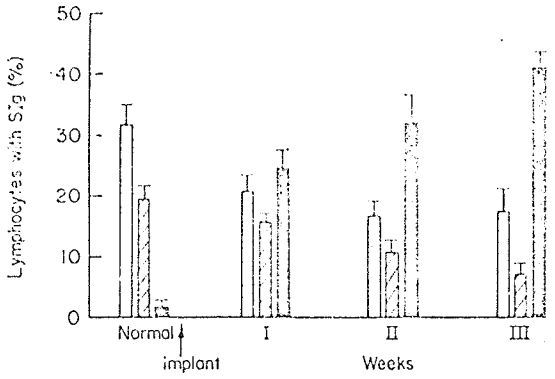
occurred in vivo and no increase of new SIg was noted in culture. The RNA extracted from spleen cells 1 hour after injection of PC-RNA was found to be 75 - 100 times less efficient in converting activity than RNA from spleen cells maintained in culture for seven days.

The converting activity of RNA from PC-RNA injected spleen cells could be transferred into other mice, although the effect disappeared after three or four transfers if the spleen was taken 1 hour after RNA injection. If this splenic RNA was cultured for seven days, the transfer capacity to convert normal immunoglobulin to plasmacytoma-idiotypic immunoglobulin was increased to at least five.

These results avail themselves to the possibility that the original active RNA replicated within the recipient cells, but more studies are required before definite conclusions can be made. The question of whether the PC-RNA has a role in intercellular transfer of imformation certainly comes to mind and is certainly strongly implicated in the above studies.

The most recent work of Bhoopalam and co-workers have confirmed the converting power of PC-RNA with the MOPC 104E plasmacytoma. This plasmacytoma has known natural activity against ∝1,3 dextran, and the RNA of this plasmacytoma confers upon the recipient of an injection the ability of the spleen cells to bind ∝1,3 dextran. This is shown in the histogram below.

Figure III-5



Change in the immunochemical characteristics of surface immunoglobulin of peripheral lymphocytes in weekly intervals after the implantation of MOPC 104E (n:6). Open columns, normal IgG; hatched columns, normal IgM; solid columns, 104E IgM (Id).

Taken from

Bhoopalam, N., et.al., Clin. Exp. Immun. 24: 357 (1976)

Also, confirming many other studies of various groups (59,67,68,69,70, 71), response of these converted lymphocytes to other antigens was lowered significantly. The immune response of mice preinjected with LPC-1 RNA, another PC-RNA, to dextran was significantly depressed relative to controls again stressing the immunological specificity induced by the RNA. As well, the transient nature of this converting phenomenon as expressed by antibody titres against dextran again became apparent, as in other studies (58). Possibly this could be explained by the elimination of these converted cells (through an immune response to a potentially hazardous invader) or by the replacement of the newly synthesized immunoglobulin by the normal immunoglobulin, through normal endogenous RNA processes (58,60,61). These studies by this group, and confirming studies of other groups, indicate several obvious facts in the RNA story. Listed below are the key issues:

1. There is a change in surface immunochemical characteristics on normal lymphocytes from circulatory or peripheral sources when the animal harbors a plasmacytoma.
2. RNA extracted from the plasmacytoma cells have been found to exert this effect, through yet an unknown manner.
3. Relatively intact RNA is required by normal lymphocytes for maintaining peak responsiveness, activity being limited to the 12S to 23S and 40 to 50S fractions.
4. Intracisternal type-A particles plus polysomes and cellular debris are contained in the active homogenate.
5. RNA extracted from the spleens of PC-RNA injected mice had ability to transfer the cell conversion phenomenon suggesting replication of the RNA.
6. Reduced immunoresponsiveness to challenge by other antigens pervaded all the studies.

The outlined facts lend themselves of course, to many interpretations but it must be kept in mind that the work concerning RNA is highly controversial. Since the bulk of this work is carried out by a very limited number of laboratories, one should await further confirmation on this subject from more independant groups of investigators. The reader is left to make his own judgement. However, the conclusion the author makes is simple and brief: The etiological agent, presumably

a virus, incorporates itself into the cell genome and thereby takes over the normal functions of the intracellular machinery, resulting in the production of "its own requirements or persuasions" and, as a result, impairs the natural ability of the normal immune armament to respond efficiently and effectively. This apparent impairment of immunological responsiveness to subsequent antigenic challenges is described and discussed in more detail in the following section.

F. Immune Response Suppression to Other Antigens in Plasmacytoma-Bearing Mice

The previous section has already made reference to the suppressive effect of plasmacytomas to the natural response to antigenic challenge. Not only has Yakulis' and Bhoopalam's work provided convincing evidence for suppression (58,61), but other groups, as well, corroborate their efforts in an attempt to explain why immune responses are frequently impaired in various malignancies. In some cases, both humoral and cellular immune responses are suppressed as with some transplantable tumors induced by several viruses and chemical carcinogens, as in the case of myeloma patients and plasmacytoma-bearing mice. However, the secondary response is much less affected, at least at the cellular level as indicated by DTH (delayed-type hypersensitivity) reactions to common antigens (64). The degree of immunosuppression is proportional to the size of the tumor borne by the hosts and cannot be related to such factors as nutritional competition of neoplastic and normal cells, feedback inhibition of the immune response by the neoplastic cells or the replacement of normal, competent cells in the lymphnodes or spleen with plasmacytoma cells. The vogue contention that a "factor" is involved in suppressing a normal response, such as that observed by Yakulis et.al. (58), was further studied by means of a millipore chamber filled with normal spleen cells and sheep red blood cells (SRBC) followed by implantation into the peritoneal cavity of plasmacytomatous mice. If a factor was indeed present in plasmacytoma-bearing mice which reduced immunological effectiveness, then the plaque assay of Jerne to follow will show reduced plaque forming cells (PFC) with respect to controls. The results indicate that only 20-35% of the PFC response is possible with implantation into plasmacytoma-bearing mice with respect to control, normal mice.

It is interesting to find that no difference is noted in PU-5 lymphoblastic lymphoma derived from thy-1 negative lymphocytes and RLσ^{7}1 thymic lymphoma, derived from thy-1 positive lymphocytes. A solid, non-lymphoid tumor S91 melanoma also had no effect.

These results, of course, do not indicate that this factor is produced by the tumor cells, such as is found in other studies implying RNA (58), or is produced by the normal cells in, or outside of, the chamber.

Other studies have found reduced responsiveness to SRBC challenge when injected purposely with Friend Leukemia virus (FLV), and plasmacytoma (MOPC 173) (or a mastocytoma). This suppression could also be manifested using cell free extracts of spleen cells, the serum fraction or ascites fraction of plasmacytomas, mastocytoma and FLV-bearing mice (68). Later studies revealed that immune responses to SRBC, BSA and peroxidase was also reduced in plasmacytoma-bearing C3H mice (69).

In an effort to determine the cellular basis of this widely reported immunosuppression, experiments aimed in this direction concluded that both the primary and secondary responses to SRBC were depressed, and that a primary response to a T-independent antigen was also depressed. This indicates that B-cells are at least one of the adversely affected populations in tumor-bearing hosts. However, the B-cell precursors of antibody-forming cells, the so called antigen binding cells, are found to be in normal quantities and seem to be able to bind the antigen. This study also shows the relationship between antigen dose and the suppressive effect of antigen challenge on the primary response: relatively high antigen doses ($>4x10^{7}$ SRBC) abrogated any suppressive effect. The study also correlated tumor size with the suppressive effect of antigen challenge on the primary response: the larger the tumor, the the higher degree of suppressed response to the antigen (69).

Further very recent studies have indicated that, although antibody-mediated immunity is impaired (59,61,67) for both thymus dependent and independent antigens (67), cell-mediated immune functions in terms of T-cell activity seem to remain intact (71). This was demonstrated through an *in vivo* method by using contact sensitivity to Dinitrofluorobenzene (DNFB) and ear swelling as a measure of reactivity and an *in vitro* method by using phytohemagglutinin, allogeneic cells and dinitrobenzene sulphonate as a measure of reactivity. (71).

It must be kept in mind that the immunosuppression discussed above is not common to all malignant conditions as indicated in the text, but is, however very impressive in the case of some plasmacytomas. The exact method of immunosuppression of immune responses upon antigenic challenge is unknown. It seems quite obvious from the foregoing discussion that a soluble mediator is involved and the previous section placed much emphasis on tumor-RNA as the incumbent suspect. Immunologists are not yet in a position to categorically conclude on these contentions.

G. Human Myelomas - An Overview

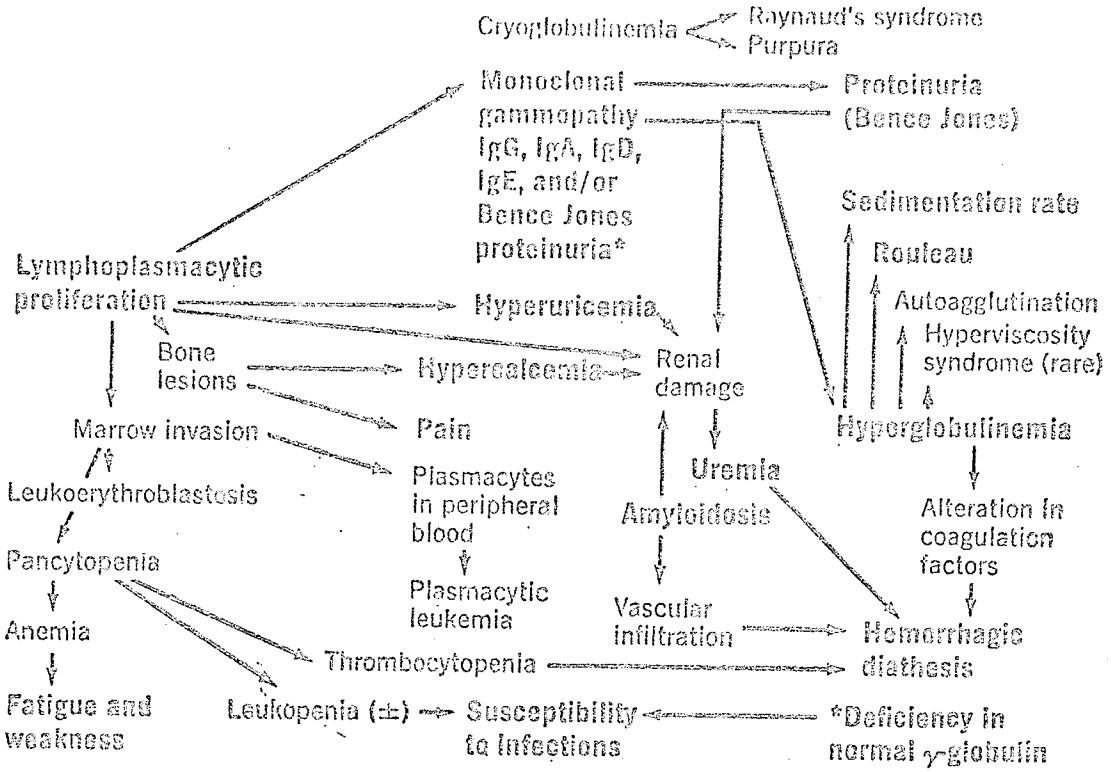
1. Introduction

Multiple myeloma is a malignant disease of plasma cells that typically involves the bone marrow but often involves other tissue as well. A myeloma may be regarded as a neoplastic proliferation of a single line of plasma cells. Many of them produce monoclonal immunoglobulins as if under constant antigenic stimulation. This protein is comprised of two heavy chains(depending on the class of protein produced)and two light chains(kappa or lambda) and is often referred to as an M or myeloma protein (or component). Some M-components were shown to have antibody activity (150). Other myelomas produce heavy chains only, or light chains only. Nonproducing tumors also exist. Multiple myeloma accounts for about 1% of all types of malignant disease and slightly more than 10% of hematologic malignancies (142).

A review of 869 cases of multiple myeloma revealed that 98% of patients were forty years of age or older and that the most common symptom of this disease is bone pain (68% of patients) as a result of osteolytic lesions most often localized in the lumbar region. Renal insufficiency was also very common. The peripheral blood often shows cytopenias (leukopenia, anemia and/or thrombocytopenia) as a result of marrow infiltration with the myeloma cell, an atypical, immature plasmacyte. Because of abnormally high protein production by these cells, proteinurea (Bence-Jones proteinurea) and abnormal electrophoretic patterns with characteristic "spikes" are common. About 60% of cases of multiple myeloma are of the IgG type and 20% are of the IgA type.

About 2% of the cases are IgD type and only 3 cases of IgE type have been reported. The remainder lack clear cut protein abnormalities (143). The pathophysiological manifestations of multiple myeloma (also referred to as plasmacytic myeloma) is shown below.

Figure III-6



Pathophysiologic manifestations of plasmacytic (or multiple) myeloma.

Taken from

Maldonato et.al., Postgrad. Med., 54: 139 (1973)

The above diagram indicates the complexity of multiple myelomas and the manifold ramifications possible, depending on the severity of the disease.

2. Immune Capability of Patients with Multiple Myeloma

Patients with diseases involving the lymphoreticular system may have a decreased resistance to infectious agents (145). It has been shown that patients with multiple myeloma have a decreased capacity to produce circulating antibody and frequently have a low serum gamma globulin concentration, the result being an increased susceptibility to bacterial infection (146). However, the delayed-type hypersensitivity skin response to several commonly encountered microbial antigens is normal or only slightly despressed. Cone and Uhr studied various parameters in 14 multiple myeloma patients and found that the primary anti- ϕX174 bacteriophage antibody response and the primary delayed hypersensitivity response to dinitro-1-fluorobenzene (DNFB) was normal in nine of eleven cases. However, delayed skin hypersensitivity could not be consistently shown with the commonly encountered microbial antigens (candida albicans, trichophyton, histoplasmin, PPD, coccidioidin, mumps, diptheria toxoid, and streptokinase-streptodornase), although each patient could respond to at least 1 of these antigens. Furthermore, eleven out of fourteen patients showed the ability to mount a secondary anti-diptheria toxoid response (145). Similarly, Fahey and his associates have found that antibody response to commonly encountered antigens (typhoid vaccine, influenza vaccine, mumps vaccine and diptheria vaccine) was below normal and added that infections were more frequent and normal gamma globulin was lower compared to control groups (147).

The above description of the immune capacities of various patients indicate that in myeloma, immune reactivity is generally at a low level, and hence complications arise with infection.

3. In Vitro Studies of Plasma Cell Myeloma Lymphocytes

Mavligit and his associates studied the reactivity of autochthonous lymphocytes against their tumor cells in ten myeloma patients by the blastogenesis method, which relies on the incorporation of ^{3}H-thymidine into the lymphocytes (148). Three patients showed positive reactions to their own myeloma cells and all of these three patients were immunocompetent as judged from the positive delayed hypersensitivity skin reaction to DNCB.

In two patients, the blastogenic response to myeloma cells occurred only with extensively washed lymphocytes resuspended in a pool of normal human serum. Resuspension into the patients own serum resulted in a complete abrogation of the effect similar to those of unwashed lymphocytes (148). This effect seemed to be tumor specific since no such effect was noted in the response of lymphocytes to phytohemagglutin. Six patients were totally unreactive to their own myeloma cells. These results demonstrate the presence of myeloma-associated antigen(s) and the in vitro cellular immune response directed against it in some cases (148). This antigen is either a distinct antigen on the surface of malignant plasma cells or perhaps related to the abnormal immunoglobulin produced by this tumor. This antigen may be similar to the transplantation antigen of mouse plasmacytomas. This study also suggests that a serum factor in some patients binds to circulating lymphocytes and renders them specifically incapable of reacting against autologous myeloma cells. Upon removal of this serum factor through extensive washing, they become reactive to myeloma cells. The nature of this factor is unknown but it was speculated that it is the myeloma protein product which blocks in vitro reactivity of lymphocytes and which also accounts for the in vivo antigenicity observed in the three patients with known reactivity (148).

The peripheral blood lymphocytes were studied in patients with multiple myeloma in vitro by immunofluorescence and differences were found when compared to the normal. Significant lowering of the percentage of B-cells having normal surface immunoglobulin (10.3% $\pm$ 6.9%) was found in the myeloma group. Normal B-cell values were 22.9% $\pm$ 7.1% of total lymphocytes (149). Also, myeloma patients in remission tended to have normal proportions of B-cells (149).

In summary, human myelomas resemble very generally mouse plasmacytomas. However, at a comparative level, much less has been done in human myeloma research except for the clinically-orientated work which is preponderant. This is quite logical since, animal models should be investigated first and then the information is extrapolated for further evaluation and application in the clinical field. There are many facets of myelomas which have to be studied in animal models for a better understanding of the human disease. The investigations described in this

thesis contribute to our knowledge regarding the immunobiology of plasmacytomas.

CHAPTER IV

IV. MATERIALS AND METHODS

Animals: Young adult (6-8 weeks old) Balb/C mice of both sexes were purchased from Jackson Laboratories (Bar Harbor, Maine, USA) and were used throughout the experiments. In transfer experiments, the donors and recipients were always the same sex. AKR and C3H/HeJ mice were obtained from Jackson Labs, also, and used in anti-thy 1 production.

New Zealand White rabbits were obtained from local breeders.

Tumors: Four plasmacytoma tumors were studied. They included MOPC104E, and IgM, K-L chain secreting plasmacytoma; J606, an IgG_3, λ -L chain secreting plasmacytoma; HOPC-1, an IgG_{2a}, K-L chain secreting plasmacytoma and S104, an IgA, λ-L chain secreting plasmacytoma. All were intitially induced through mineral oil injections at Jackson Labs, Maine, and were kindly supplied by Dr. B.G. Carter of this department.

Media: All culture and suspension media such as Eagle's minimal essential medium (MEM), trypsin, MEM Spinner modified, and RPMI 1640 were purchased in powder form from Difco Laboratories, (Detroit, Michigan, USA). N-2-hydroxyethylpiperozine N-2-ethanesulphonic acid (HEPES) for the addition to MEM (20mM) was supplied by Fisher Chemical co., N.J.

Fetal Calf Serum: Fetal calf serum (FCS) was supplied by Grand Island Biological Co., Grand Island, New York, USA.

Complete Freunds Adjuvant (CFA): CFA with 1mg/ml Mycobacterium butyricum was supplied by Difco Labs, and used in immunization of rabbits.

Antisera: (a)AKR anti-C3H antiserum (anti-thy-1/theta) was produced according to the method of Reif and Allen (129). Briefly, 6 intraperitoneal injections of 10^7 C3H thymocytes in phosphate-buffered saline were given to AKR mice at weekly intervals. One week following the last injection, the mice were bled from the tail and the serum titrated. The titre, as determined by the ^{51}Cr-release assay was found to be high on thymocytes (50% kill at 1:1000 dilution) but within expected range on spleen cells (50% kill at 1:5 dilution).

(b) Rabbit anti-mouse brain (anti-T cell antiserum) was purchased from Cedarlane laboratories in London, Ontario and used in a 1:64 dilution.

(c) Rabbit anti-mouse gamma globulin antiserum was produced in NZW rabbits. 1mg of mouse gamma globulin was mixed with CFA and injected in a total volume of 1ml into the four footpads of the rabbits. The rabbits were challenged with 1 mg of mouse gamma globulin in Incomplete Freunds Adjuvant subcutaneously (s.c.) twice, two weeks apart. The antiserum reacted strongly upon immunoelectrophoresis with mouse gamma globulin.

BCG: Bacillus Calmette-Guerin was obtained from Connaught Labs, in Toronto and administered in 0.1 ml quantity together with the appropriate cell suspension.

X-irradiation: For the irradiation of cell suspensions, 3000 R was administered by a Cobalt 60 apparatus. Animals were irradiated from the same source and received a total body dose of 850 R. One day after irradiation, the mice received 10^7 bone marrow cells i.v., through a tail vein.

Complement: For cytotoxic tests, all complement was from normal guinea pigs and was absorbed with agarose (Fisher Chemical Co.) (80mg/3ml of a 1:3 dilution of serum) before use. The absorbed 1:3 diluted serum was further diluted 2X immediately before use.

Preparation of Tumor Cell Suspensions:

All plasmacytomas, which were all subcutaneously grown, until 1.5 - 2.0 cm in diameter, were aseptically removed from the mice and placed into plastic petri dishes. The necrotic tissue was separated from the viable tissue with forceps and scalpel. The non-necrotic tissue was then finely minced with the aid of two sharp scalpels until sizes of the minced chunks were approximately 2mm in diameter. This minced tumor tissue was then placed into an Erlenmeyer flask with a magnetic bar and approximately 12-15 ml of either Spinner-modified MEM or simply phosphate buffered saline (consisting of 0.01 M phosphate buffer and 0.15 M NaCl, pH 7.2) containing 0.25% trypsin was added. Trypsinization was allowed to proceed for 30-45 minutes at room temperature, after which the supernatant was removed, HEPES-MEM added,

centrifuged (1000 rpm, 5-7 min.) twice for washing the suspension. All Procedures were carried out under sterile conditions.

The use of trypsin as a means to prepare the tumor cells may have some effect on the vitality of the tumor cells, at least temporarily. Since trypsin is a proteolytic enzyme acting at the cell surface, some damage may occur to the surface tumor antigens. However, it is thought that these surface antigens regenerate relatively quickly (12-24 hr.) and so the effect on the experiment of the trypsinized tumor cells should be minimal (153).

Preparation of Lymphoid Cell Suspensions: In all studies, spleens from normal and tumor-bearing mice were used. The spleens were aseptically removed and placed into a sterile plastic petri dish. They were then cut into 4-5 pieces with scissors or scalpel and placed into a cell-suspender, with 3-5 ml of medium. The cell-suspender was simply a glass barrel tissue homogenizer with the teflon plunger reduced in diameter to where it was loosely fitting into the glass homogenizer. The resultant suspension was filtered through a platinum-coated screen into sterile plastic tubes and this suspension was washed twice by centrifugation (1000 rpm for 5 min). All cell suspensions were kept at 4 ^{o}C or on a ice bath for the entire preparation period, usually between 2-5 hours. All lymphoid cell suspensions were treated with 0.17 M NH_4Cl for 10 min. at 4^{o}C (to remove RBC's) (151).

Determination of Cell Number and Viability: Trypan blue was used as an indicator for cell viability. Usually, 0.1 ml of cell suspension was added to 1.9 ml of PBS. This was thoroughly mixed and then 0.1 ml of this suspension was added to 0.1 ml of 1% Trypan blue. An appropriate amount of this suspension was placed under a cover glass on a hemocytometer and counted. Cells which stained blue were considered dead. Those cells which excluded the dye were considered viable. Viabilities of tumor cells prepared by trypsinization were greater than 95% and of spleen cells prepared by this tissue homogenizer were 80-95%.

Nylon Wool Separation of Lymphoid Cells: The method of Julius et.al. (130) has been largely adopted for our separation method using nylon wool columns with some minor changes. Briefly, the nylon wool (3gm/30ml syringe column) was boiled for 30 min. in 0.2N HCl. It was washed with distilled water immediately after boiling and then soaked for a period

of 5 days in triple distilled water at 37°C, with a water change daily. The nylon wool was then dried at 37°C for 3 days. The nylon wool (3gm) was then loaded into the barrel of a 30cc syringe and autoclaved. Before use, the column was flushed with RPMI1640 with 10% FCS and then incubated at 37°C for 1 hour to remove air pockets. After incubation, the nylon wool was flushed with copious amounts of RPMI1640-FCS. The lymphoid cells were then loaded onto the column in a total of 5ml at a concentration of 1-2 x 10^8 cells/ml. The suspension was slowly allowed to penetrate the nylon wool after which the cells were incubated for 45 min. at 37°C. After incubation, about 5ml of RPMI1640-10% FCS was added and allowed to penetrate into the nylon wool, followed by a further 15 min. incubation. This procedure was repeated two times. The column was then ready for elution: 50ml. of media (RPMI1640 with 10% FCS) was very slowly added to the column and allowed to drip into a sterile conical centrifuge tube over the period of about 30-40 minutes. This was considered the "T-Cell Fraction". The nylon wool was then compressed with the barrel plunger in order to force out any loosely attached cells. This was considered the "B-Cell Fraction" After this forced elution about 30 ml of media was added and the column was again subjected to pressure with the barrel plunger. After this procedure the total yield was 50ml of cell suspension. The tubes were then centrifuged and the cells washed twice in RPMI, resuspended in about 2 ml of RPMI1640 for counting and determination of viability. Each fraction i.e. unattached effluent cells and the "forced out" cells, was evaluated for T cell content either by anti-theta serum or by rabbit anti-mouse brain serum plus complement in the dye exclusion test. Killed cells (staining blue) were considered as T-cells, and the surviving cells were considered to be B-cells. After many experiments, it was found that the nylon wool columns yielded 65-75% T-cells in the effluent fraction, and that "forced out" cells were 80-85% B-cells (i.e. non T-cells). Cell yields were approximately 40-50% of the total number of cells loaded onto the column. These results were very reproducible.

However, it must be kept in mind that the "B-cell fraction" as judged by being resistant to lysis by anti-theta serum may contain not just B-cells but other cell types as well. This is shown by the fact that 15-20% of these cells were lysed by anti-theta serum. Also, other cells with no detectable thy-1 antigen or surface immunoglobulin

referred to as "null" cells may be part of the cells which are not sensitive to anti-theta serum (154). In fact, using immunofluorescence in our laboratory, it has been shown (personal communication with Mr. K.C. Wang) that 60% of the B-cell fraction stains positive for surface immunoglobulin and, in the T-cell fraction, 97% of the cells stain positive for the thy-1 antigen. Therefore, the B-cell fraction contains 60% immunoglobulin positive cells, 15-20% thy-1 positive cells and the remaining 20% comprising "null" cells perhaps as well as other cell types such as macrophages. The T-cell fraction, therefore, contains 65-75% thy-1 positive cells as judged by the anti-theta serum cytotoxicity test, as opposed to 97% thy-1 positive cells as judged by immunofluorescence. The remaining cells then comprise "null" cells perhaps as well as other cells such as macrophages for example but in any case the remaining cells comprise a group which are not detected by a anti-theta antiserum cytotoxicity test or by immunofluorescence. The discrepancy in quantitating T-cells with the two different methods may be based on the fact that immunofluorescence is a much more sensitive measure of the presence or absence of the thy-1 antigen. It is likely that much more antibody and thy-1 antigen is necessary to indicate the presence of thy-1 antigen using the cytotoxicity test. Immunofluorescence, on the other hand, requires less antibody and thy-1 antigen to visually represent the presence of the thy-1 antigen through fluorescence. It is for this reason that immunofluoresence shows that almost all the T-cells are indeed thy-1 positive whereas with the cytotoxic test using anti-theta serum only 65-75% of the cells are indicated as thy-1 positive cells.

Cr^{51} - Release Cytotoxicity Assay (152): This assay was used to quantitate the titre of anti-theta antiserum produced in AKR mice. Briefly, the target cells (thymocytes, bone marrow, spleen) ($2\text{-}5\times10^6$ cells) were prepared as already described and were incubated with 100μCi of $Na_2{}^{51}CrO_4$ (New England nuclear cat. no. NEZ030) for 45 minutes at 37°C. Following incubation, the Cr^{51} labelled suspension was thoroughly washed four times to remove residual Cr^{51}. A constant number of labelled target cells ($5\text{-}10\times10^5$ cells) in 0.1ml was added to a microtitre plate (Falcon Plastics, Cat. no. 3046) after which various dilutions of 0.1ml anti-theta antiserum was added. Finally, 0.1ml of appropriately prepared guinea pig complement (1:6 dilution) was added to each well. Suitable controls (0% and 100% lysis wells using complement alone

and 1N HCl alone with the cells, respectively and also normal sera) were also included in the microtitre plate. The plate was then incubated for 30-40 min at 37°C for cytolysis to take place. After incubation, the plate was centrifuged at 1000 rpm for 10 min. and 0.1ml of supernatant very carefully removed for gamma-irradiation counting. After counting, % lysis with respect to the 0% and 100% controls was calculated for each cell type at each anti-theta serum dilution according to the following formula:

$$\% \text{ specific lysis} = \frac{E-C}{T-C} \times 100,$$

WHERE: E=counts per minute (cpm) in the supernatant of the experimental samples; C=cpm in the supernatant of the control samples containing complement alone; T=total radioactivity released into the supernatant by addition of 0.1ml of 1N HCl.

Titres were expressed as a dilution which kills 50% of the target cells. The anti-theta antiserum used in these experiments had a titre of 1/1000 on thymocytes and a titre of 1/5 on normal splenocytes (T-cells taken to be 35% of the whole spleen cell population).

Trypan Blue Cytotoxicity Test: This test was used to determine the % of cells sensitive to anti-theta or to rabbit anti-mouse brain antisera after nylon wool column separation of spleen cells. Briefly, the lymphoid cell fractions were adjusted to a concentration of 5×10^6 cells/ml. 0.1ml of this suspension was removed and added to either 0.1ml of undiluted anti-theta serum or to 0.1ml of 1:64 diluted rabbit anti-mouse brain serum. This was followed by the addition of 0.1ml of appropriately prepared and diluted normal guinea pig serum as a source of complement. The treated cells were then incubated at 37°C for 45 min. after which the number of dead cells was calculated by the addition of 0.1ml Trypan blue to a suitably diluted aliquot of cell suspension. Results are then expressed as the % T-cells (i.e. % of cells killed by either anti-theta or rabbit anti-mouse brain antisera) or % B-cells, the remaining unstained, living cells.

Statistical Methods: All statistical analysis computed using the student's t-test for comparing two populations. Statistical significance is considered to be $p \leq 0.05$.

Normally, 3 points on the curves were taken and compared using the student's t-test. It was felt that it would be sufficient to generally select 3 points at times where statistical significance was most likely to occur as judged by observing the entire graph. It seemed reasonable to assume that analyzing any more than 3 points would not result in a substantial increase in accuracy in reporting the data.

Measurement of Tumors: The tumors were measured 2-3 times per week using calipers equipped with a Vernier scale. Two measurements of the diameter of the tumor were taken, one at right angles to the other and the two readings were then averaged. The measurements were made in centimeters and averaged to 1 decimal place accuracy. When more than one tumor was present, the same procedure was followed and the averaged tumor diameters of all tumors were added together to give a sum total measurement.

CHAPTER V

V. EXPERIMENTAL RESULTS:

A. MOPC104E Plasmacytoma

1. Estimation of Minimal Lethal Dose of MOPC104E

Prior to immunological studies, it is necessary to estimate the approximate minimal lethal dose i.e. the number of cells required per injection to initiate a tumor. The reason is to establish a consistent protocol and to approximately determine the fine line between tumor rejection and tumor growth. Large numbers of tumor cells over the minimum dose can easily overwhelm the immune system and no detectable difference in tumor appearance time or growth rate would probably be seen. For all experiments, the tumor cells were prepared by trypsinization as described earlier.

To determine the minimal lethal dose of tumor cells, groups of three mice each were given $1x10^5$, $2.5x10^5$, $5x10^5$, $7.5x10^5$, and $1x10^6$ MOPC104E cells, subcutaneously, in 1.0ml. CMEM. MOPC104E tumors, as all plasmacytomas investigated, grow subcutaneously as a solid mass (but can also be grown intraperitoneally as a solid mass or ascites). The tumor incidence was checked regularly and the results are shown below in Table V-1.

Table V-1. MOPC104E Minimum Dose Study

Group No.	1	2	3	4	5
Cell Dose	1×10^6	7.5×10^5	5×10^5	2.5×10^5	1×10^5
Number of Animals	3	3	3	3	3
% Tumor Incidence (after 45 days)	100	100	100	100	67

From the table, $5x10^5$ cells were chosen as the dose required to consistently induce tumors, and this dose was used throughout experiments involving MOPC104E.

2. Investigation of the Antigenicity of MOPC104E

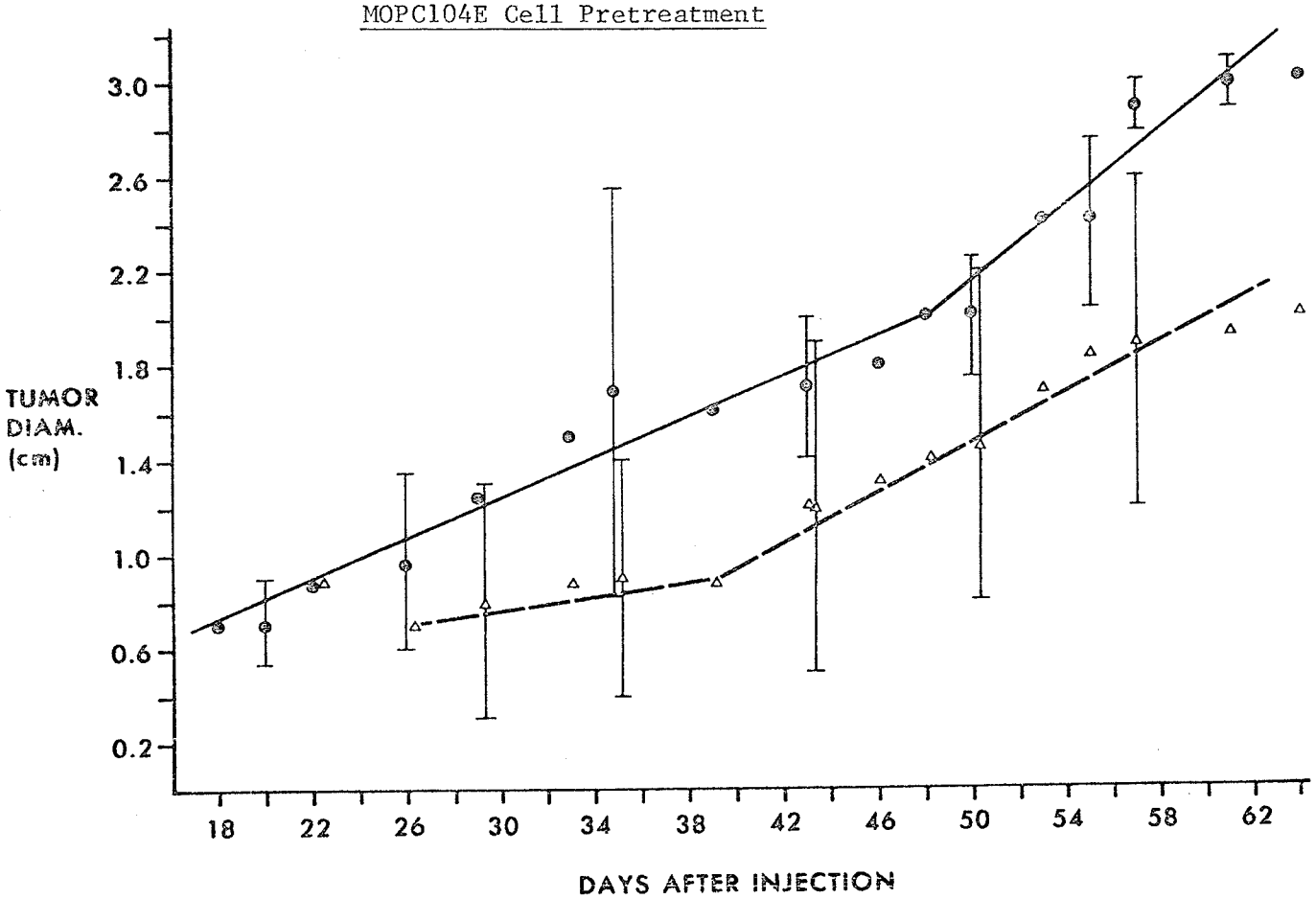
The antigenicity of plasmacytomas seems to be highly variable (39). Therefore, we undertook studies to determine the antigenicity of the four plasmacytomas investigated. Only one experiment was undertaken to indicate the antigenicity of MOPC104E since, from previous Winn Assays, protective effects were noticed, and hence antigenicity clearly represented. We attempted to substantiate these results by pretreating Balb/C mice with X-irradiated MOPC104E cells. Mice received s.c. $5x10^6$ MOPC104E cells, irradiated with 3000 rads, 21 days prior to the MOPC104E challenge. The results are indicated in Fig. V-1. Although no significant difference is noted in the tumor diameters between the treated and control groups, significant ($p=0.05$) lengthening of survival time in the treated group is shown. Furthermore, only 75% tumor incidence is noted in the experimentally treated group and one animal from this group grew the tumor and later, after 81 days, rejected it.

3. The Effect of Adoptive Transfer of Lymphocytes on MOPC104E

In all adoptive transfer studies, the Winn assay was employed (38). This assay simply involves mixing the tumor cells with the lymphocyte or other cell fraction and injecting the mixture subcutaneously. Initial Winn assays involving the mixture of tumor cells with spleen cells from MOPC104E-bearing mice were encouraging and, the results are shown in Figure V-2. Although no survival time data was possible, the growth rate of the tumors indicated the retarded growth by the group treated with spleen cells from tumor bearing donors. Further experiments indicated no significant difference in mice treated with either spleen cells from normal or MOPC104E-bearing mice. In fact, in terms of survival times, both groups died significantly earlier ($p=0.05$) with respect to the control group which received only MOPC104E. This is shown in Fig. V-3.

In experiments using separated T-cells and B-cells from the spleens of normal and tumor-bearing (MOPC104E) mice, we attempted to illustrate which cell type(s) was actually involved in conferring resistance and enhancement to tumor induction indicated in the previous experiments.

Fig. V-1. Induction of Immunity with MOPC104E using X-irradiated MOPC104E Cell Pretreatment



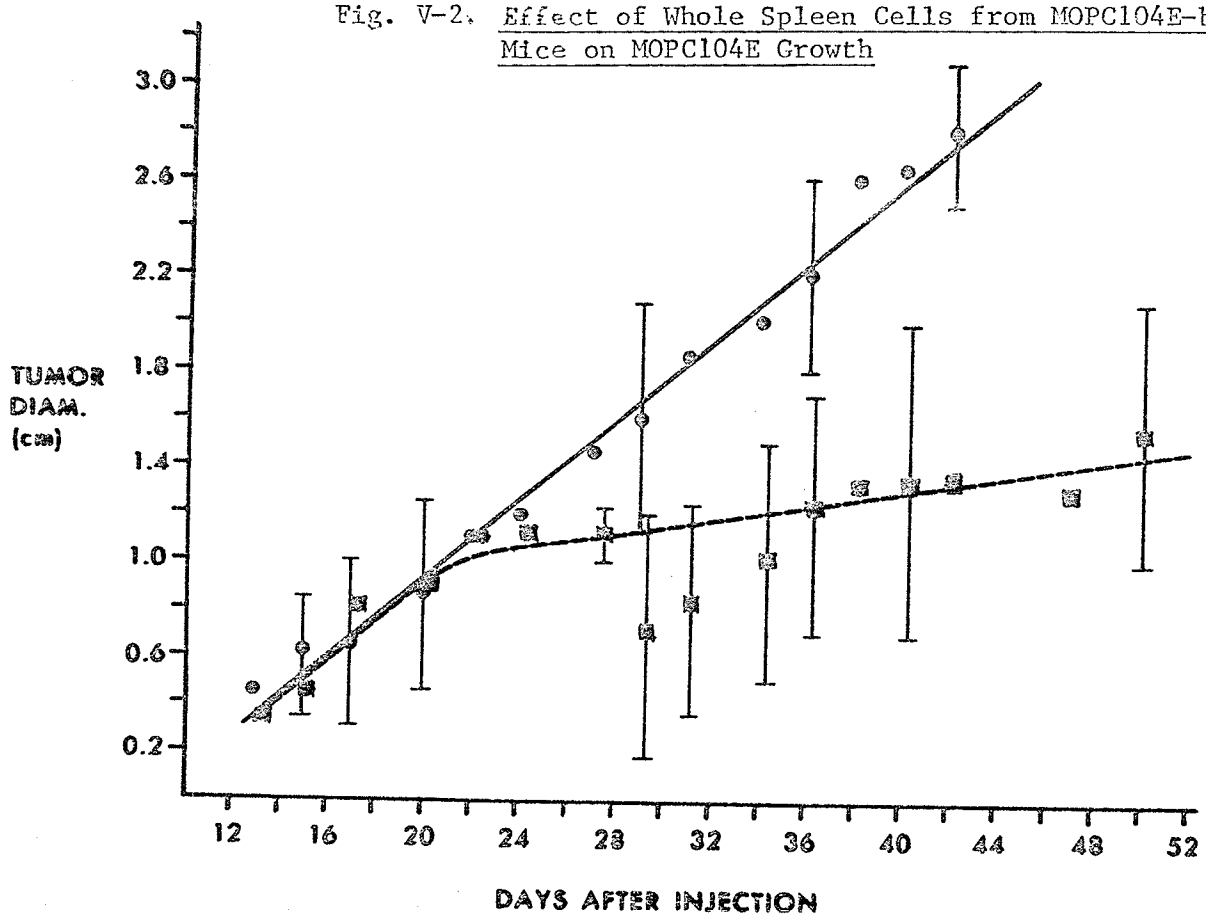
LEGEND

Group No.	1	2
Symbol in Graph	●——●——●	△——△——△
No. Animals	5	4
Treatment*	MOPC 104E only	MOPC 104E Pretreated with X-irrad. MOPC 104E 1.
Survival Time (days ±S.E.)	58±18	102±20 (p=0.05)
% Tumor Take	100	75

*All groups received 5×10^5 MOPC 104E cells S.C. in 1.0 ml.

1. This group received 5×10^6 MOPC 104E cells S.C. irradiated with 3000 Rads 21 days prior to MOPC 104E challenge.

Fig. V-2. Effect of Whole Spleen Cells from MOPC104E-bearing Mice on MOPC104E Growth



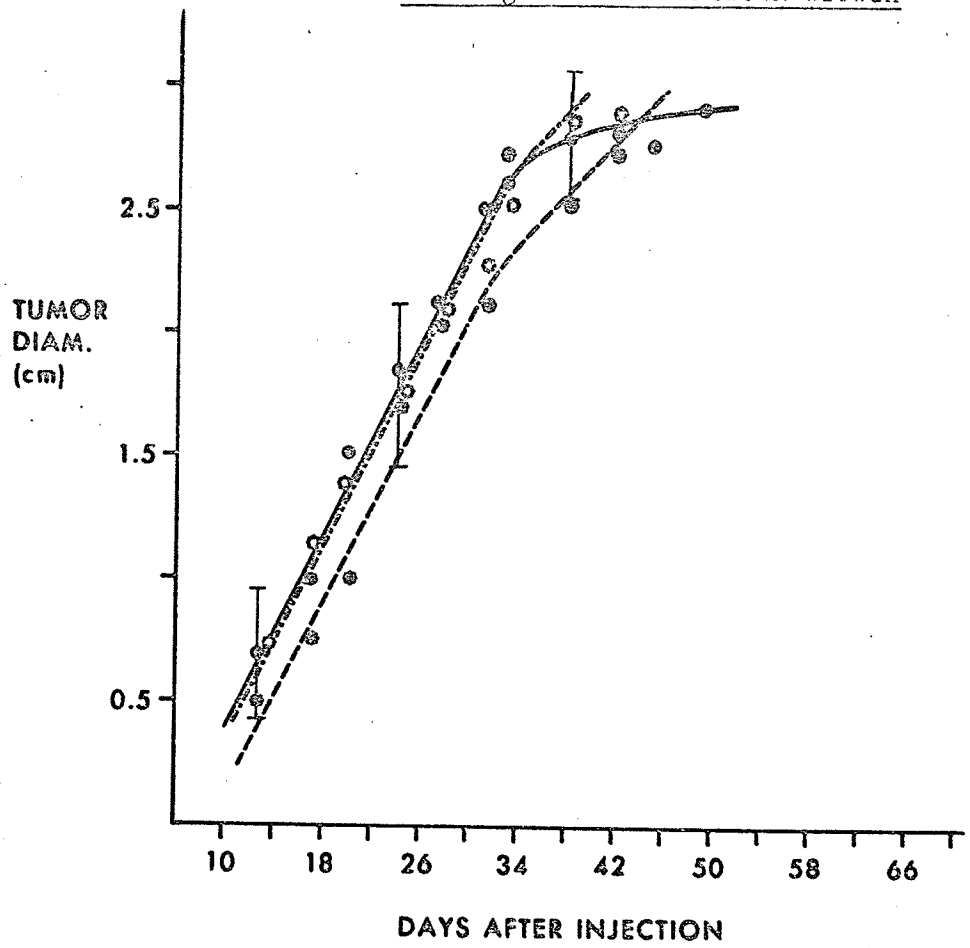
LEGEND

Group No.	1	2
Symbol in Graph	●——●——●	■- - -■- - -■
No. Animals	3	7
Treatment *	MOPC 104E only	MOPC 104E + spleen cells (10^7) i.
Survival Time (days + S.E.)	N.A.	N.A.
% Tumor Take	100	100

* All groups received 5×10^5 MOPC 104E cells S.C. in 1 ml. volume
In appropriate groups the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

1. Spleen cells were taken from MOPC 104E-bearing mice

Fig. V-3. Effect of Whole Spleen Cells from MOPC104E-bearing Mice on MOPC104E Growth



LEGEND

Group No.	1	2	3
Symbol in Graph	——o——	——o——	o---o---o
No. Animals	8	8	8
Treatment*	MOPC 104E only	MOPC 104E + Spleen Cells (10^7) 1.	MOPC 104E + Spleen Cells (10^7) 2.
Survival Time (days±S.E.)	52±4	38±12 (p=0.05)	38±6 (p=0.05)
% Tumor Take	100	100	100

*All groups received 5 x 10^5 MOPC 104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

1. Spleen cells were prepared from the spleens of MOPC 104E-bearing mice.
2. Spleen cells were prepared from the spleens of normal mice.

In initial experiments, it was found that the T-cell fraction of spleens from MOPC104E-bearing mice significantly ($p=0.05$) reduced the size of the tumors throughout the majority of the experiment ($p=0.05$ at days 23 and 30) whereas B-cells and whole spleen cells from the same animals had no effect. This is shown in Fig. V-4. In another experiment, T-cells also significantly reduced tumor diameters as well as whole spleen cells, B-cells and T-cells + B- cells together ($p \leq 0.05$ at day 36 and $p \leq 0.05$ at day 44). Also, in the same experiment, it was found that all treated animals except the group treated with B-cells lived significantly longer with respect to the control group. This is shown in Fig. V-5.

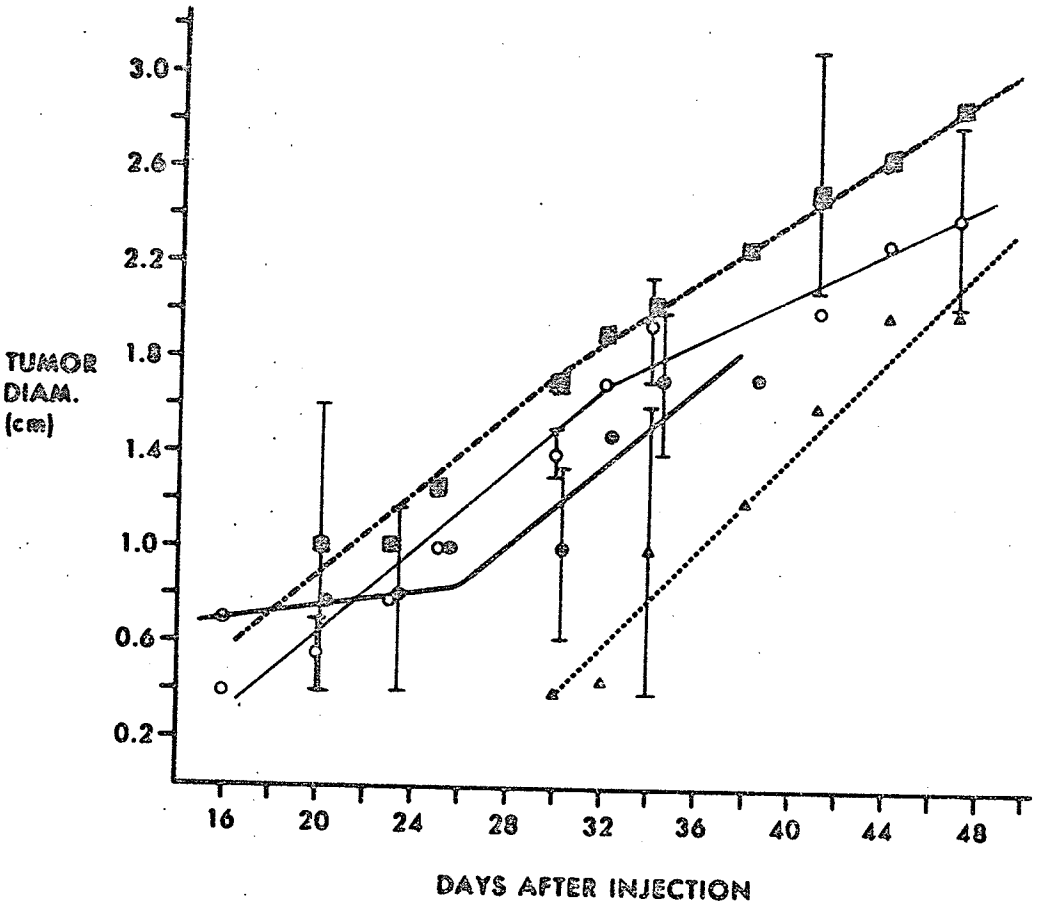
In experiments designed to show the involvement of normal spleen cell populations, T-cells were found to have no effect on survival time or growth of the tumor. In one experiment, however, normal whole spleen cells were found to actually increase tumor growth and normal B-cells found to inhibit tumor growth. These results are shown in Fig. V-6. In another experiment using normal spleen cell subpopulations, no effect was demonstrated in the T-cell or B-cell treated group either in terms of survival time or tumor growth. This is shown in Fig. V-7.

Experiments were also designed to see whether lethal X-irradiation of the host followed by bone-marrow reconstitution produced any effect on tumor growth by subpopulations of spleen cells of MOPC104E-bearing mice. The experiments of Rollinghoff and his associates (39) largely employed the use of sublethal (450 Rads) irradiation in all their experiments in an attempt to immunologically reduce all mice to the same level. We found, however, in our experiments no significant differences in terms of tumor growth or survival time with respect to the control group in this experiment in any group. The results are shown in Fig. V-8.

4. Effect of Thymocytes on MOPC104E Induction

Experiments were designed to investigate the contribution of thymocytes on tumor induction. Four groups of animals were used. One group received only MOPC104E cells and served as the control. The second group received, in addition to tumor cells, whole spleen cells from MOPC104E-bearing mice and the final group received, in addition to tumor cells, thymocytes and whole spleen cells from MOPC104E-bearing mice. Figure V-9 shows the results to indicate no significant difference between any of the four groups in terms of tumor growth and only the group treated with whole spleen cells survived significantly ($p=0.05$)

Fig. V-4. Effect of Fractionated Cells from MOPC104E-bearing Mice on MOPC104E Growth



LEGEND

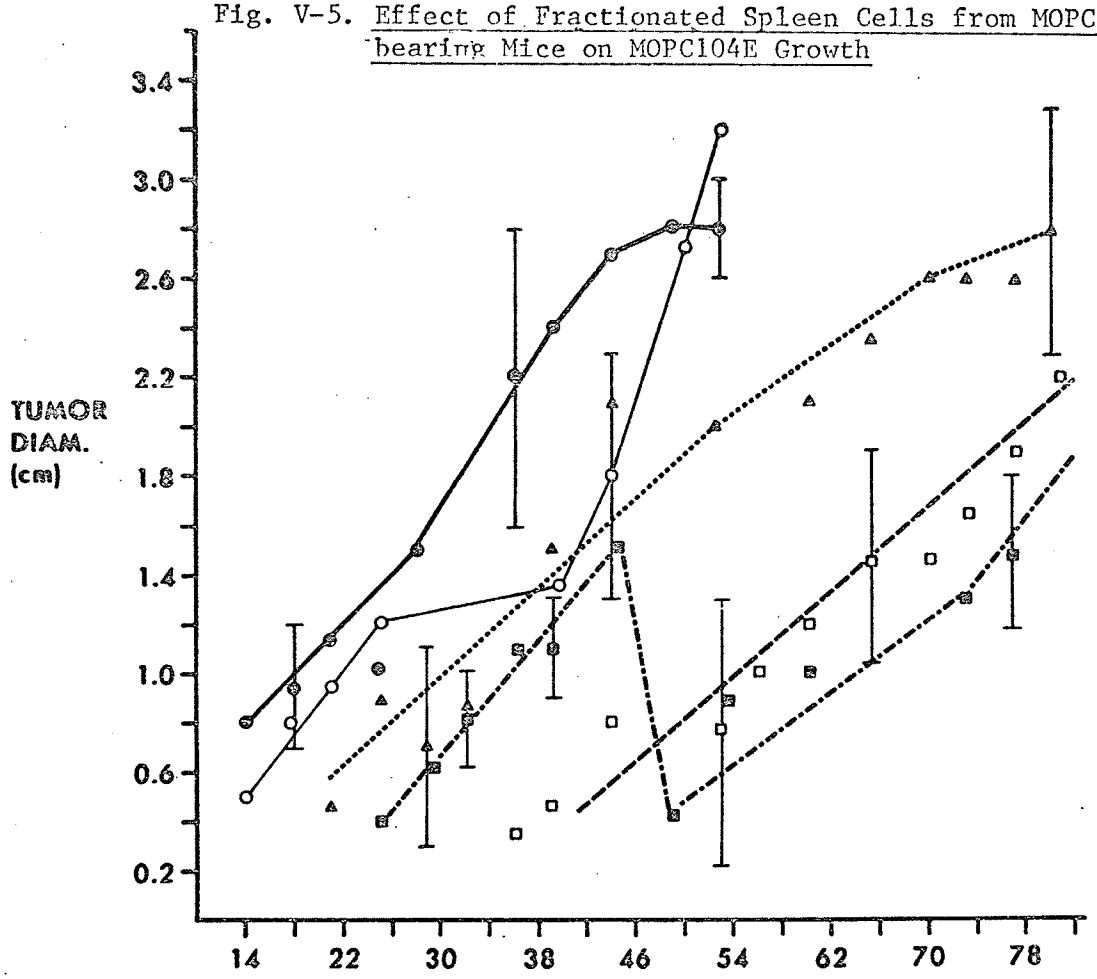
Group No.	1	2	3	4
Symbol in Graph	●——●——●	▲·····◇·····▲	○——○——○	■——■——■
No. Animals	5	5	5	5
Treatment *	MOPC104E only	MOPC104E + T-cells (10^7)	MOPC104E + B-cells (10^7)	MOPC104E + Whole Spleen (10^7)
Survival Time (days ± S.E.)	N.A.	N.A.	N.A.	N.A.
% Tumor Take	100	100	100	100

* All groups received 5×10^5 MOPC104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cell and B-cell fractions were prepared from spleens of MOPC104E-bearing mice.

Fig. V-5. Effect of Fractionated Spleen Cells from MOPC104E-bearing Mice on MOPC104E Growth



LEGEND

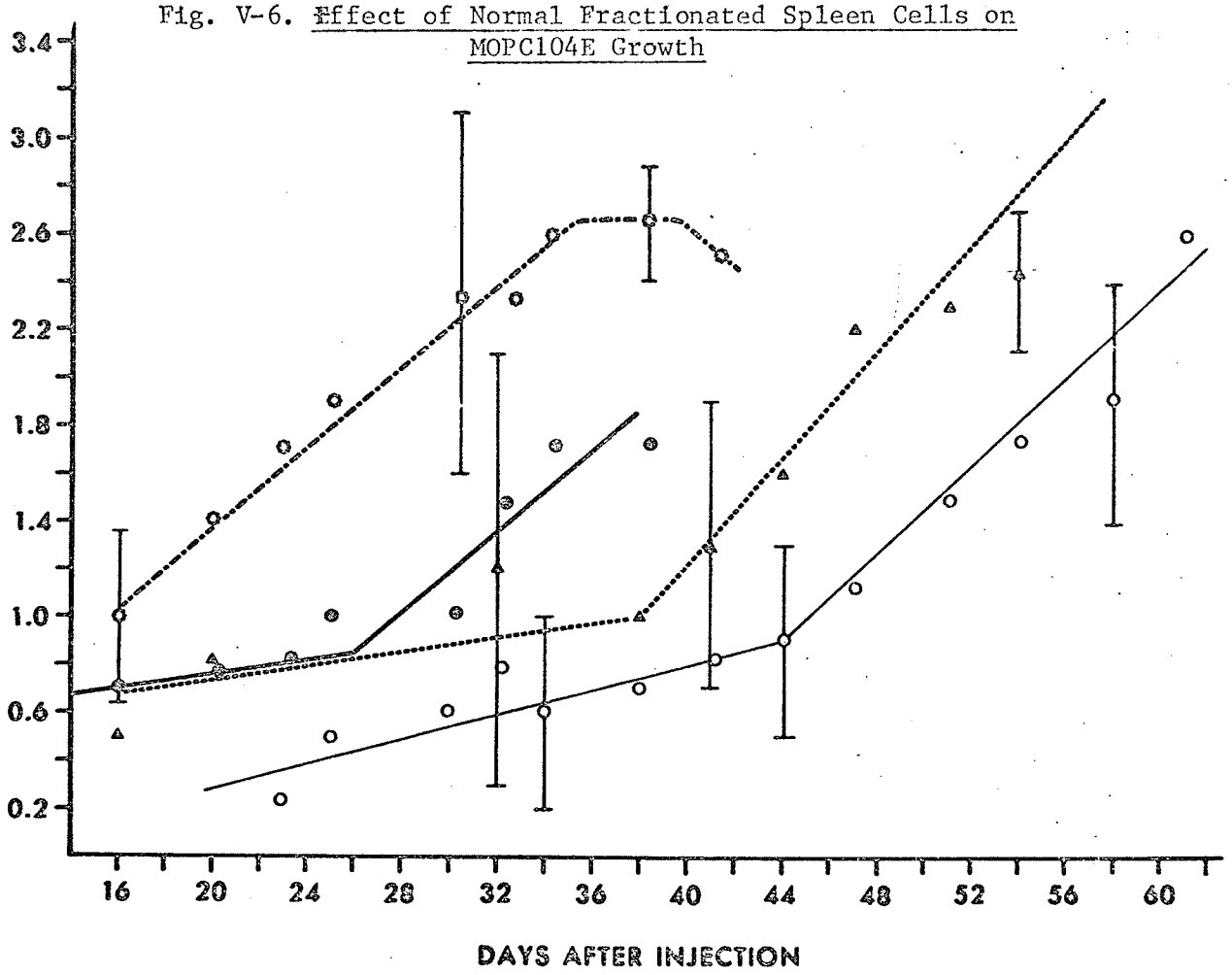
Group No.	1	2	3	4	5
Symbol in Graph					
No. Animals	5	5	5	5	5
Treatment *	MOPC 104E only	MOPC104E + T-cells (10^7)	MOPC104E + B-cells (10^7)	MOPC104E + T-cells (10^7) + B-cells (10^7)	MOPC104E + whole spleen cells (10^7)
Survival Time (days±S.E.)	46±8	78±20 (p=0.05)	51±10 (p=0.5)	87±12 (p=0.01)	76±22 (p=0.05)
% Tumor Take	100	100	100	100	100

* All groups received 5×10^5 MOPC104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection

All T cell, B-cell, and whole spleen fractions were prepared from spleens of MOPC104E-bearing mice.

Fig. V-6. Effect of Normal Fractionated Spleen Cells on MOPC104E Growth



LEGEND

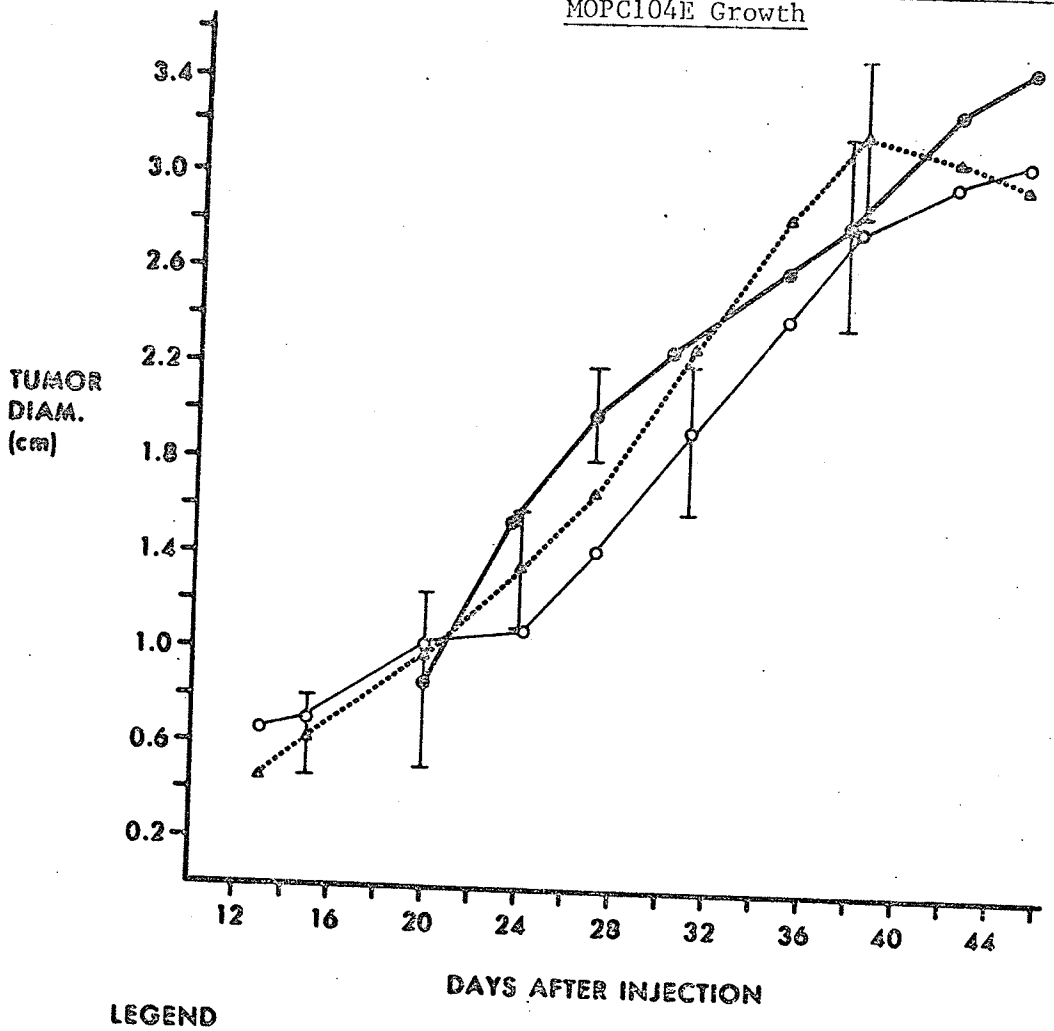
Group No.	1	2	3	4
Symbol in Graph	○—○—○	△·····△·····△	○—○—○	○-·-○-·-○
No. Animals	5	5	4	5
Treatment*	MOPC 104E only	MOPC 104E + T-cells (10^7)	MOPC 104E + B-cells (10^7)	MOPC 104E + whole spleen cells (10^7)
Survival Time (Days±S.E.)	N.A.	N.A.	N.A.	N.A.
% Tumor Take	100	100	100	100

*All groups received 5×10^5 MOPC 104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cell, B-cell and whole spleen fractions were prepared from the spleens of normal mice.

Fig. V-7. Effect of Normal Fractionated Spleen Cells on MOPC104E Growth



LEGEND

Group No.	1	2	3
Symbol in Graph	⊙——⊙	△·····△	○——○
No. Animals	4	5	5
Treatment *	MOPC104E only	MOPC104E + T-cells (10^7)	MOPC104E + B-cells (10^7)
Survival Time (days ± S.E.)	43±3	43±5 (p=0.5)	44±5 (p=0.5)
% Tumor Take	100	100	100

* All groups received $5x10^5$ MOPC104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cell and B-cell fractions were prepared from spleens of normal mice.

Fig. V-8. Effect of Fractionated Spleen Cells from MOPC104E-bearing Mice on MOPC104E Growth in X-irradiated, Bone Marrow Reconstituted Mice.

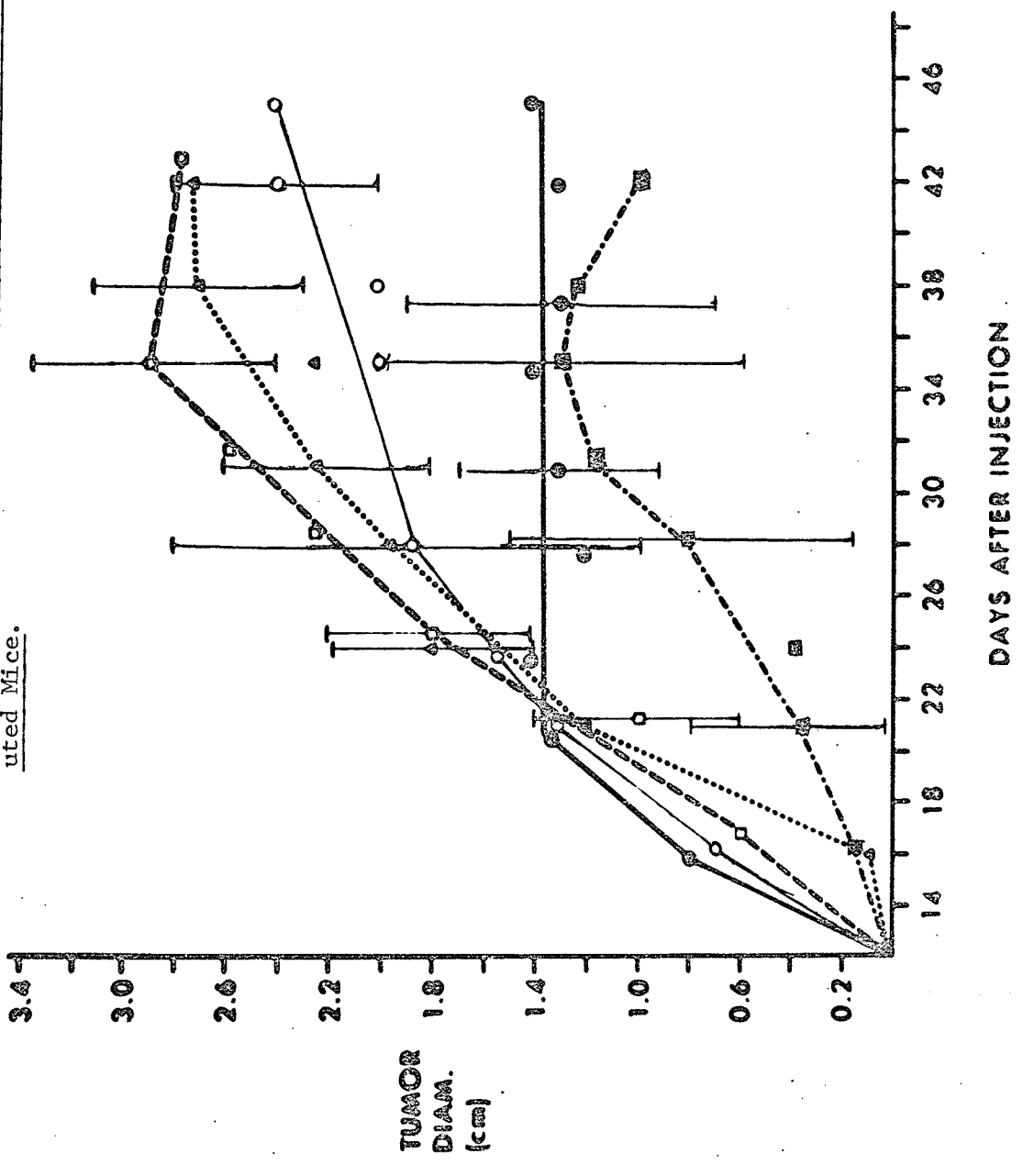
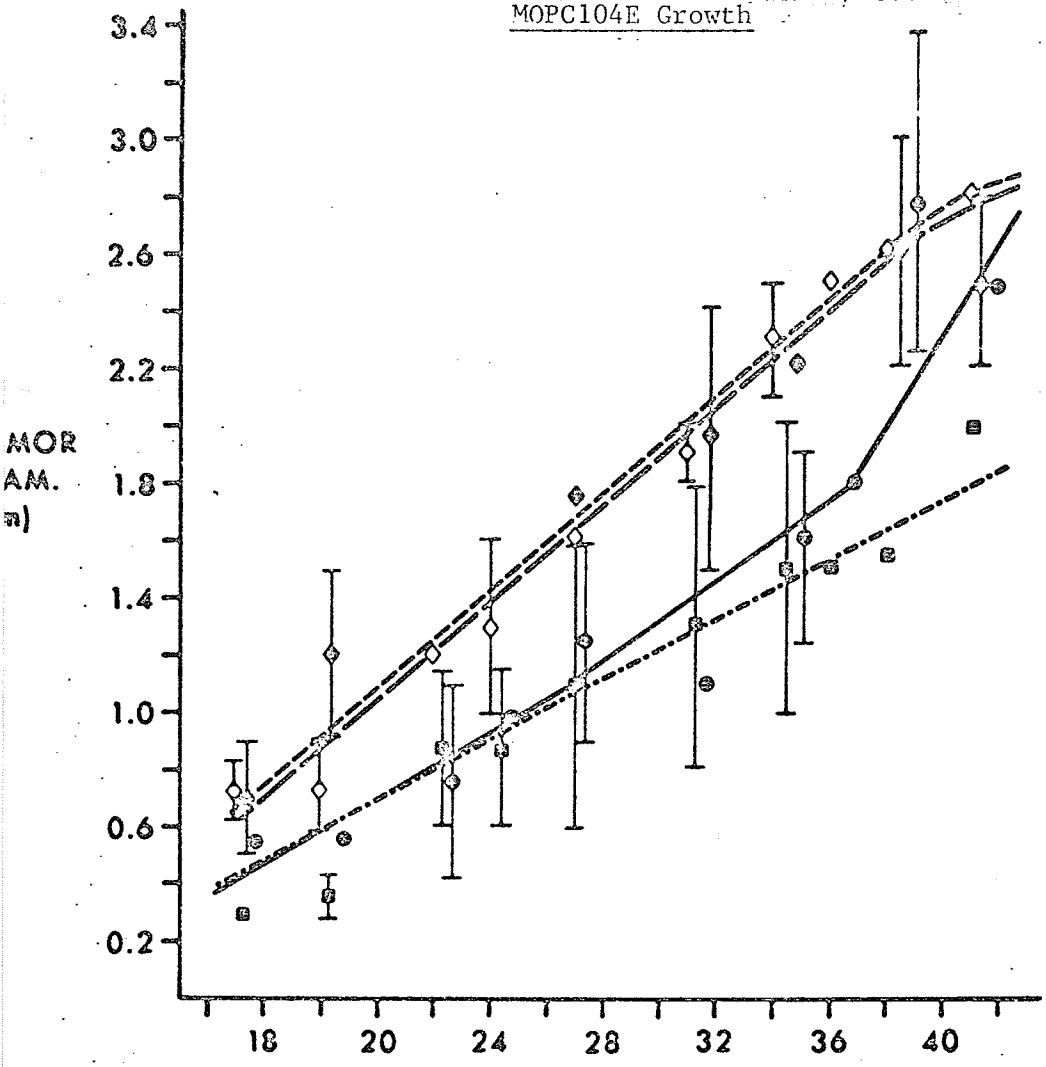


Fig. V-9. Effect of Thymocytes from MOPC104E-bearing Mice on MOPC104E Growth



LEGEND

Group No.	1	2	3	4
Symbol in Graph	—●—	—◇—	·●·●·	—◆—
No. Animals	6	4	5	5
Treatment*	MOPC 104E only	MOPC 104E + Thymocytes (10^7)	MOPC 104E + Spleen cells (10^7)	MOPC 104E + Thymocytes (10^7) + Spleen Cells (10^7)
Survival Time (days±S.E.)	38±5	38±8 (p=0.5)	48±8 (p=0.05)	53±17 (p=.2)
% Tumor Take	100	100	100	100

*All groups received 5×10^5 MOPC 104E cells S.C. in 1 ml volume.

In appropriate groups, the thymocyte fractions and/or spleen fractions were mixed with tumor cells prior to S.C. injection.

All spleen cells and thymocytes were taken from MOPC 104E-bearing mice.

longer, as in other experiments. At one point (day 45) there was a significant difference between the groups treated with whole spleen cells and the group treated with thymocytes and whole spleen cells together.

Another experiment was performed to investigate the effect of sub-populations of spleen cells (T-cells, B-cells) from MOPC104E-bearing mice together with either normal thymocytes or thymocytes from MOPC104E-bearing mice on tumor growth and survival. In looking at the T-cell involvement together with normal thymocytes and thymocytes from tumor-bearing animals (see Fig.V-10) no significant differences were found in terms of tumor growth or survival times with respect to the group treated with MOPC104E. However, it was found that there was a significant ($p=0.05$) difference at days 23 and 31 between groups treated with thymocytes from tumor-bearing animals and normal thymocytes. The group treated with thymocytes from tumor-bearing mice lived significantly ($p=0.05$) shorter (41 ± 8 days) lives and the tumor grew comparitively larger than the group treated with normal thymocytes, together with MOPC104E cells which lived 59 ± 9 days.

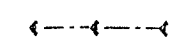
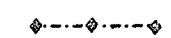
In looking at the B-cell involvement together with normal thymocytes and thymocytes from tumor-bearing animals (see Fig. V-11), no significant difference was found when compared to the group treated with just MOPC104E. However, a significant ($p=0.05$) difference was found at two points (days 31 and 41) between groups treated with thymocytes from tumor-bearing animals together with B-cells and groups treated with normal thymocytes together with B-cells. The group treated with thymocytes from tumor-bearing animals together with B-cells grew tumors significantly larger than the group treated with normal thymocytes together with B-cells. Furthermore, the group treated with thymocytes from tumor-bearing mice together with B-cells also grew tumors significantly larger at one point (day 23) than the group treated with just B-cells (together with MOPC104E). These results are compatible with those obtained in Fig. V-10.

B. J606 Plasmacytoma

1. Estimation of Minimal Lethal Dose of J606

For reasons already mentioned, it is necessary to establish the minimum dose of tumor cells required to consistently produce a tumor.

LEGEND TO FIGURE V-10

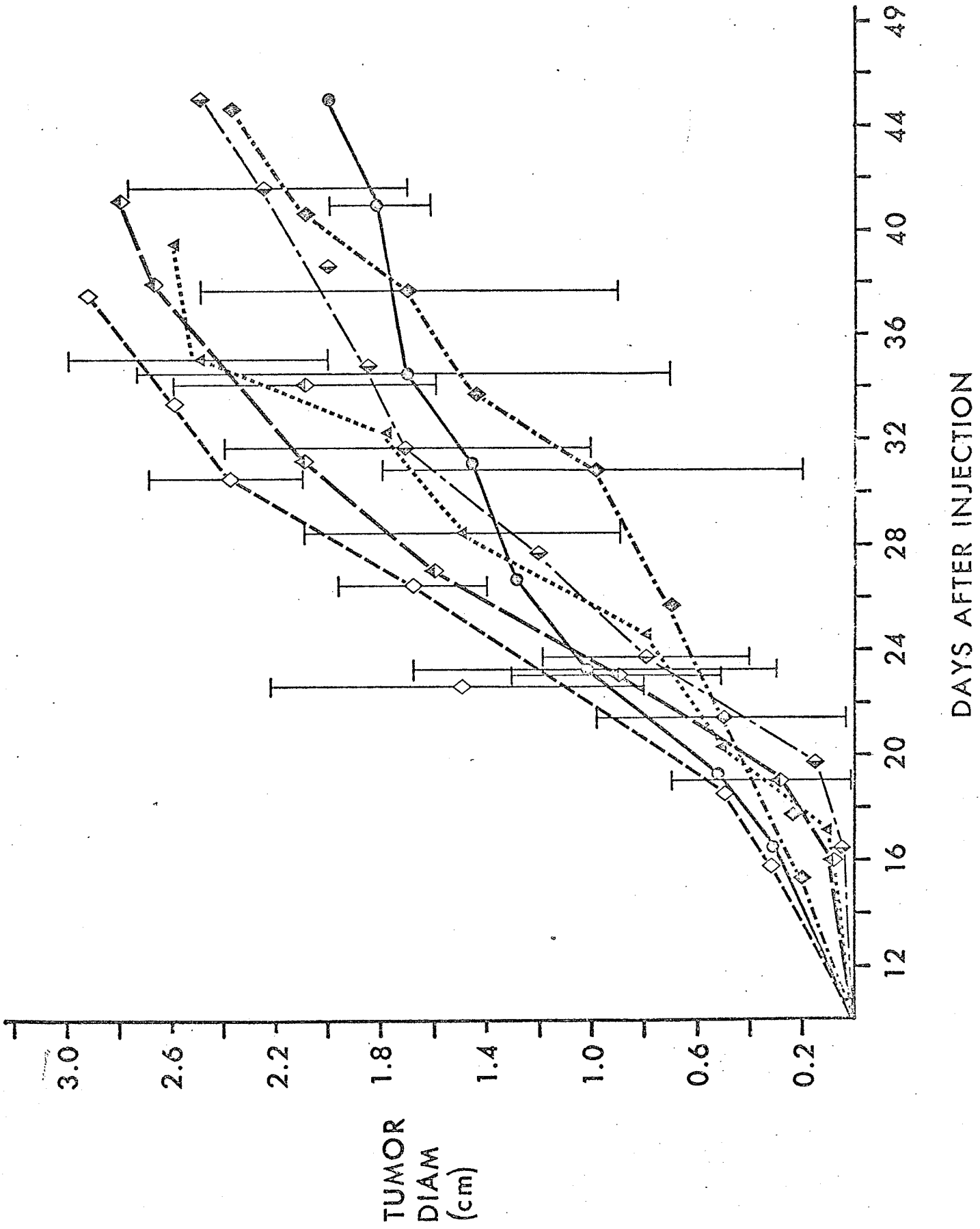
Group No.	1	2	3	4	5	6
Symbol in Graph						
No. Animals	4	4	5	5	5	5
Treatment *	MOPC 104E only	MOPC 104E + Thymocytes (tumor) + T-cells 1.	MOPC 104E + Thymocytes (tumor) 2.	MOPC 104E + Thymocytes (normal) + T-cells 3.	MOPC 104E + Thymocytes (normal) 4.	MOPC 104E + T-cells 5.
Survival Time (days±S.E.)	46±9	44±2 (p=0.5)	41±8 (p=0.5)	51±13 (p=0.5)	59±9 (p=0.2)	42±8 (p=0.5)
% Tumor Take	100	100	100	100	100	100

*All groups received 5 x 10^5 MOPC 104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions and/or thymocyte fractions were mixed with tumor cells prior to S.C. injection.

All lymphocytes originated from the spleen and thymocytes from the thymus.

1. This group received 1 x 10^7 thymocytes together with 1 x 10^7 T-cells, both from MOPC 104E -bearing animals, in addition to 5 x 10^5 MOPC 104E cells.
2. This group received 1 x 10^7 thymocytes from MOPC 104E-bearing animals in addition to 5 x 10^5 MOPC 104E cells.
3. This group received 1 x 10^7 thymocytes from normal animals together with 1 x 10^7 T-cells from MOPC 104E-bearing animals, in addition to 5 x 10^5 MOPC 104E cells.
4. This group received 1 x 10^7 thymocytes from normal animals in addition to 5 x 10^5 MOPC 104E cells.
5. This group received 1 x 10^7 T-cells from MOPC 104E-bearing animals in addition to 5 x 10^5 MOPC 104E cells.


TUMOR DIAM (cm)
3.0
2.6
2.2
1.8
1.4
1.0
0.6
0.2
12
16
20
24
28
32
36
40
44
49
DAYS AFTER INJECTION

LEGEND TO FIGURE V-11

Group No.	1	2	3	4	5	6
Symbol in Graph						
No. Animals	4	5	5	5	5	5
Treatment *	MOPC 104E only	MOPC 104E + Thymocytes (tumor) + B-cells 1.	MOPC 104E + Thymocytes (tumor) 2.	MOPC 104E + Thymocytes (normal) + B-cells 3.	MOPC 104E + Thymocytes (normal) 4.	MOPC 104E + B-cells 5.
Survival Time (days±S.E.)	46±9	37±10 (p=0.3)	41±8 (p=0.5)	59±21 (p=0.4)	59±9 (p=0.2)	50±7 (p=0.5)
% Tumor Take	100	100	100	100	100	100

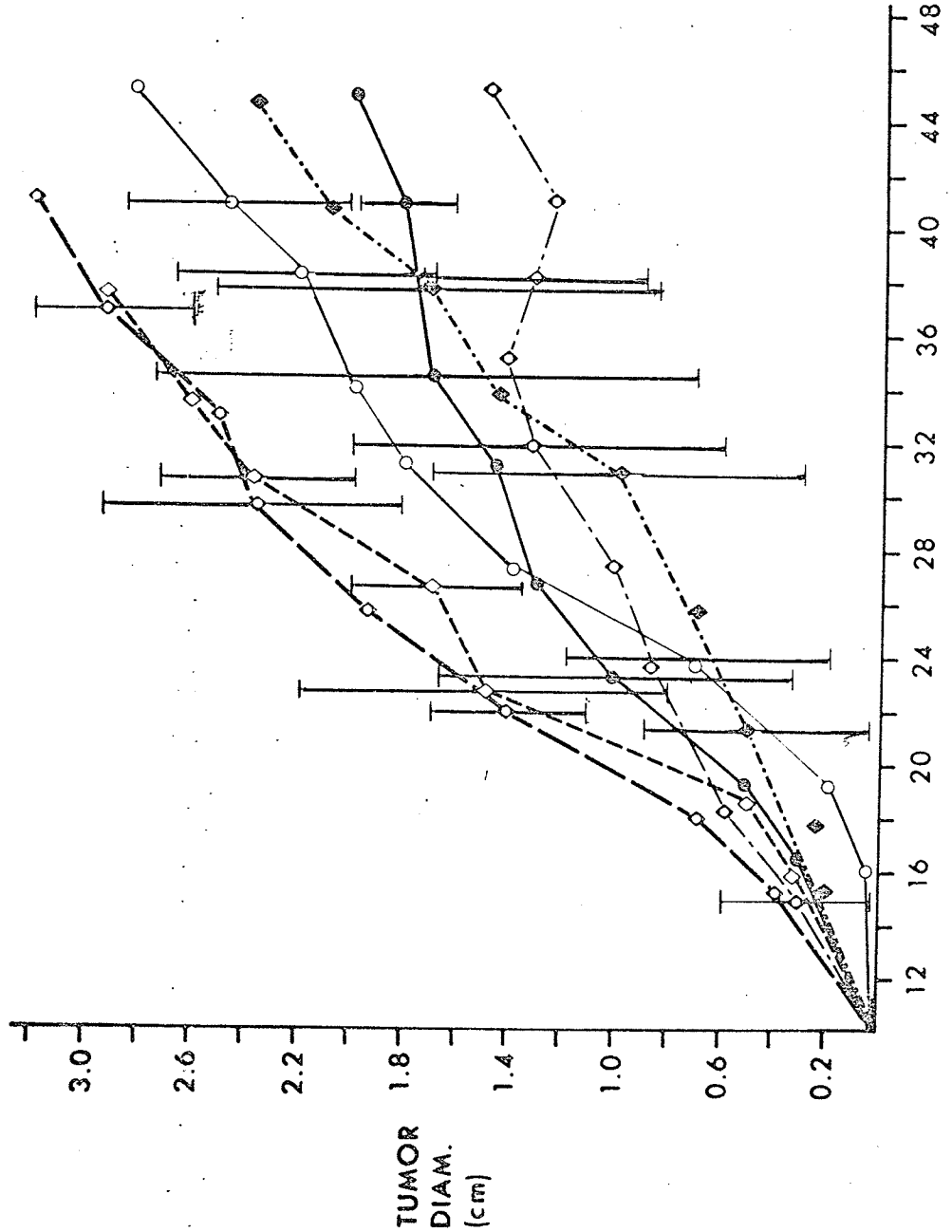
*All groups received 5 x 10^5 MOPC 104E cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions and/or thymocyte fractions were mixed with tumor cells prior to S.C. injection.

All lymphocytes originated from the spleen & thymocytes from the thymus.

1. This group received 1 x 10^7 thymocytes together with 1 x 10^7 B-cells, both from MOPC 104E-bearing animals, in addition to 5 x 10^5 MOPC 104E cells.
2. This group received 1 x 10^7 thymocytes from MOPC 104E-bearing animals in addition to 5 x 10^5 MOPC 104E cells.
3. This group received 1 x 10^7 thymocytes from normal animals together with 1 x 10^7 B-cells from MOPC 104E-bearing animals, in addition to 5 x 10^5 MOPC 104E cells.
4. This group received 1 x 10^7 thymocytes from normal animals, in addition to 5 x 10^5 MOPC 104E cells.
5. This group received 1 x 10^7 B-cells from MOPC 104E-bearing animals in addition to 5 x 10^5 MOPC 104E cells.

Fig. V-11. Effect of B-cells and Thymocytes on MOPC104E Growth



To accomplish this, four groups of three mice each received $1x10^6$, $1x10^5$, $1x10^4$ and $1x10^3$ J606 cells in 1.0ml CMEM, subcutaneously.

Tumor incidence was checked regularly and the results are shown below in Table V-2.

Table V-2. J606 Minimum Dose Study

Group No.	1	2	3	4
Cell Dose	1×10^6	1×10^5	1×10^4	1×10^3
Number of Animals	3	3	3	3
% Tumor Incidence (after 30 days)	100	33	0	0

From this table, $1x10^6$ J606 cells was chosen as the minimum lethal dose and this dose was used in all experiments involving J606 plasmacytomas.

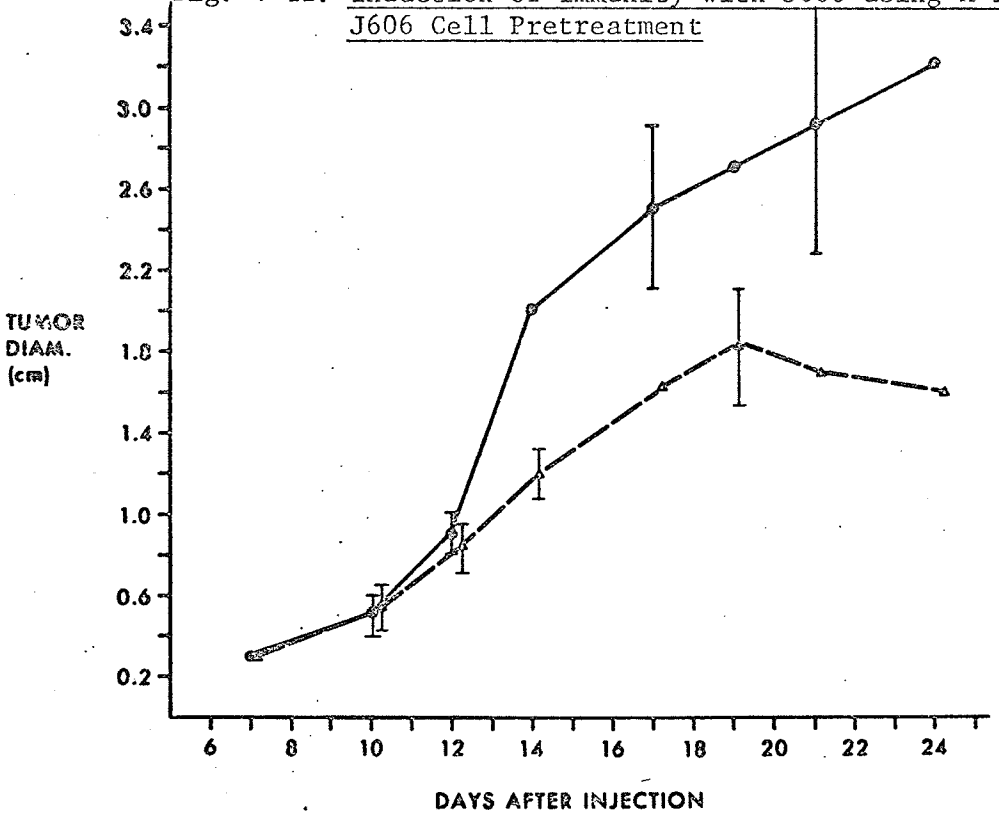
2. Investigation of the Antigenicity of J606

To investigate how antigenic J606 was, the following experiment was performed. One group of five animals was given $5x10^6$ J606 cells, s.c., irradiated with 3000 rads. After 21 days, these animals were challenged with $1x10^6$ viable J606 tumor cells and the tumor growth monitored with respect to a control group which received no pretreatment and only $1x10^6$ J606 tumor cells on the day of the challenge. The results are shown in Fig. V-12. At one point (day 18), a significant reduction ($p=0.05$) was noted in the growth of the tumor in the pretreated group with respect to the control group. At two other points (day 11 and day 15), no significant difference was found.

3. Effect of Adoptive Transfer of Lymphocytes on J606

In studies using separated spleen cells from tumor-bearing mice,

Fig. V-12. Induction of Immunity with J606 using X-irradiated J606 Cell Pretreatment



LEGEND

Group No.	1	2
Symbol in Graph	⊙——⊙——⊙	△- - -△- - -△
No. Animals	3	5
Treatment *	J606 Only	J606 pretreated with x-irrad J606 1.
Survival Time (days + S.E.)	N.A.	N.A.
% Tumor Take	100	100

* All groups received 1×10^6 J606 cells S.C. in 1 ml. volume.

1. This group received 5×10^6 J606 cells S.C. irradiated with 3000 Rads 21 days prior to J606 challenge.

T-cells, B-cells, T-cells together with B-cells as well as whole spleen cells had very little effect with respect to tumor growth when compared to the J606 control group as can be seen in Fig. V-13. Although there are no statistically significant differences, there may, realistically, be some tendencies. Using statistics, large standard errors are incurred in this experiment because of the variation in the appearance of the tumor. As a result, statistics, through the students t-test, dictates that there is no significant difference. With this in mind, figure V-13, may indicate that T-cells actually enhance tumor growth and that whole spleen cells actually suppress tumor growth. However, the group treated with whole spleen cells from tumor-bearing mice developed tumors in only 80% (4/5) of the group.

In experiments using normal spleens for separation into T and B-cell components, similar results were obtained, as seen in Fig. V-14. However, it was found that the group treated with B-cells and J606 cells lived significantly ($p=0.05$) shorter lives (31 ± 2 days) when compared to the control group which received only J606 cells and survived 42 ± 6 days.

C. HOPC-1 Plasmacytoma

1. Estimation of Minimal Lethal Dose of HOPC-1

For reasons already mentioned, it was necessary to establish the minimum lethal dose of tumor cells required to consistently produce a tumor.

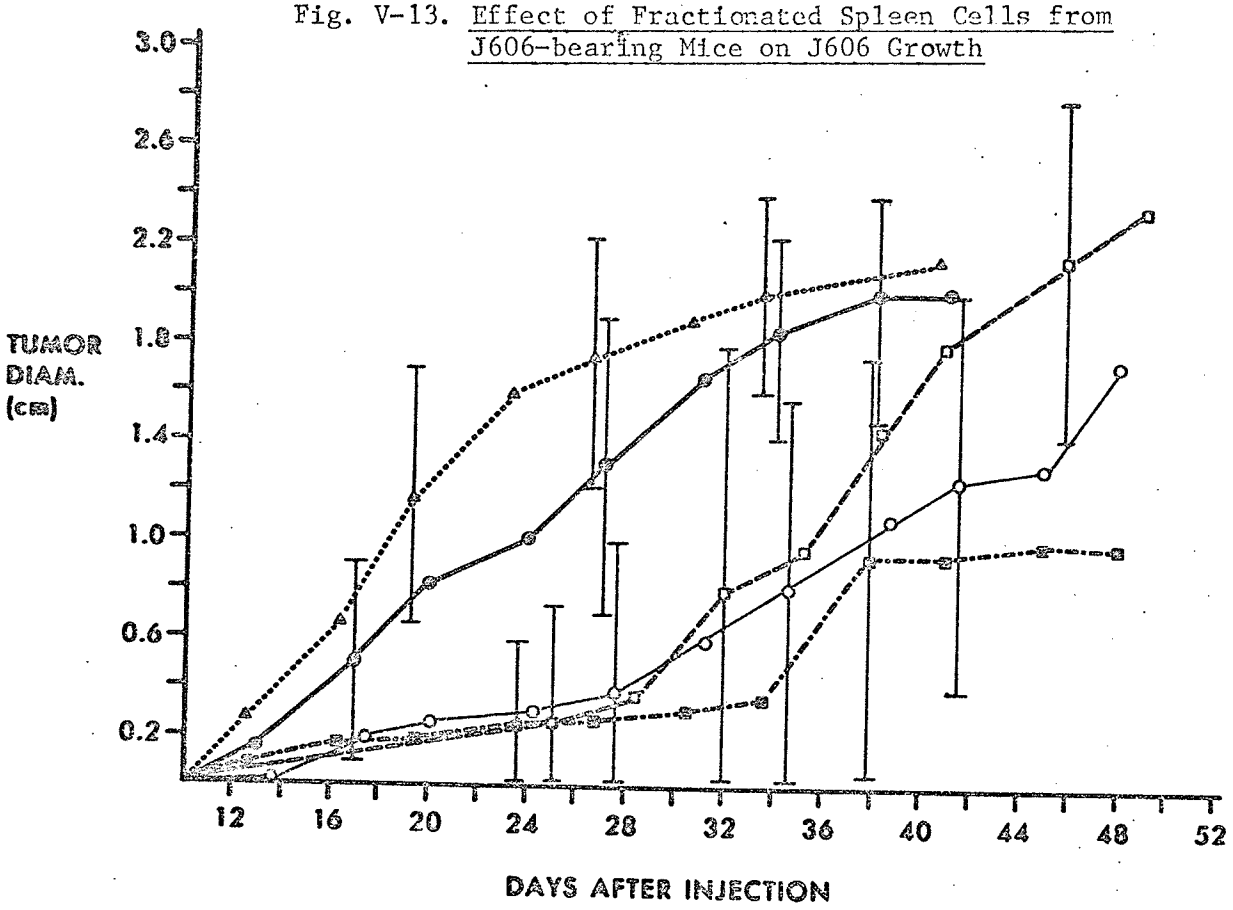
Four groups of three mice each received $1x10^7$, $1x10^6$, $1x10^5$ and $1x10^4$ HOPC-1 cells, subcutaneously, in 1 ml. CMEM.

Tumor incidence was observed regularly and the results are shown below in Table V-3.

Table V-3. HOPC-1 Minimum Dose Study

Group No.	1	2	3	4
Cell Dose	1×10^7	1×10^6	1×10^5	1×10^4
Number of Animal	3	3	3	3
% Tumor Incidence (after 30 days)	100	100	100	0

Fig. V-13. Effect of Fractionated Spleen Cells from J606-bearing Mice on J606 Growth



LEGEND:

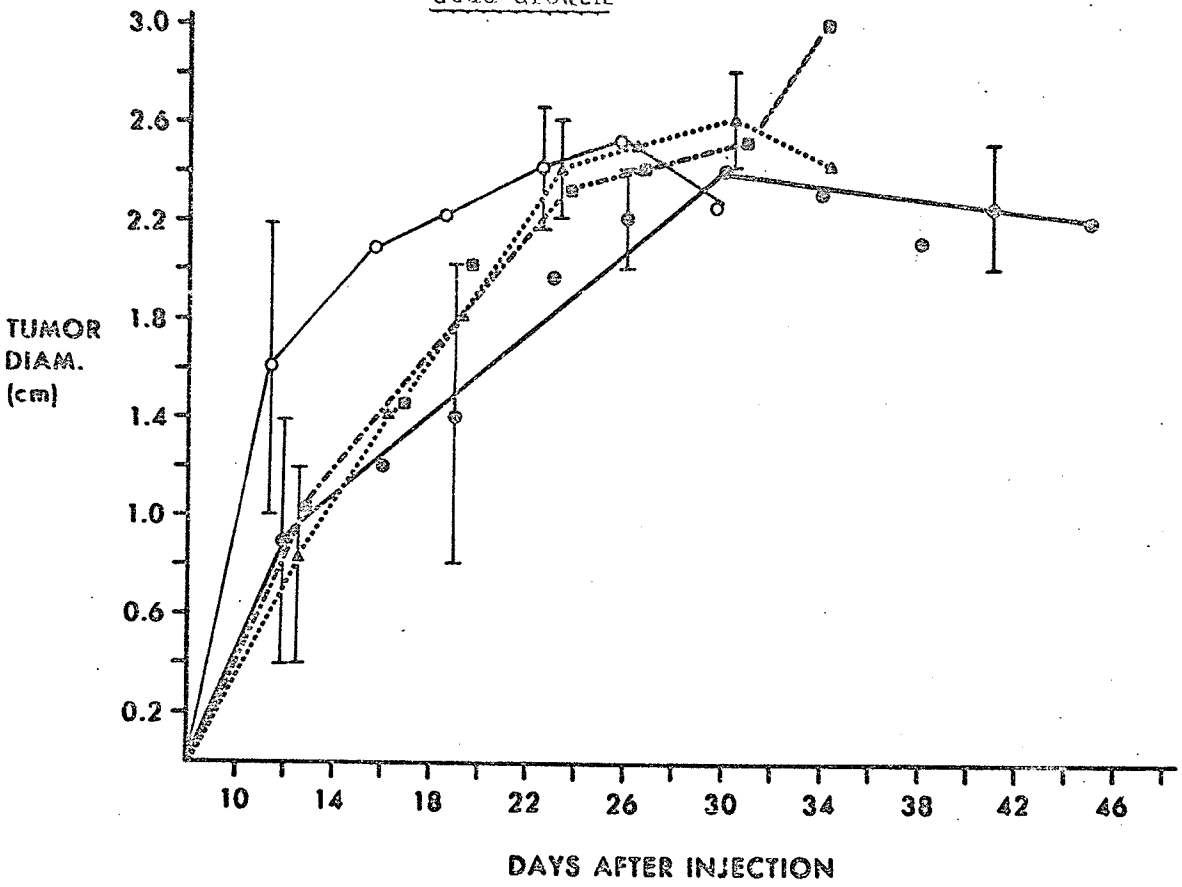
Group No.	1	2	3	4	5
Symbol in Graph					
No. Animals	5	5	5	5	5
Treatment	J606 only	J606 + T-cells (10^7)	J606 + B-cells (10^7)	J606 + T-cells (10^7) + B-cells (10^7)	J606 + whole spleen cells (10^7)
Survival Time (days ± S.E.)	50±10	46±10 (p=0.5)	62±12 (p=0.2)	54±5 (p=0.5)	58±8 (p=0.5)
% Tumor Take	100	100	100	100	80

All groups received 1×10^6 J606 cells S.C. in 1 ml. volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C, injection.

All T-cells, B-cells, and whole spleen cell fractions were prepared from the spleens of J606-bearing mice.

Fig. V-14: Effect of Normal Fractionated Spleen Cells on J606 Growth



LEGEND

Group No.	1	2	3	4
Symbol in Graph	○——○——○	△·····△·····△	○——○——○	■-·-■-·-■
No. Animals	5	5	5	5
Treatment*	J606 only	J606 + T-cells (10^7)	J606 + B-cells (10^7)	J606 + Whole Spleen Cells (10^7)
Survival Time (days±S.E.)	42±6	37±1 (p=0.2)	31±2 (p=.05)	35±2 (p=0.1)
% Tumor Take	100	100	100	100

*All groups received 1 x 10^6 J606 cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cells, B-cells, and whole spleen cells fractions were prepared from the spleens of normal mice.

From this table, $1x10^5$ HOPC-1 cells was chosen as the minimum lethal dose. However, after extensive tests, it was found that $2x10^5$ HOPC-1 cells had a higher reliability to induce a tumor in 100% of the animals. Therefore, in all experiments involving HOPC-1 plasmacytoma, $2x10^5$ cells were given.

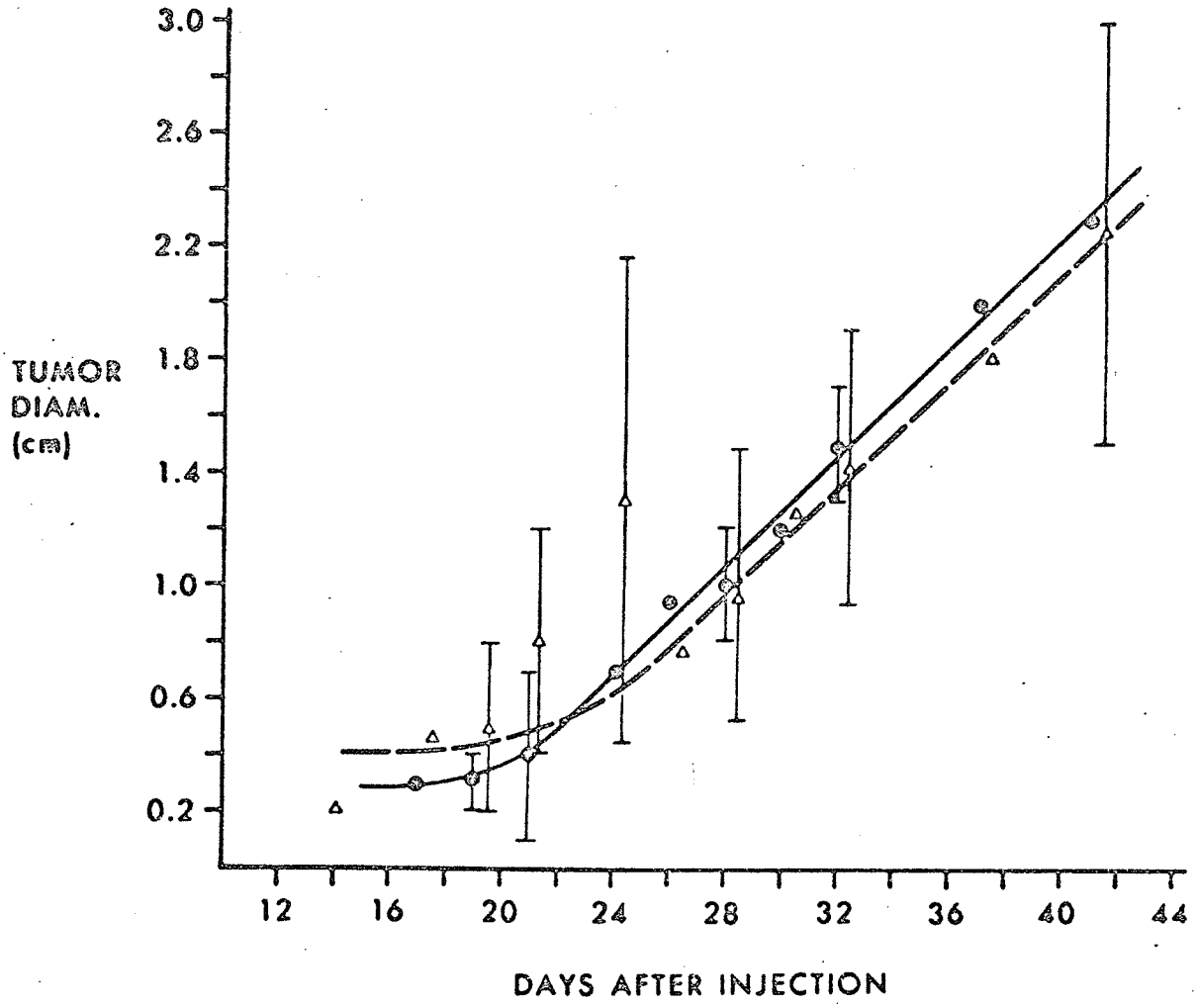
2. Investigation of the Antigenicity of HOPC-1

To assess the antigenicity of HOPC-1, several experiments were done. Using a method already described, i.e. pretreatment of mice with X-irradiated HOPC-1 cells followed by HOPC-1 challenge 21 days later, the results recorded are as shown in Fig. V-15. No significant difference was apparent throughout the entire length of the experiment in terms of tumor growth and survival time.

In another experiment, interesting results were obtained. In this experiment, one group of mice received a pretreatment involving X-irradiated HOPC-1 cells. These cells were injected together with 0.1mg BCG. Twenty one days later, these animals were challenged with $2x10^5$ viable HOPC-1 cells as well as a control group which also received $2x10^5$ viable HOPC-1 cells only. The results are shown in Fig. V-16. Each animal was represented separately, because, if the animals were represented as a group, misleading data resulted because of the high degree of variability in survival time as a result of the high degree of variability in tumor growth. It can be seen from the Figure that one control animal grew the tumor but later at day 38, regressed the tumor completely until day 108, when it subsequently began to grow again. The remaining three control animals grew the tumors in a regular manner without incidence of rejection or vast difference in survival time. In the treated group, one animal failed to develop a tumor until very late (day 84) and another animal grew the tumor and maintained it at a relatively small size up until day 72, whereupon it regressed completely until day 84. At this time, then, the tumor grew at the normal rate (with respect to controls) until the animal died. The remaining two treated animals grew the tumor similar to the control group, and showed no significant differences in terms of tumor growth or survival times.

In another experiment, we attempted to induce HOPC-1 tumors in the subcutaneous tissues of the tail. However, because of probable technical limitations these tumors failed to grow. We then decided to test whether

Fig. V-15. Induction of Immunity with HOPC-1 using X-irradiated HOPC-1 Cell Pretreatment



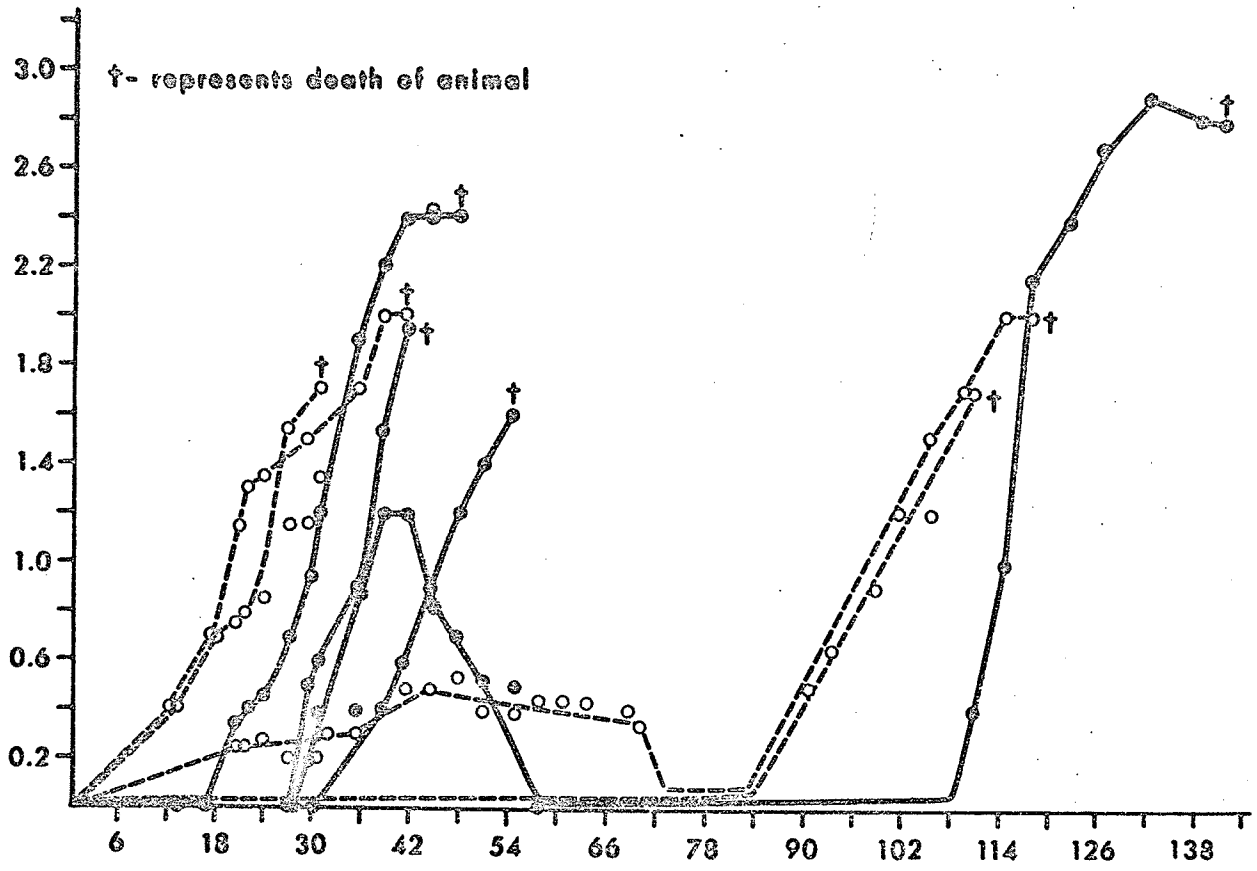
LEGEND

Group No.	1	2
Symbol in graph	●——●——●	△- - -△- - -△
No. Animals	3	5
Treatment *	HOPC-1 only	HOPC-1 pretreated with x-irrad. HOPC-1 1.
Survival Time (days+S.E.)	N.A.	N.A.
% Tumor Take	100	100

* All groups received 2×10^5 HOPC-1 cells S.C. in 1 ml volume

1. This group received 5×10^5 HOPC-1 cells S.C. irradiated with 3000 Rads 21 days prior to HOPC-1 challenge.

Fig. V-16. Induction of Immunity with HOPC-1 using X-irradiated HOPC-1 Cell Pretreatment + BCG



LEGEND

Group No.	1	2
Symbol in Graph	—●—	○---○---
No. Animals 2.	1	1
Treatment*	HOPC-1 only	HOPC-1 after pretreatment with X-irrad HOPC-1 cells & BCG. 1.
Survival Time (days±S.E.) 3.	61±18	66±25 (p=0.5)
% Tumor Take	100	100

*All groups received 2 x 10^5 HOPC-1 cells S.C. in 1 ml volume.

1. This group received 5 x 10^5 HOPC-1 cells irradiated with 3000 Rads given together with 0.1 mg BCG S.C. 21 days prior to the HOPC-1 challenge.
2. In both groups, each mouse was plotted separately for a total of 4 in each group.
3. In both groups, this survival time represents the average of the 4 mice/group.

it was possible to induce a tumor in the normal manner (subcutaneously on the back) in spite of this pretreatment. However, in the control group, only 50% of the tumors grew. Nevertheless, in the pretreated group, 100% of the animals took the tumor. In terms of survival time the pretreated animals died sooner ($p=0.05$) than the control animals. The results are shown in Fig. V-17. These results indicate that the injection of HOPC-1 cells into the subcutaneous tail tissue resulted in immunological enhancement, both in terms of tumor induction and survival time.

3. Effect of Adoptive Transfer of Lymphocytes on HOPC-1

The effects of adoptive transfer of lymphocytes were investigated by separating T-cells and B-cells with nylon wool. Winn Assays were then used, as mentioned before, and tumor growth and survival time were used to express the effects of each fraction. Unfortunately, the separation of spleen cells from HOPC-1-bearing animals was not possible because of time limitations but normal spleen subpopulations had interesting effects. The results are shown in Fig. V-18. In terms of survival times, B-cell treated animals lived significantly ($p=0.01$) longer (106 ± 23 days) than the control animals (63 ± 16 days). Furthermore, 40% (2/5) of the B-cell treated group, rejected the tumor. In the T-cell treated group, 20% (1/5) of the group rejected the tumor and 20% (1/5) of the group didn't grow the tumor until late in the experiment (day 115). However, no difference was noted in survival times or tumor growth as compared to the control group in all other groups. It is also of interest to note that 40% (2/5) of the T-cell + B-cell treated group rejected the tumor.

B. S104 Plasmacytoma

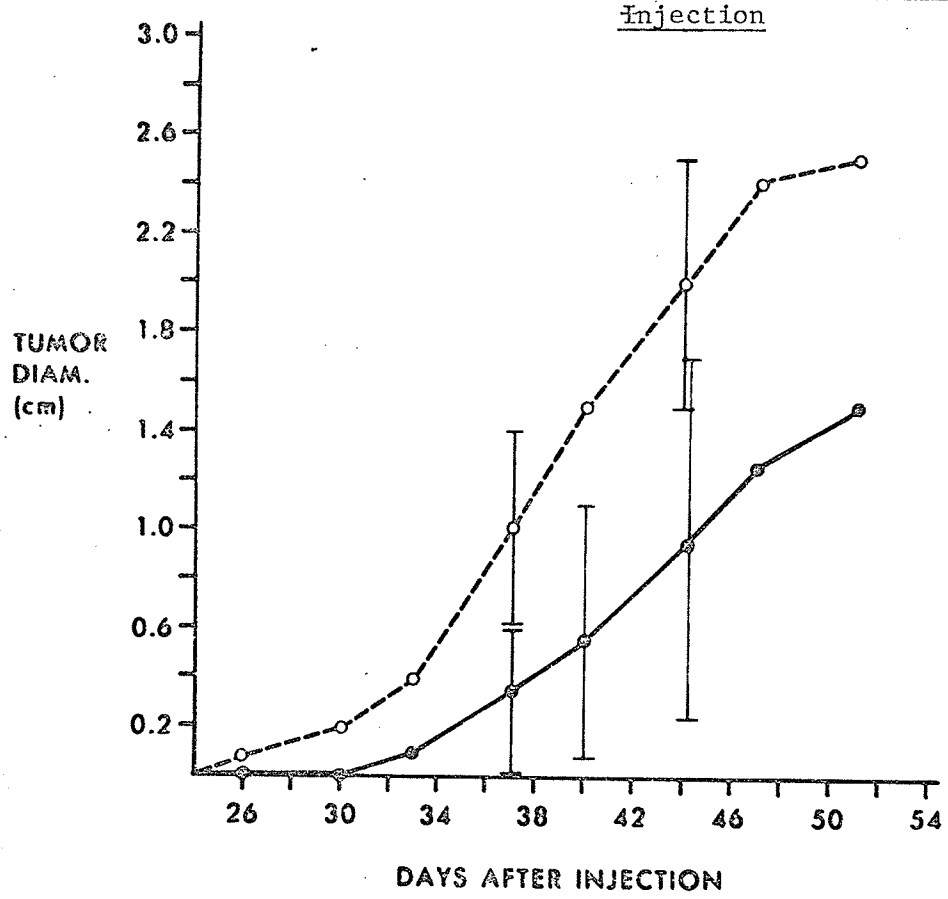
1. Estimation of Minimal Lethal Dose of S104

For reasons already mentioned, it is necessary to establish the minimum dose of tumor cells required to consistently produce a tumor.

To accomplish this, three groups of three mice each received $1x10^6$, $1x10^5$ and 10^4 S104 cells in 1.0ml; subcutaneously.

Tumor incidence was checked regularly and the results are shown below in Table V-4.

Fig. V-17. Induction of Immunity in HOPC-1 after Tail Injection



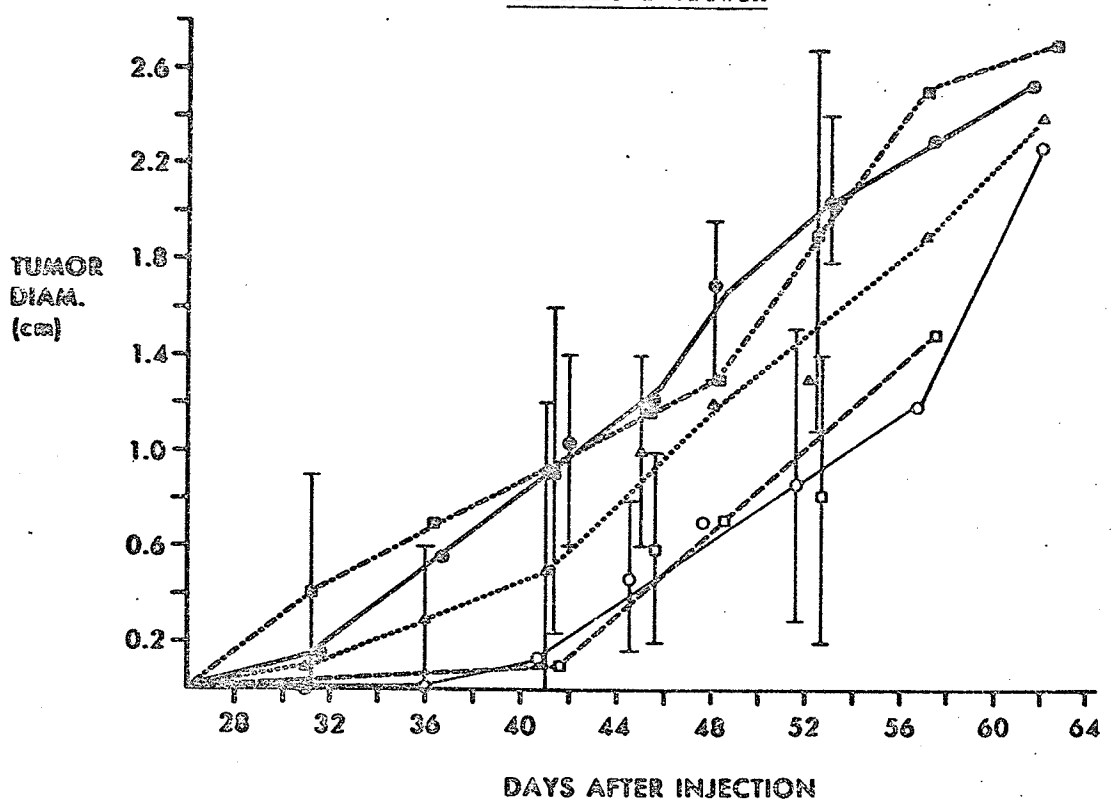
LEGEND

Group No.	1	2
Symbol in Graph	—●—	—○—
No. Animals	6	6
Treatment*	HOPC-1 only	HOPC-1 Pretreated with HOPC-1 [1.]
Survival Time (days±S.E.)	82±15	60±12 (p=.05)
% Tumor Take	50	100

*All groups received 2×10^5 HOPC-1 cells S.C. in 1 ml volume.

1. This group received 2×10^5 HOPC-1 cells S.C. into the tail, but failed to develop a tumor there and was therefore challenged 21 days later with HOPC-1 S.C. on the backs of the animals.

Fig. V-18. Effect of Normal Fractionated Spleen Cells on HOPC-1 Growth



LEGEND:

GROUP NO.	1	2	3	4	5
SYMBOL IN GRAPH	●—●—●	△·····△·····△	○—○—○	□—□—□	■·····■·····■
NO. ANIMALS	5	5	5	5	5
TREATMENT*	HOPC-1 only	HOPC-1 + T-cells (10^7)	HOPC-1 + B-cells (10^7)	HOPC-1 + T-cells (10^7) + B-cells (10^7)	HOPC-1 + whole spleen cells (10^7)
SURVIVAL TIME (DAYS ± S.E.)	63±16	79±30 (p=0.4)	106±23 (p=0.01)	87±23 (p=0.2)	58±13 (p=.5)
% TUMOR TAKE	100	80	60	60	100

*All groups received 2×10^5 HOPC-1 cells S.C. in 1ml. volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cells, B-cells, and whole spleen fractions were prepared from the spleens of normal mice.

Table V-4. S104 Minimum Dose Study

Group No.	1	2	3
Cell Dose	1×10^6	1×10^5	1×10^4
Number of Animals	3	3	3
% Tumor Incidence (after 50 days)	100	67	0

From this table, $1x10^6$ S104 cells was chosen as the minimum lethal dose and this dose was used in all experiments involving S104 plasmacytoma.

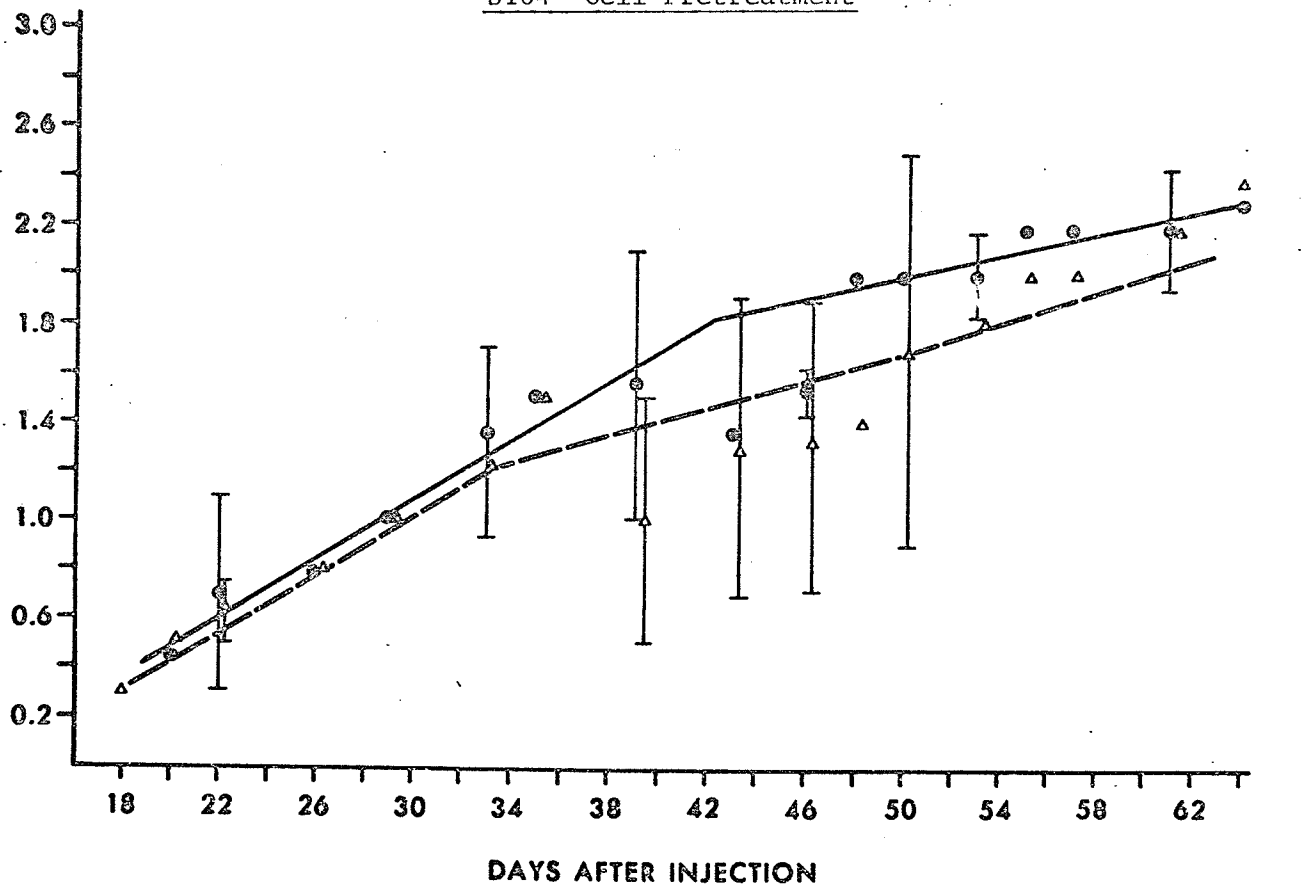
2 . Investigation of the Antigenicity of S104

The investigation of the antigenicity of S104 was similar to that of HOPC-1. In the experiment where animals are pretreated with X-irradiated S104 cells and then challenged 21 days later, the results are shown in Fig. V-19. It is apparent from this figure that no detectable difference either in tumor growth or survival time was present when compared to the control which received only S104 cells on the day of challenge.

In another experiment, involving the pretreatment of mice with X-irradiated S104 cells but in addition treated simultaneously with 0.1mg BCG followed by a challenge 21 days later, similar results were obtained. Again, as is shown in Fig. V-20, no detectable difference is noted in survival time or tumor growth when compared to a control which received S104 cells only.

In an experiment where one group of mice is pretreated with a viable subcutaneous tail injection and followed by a normal subcutaneous injection on the back 21 days later, there is a difference noted. When compared to tumor growth of the control group a significant ($p=0.05$)

Fig. V-19. Induction of Immunity with S104 using X-irradiated S104 Cell Pretreatment



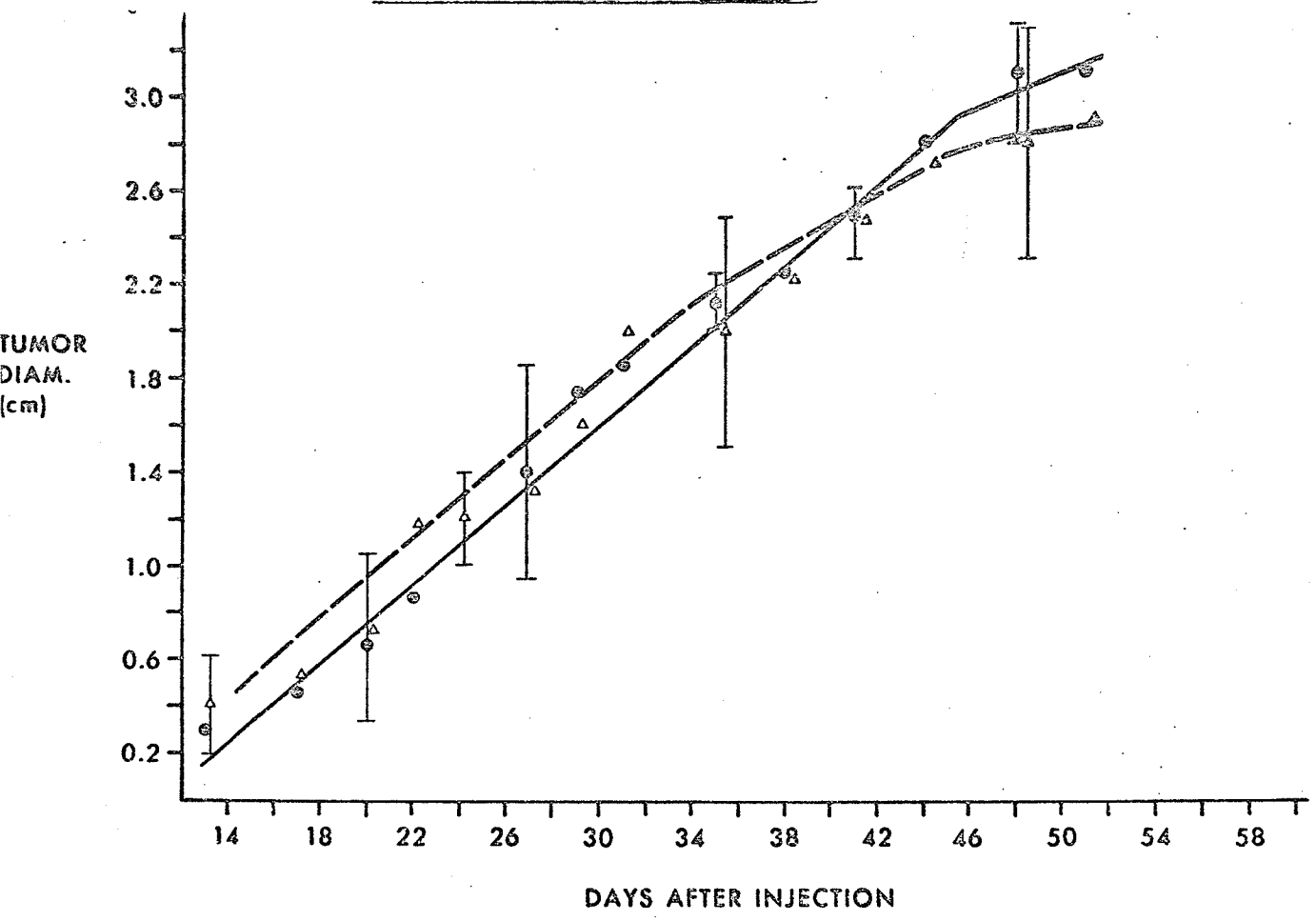
LEGEND

Group No.	1	2
Symbol in Graph	●—●—●	△—△—△
No. Animals	4	5
Treatment *	S104 only	S104 Pretreated with X-irrad. S1041.
Survival Time (days±S.E.)	66±24	75±20 (p=0.5)
% Tumor Take	100	100

*All groups received 1 x 10^6 S104 cells S.C. in 1 ml volume.

1. This group received 5 x 10^6 S104 cells S.C. irradiated with 3000 Rads 21 days prior to S104 challenge.

Fig. V-20. Induction of Immunity with S104 using X-irradiated S104 Cell Pretreatment + BCG.



LEGEND

Group No.	1	2
Symbol in Graph	●——●——●	△– –△– –△
No. Animals	5	5
Treatment*	S104 only	S104 after Pretreatment with X-irrad. S104 Cells & B.C.G. 1.
Survival Time (days±S.E.)	82±15	76±12 (p=0.5)
% Tumor Take	100	100

*All groups received 1 x 10^6 S104 cells S.C. in 1 ml volume.

1. This group received 5 x 10^6 S104 cells irradiated with 3000 Rads given together with 0.1 mg BCG S.C. 21 days prior to the S104 challenge.

increase in tumor diameters is noted in the pretreated group at day 35 and day 52. These results are shown in Fig. V-21. However, in the final stages of the experiment, no difference is noted in either survival times or tumor growth.

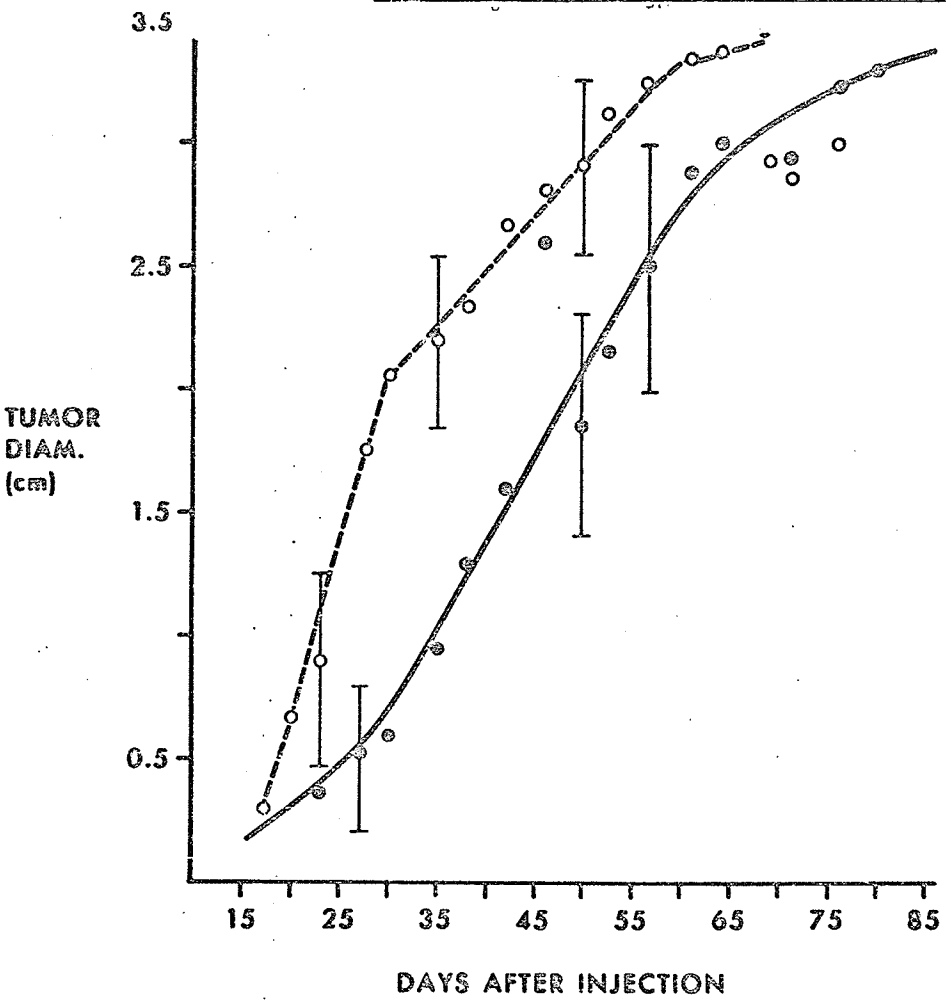
3 . The Effect of the Adoptive Transfer of Lymphocytes on S104

The effect of adoptive transfer of lymphocytes through nylon wool separation columns is measured through the use of Winn Assays, as mentioned before.

In using spleen cells from S104-bearing mice, the separated cells yielded no difference in either survival time or tumor growth except in the T-cell treated group. This group showed significant ($p=0.05$) decrease in survival time when compared to the control group which received only S104 cells. These results are shown in Fig. V-22.

In separating normal spleen cells into the T- and B-cell components, interesting results were obtained. These results are shown in Fig. V-23. Here it is found again that B-cells significantly ($p=0.05$) lower tumor diameters at two points (days 15 and 26) with respect to the control group. Also, 20% (1/5) of this group rejected the tumor and another 20% (1/5) of this group grew the tumor only after the experiment had been terminated (day 85). Similarly, 20% (1/5) of the T-cell treated group grew the tumor but rejected it after 34 days. No significant differences were found in the groups treated with whole spleen cells from S104-bearing animals or T-cells + B-cells from S104-bearing animals.

Fig. V-21. Induction of Immunity with S104 after Tail Injection



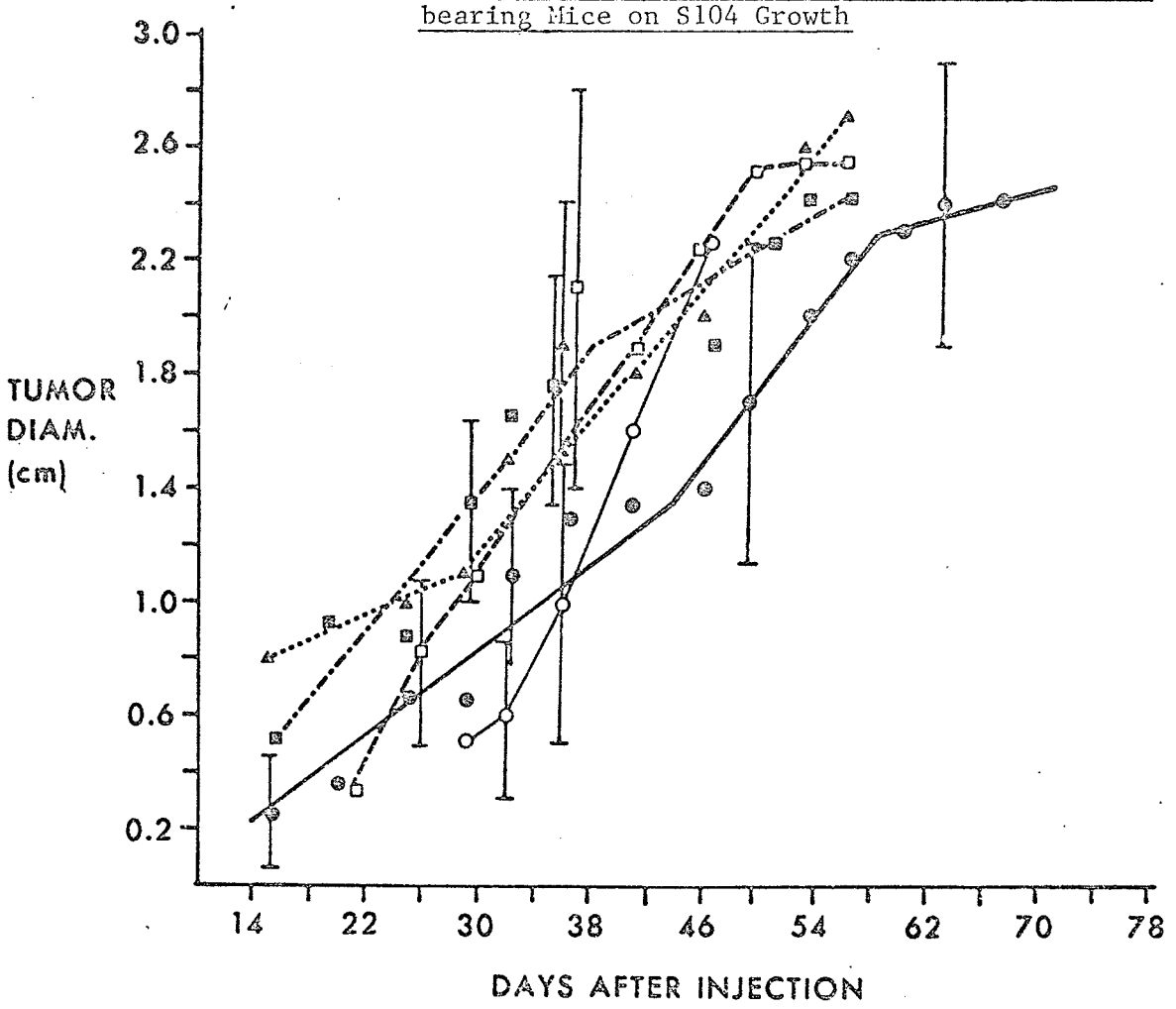
LEGEND

Group No.	1	2
Symbol in Graph	●——●——●	o- - -o- - -o
No. Animals	5	5
Treatment *	S104 only	S104 Pretreated with S104 1.
Survival Time (days±S.E.)	70±16	73±7 (p=0.5)
% Tumor Take	100	100

*All groups received 1 x 10^6 S104 cells S.C. in 1 ml volume.

1. This group received 1 x 10^6 S104 cells S.C. into the tail, but failed to develop a tumor there, and therefore was challenged 21 days later with S104 S.C. on the backs of the animals.

Fig. V-22. Effect of Fractionated Spleen Cells from S104-bearing Mice on S104 Growth



LEGEND

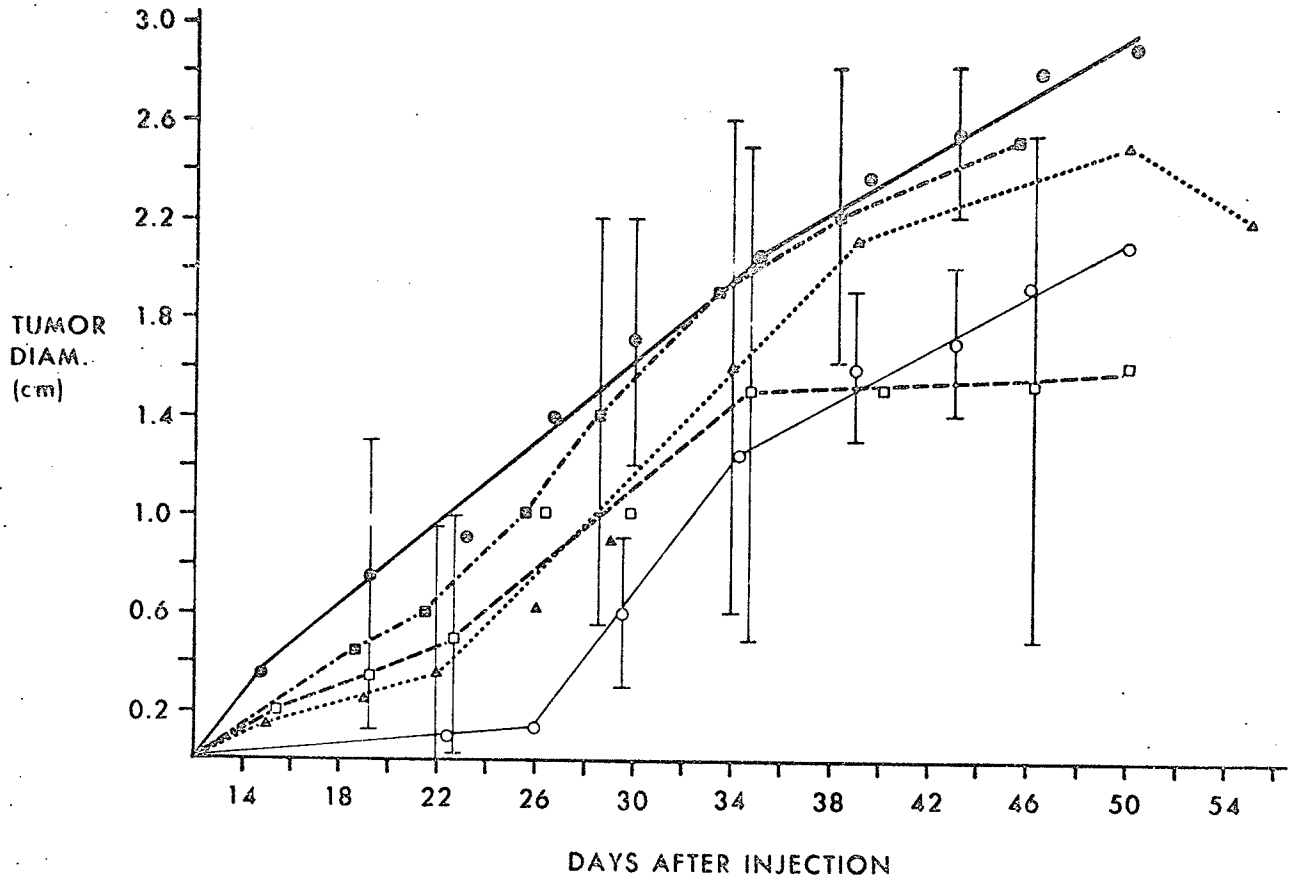
Group No.	1	2	3	4	5
Symbol in Graph					
No. Animals	5	5	5	5	5
Treatment *	S104 only	S104 + T-cells (10^7)	S104 + B-cells (10^7)	S104 + T-cells (10^7) + B-cells (10^7)	S104 + whole spleen cells (10^7)
Survival Time (days±S.E.)	75±14	51±4 (p=0.05)	64±14 (p=0.4)	55±17 (p=0.2)	N.A.
% Tumor Take	100	100	100	100	100

* All groups received 1×10^6 cells S.C. in 1 ml volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cells, B-cells, and whole spleen fractions were prepared from the spleens of S104-bearing animals

Fig. V-23. Effect of Normal Fractionated Spleen Cells on S104 Growth



LEGEND:

Group No.	1	2	3	4	5
Symbol in Graph	⊗—⊗—⊗	△·····△·····△	○—○—○	□—□—□	⊠-·-⊗-·-⊠
No. Animals	5	5	5	5	5
Treatment*	S104 only	S104 + T-cells (10^7)	S104 + B-cells (10^7)	S104 + T-cells (10^7) + B-cells (10^7)	S104 + whole spleen cells (10^7)
Survival Time (days±S.E.)	50±15	94± 35 (p=0.1)	79±38 (p=0.3)	62±24 (p=0.5)	52±15 (p=0.5)
% Tumor Take	100	100	80	100	100

*All groups received $1x10^6$ S104 cells S.C. in 1 ml. volume.

In appropriate groups, the lymphocyte fractions were mixed with tumor cells prior to S.C. injection.

All T-cells, B-cells and whole spleen fractions were prepared from the spleens of normal mice.

CHAPTER VI

VI. GENERAL DISCUSSION

The study of tumor immunology fundamentally attempts to describe, in as much detail as possible and as accurately as possible, immunological mechanisms underlying the tumor-host relationship. The elucidation in recent years of fundamental mechanisms has led to the belief that effector cells of various types and antibody of various types are responsible for the eradication of neoplasms. Antibodies cytotoxic to tumor cells in the presence of complement in the host have been shown, for example, to be involved in destruction of malignant melanomas and in Burkitt's lymphoma (90). Where cytotoxic antibody in the presence of complement apparently plays no role, antibody may act in concert with cell mediated effector arms (antibody-dependant-cell-mediated cytotoxicity) such as in the case of sarcomas, which usually only poorly elicit cytotoxic antibody (91). Cellular mechanisms responsible for tumor cell destruction, are also involved in a tumor-host relationship. T-cells have been found by many investigators to play an extremely important role in tumor immunity. Plasmacytomas have been shown by various authors to elicit T-cell responses in the host (15). However, these responses by T-cells may be varied because of the many subpopulations of T-cells which exist according to Boyse and associates (95). The most controversial T-cell subset is the suppressor T-cell of the spleen and thymus which has been strongly implicated as the cell type central in contributing to tumor escape (96). B-cells have also been implicated as being important cytotoxic effectors in tumor immunity (101,102). Furthermore, macrophages are also known to be involved (106). Not only are the above effector mechanisms active after exposure to tumor cells in experimental animals, but immunity has been detected in <u>normal</u> animals who have never been exposed experimentally to the tumor cells before and may, in this sense, be considered "natural killer mechanisms", (11,51).

However, effector mechanisms are not always effective and therefore tumors escape immunological attack. Suppressor cells have already been mentioned. Enhancing mechanisms, other than the suppressor-cell mediated modes may result from antibody though as yet, poorly understood mechanisms (115, 117). With antibody implicated in enhancing mechanisms, a logical extension would have to, as well, implicate B-cells as

enhancing cell types (directly or indirectly) since B-cells are the antibody producers. Soluble antigens and immune complexes also play a role in the escape of tumors (136).

The major complicating factors of a tumor-host relationship include the diverse biological and antigenic characteristics of tumors, the extensive integration of many cell types in ultimately manifesting an immune response to the tumor and the poorly understood, but manifold escape mechanisms available to tumors. For these reasons, the majority of tumor immunology revolves around these focal points, as do in part the investigations contained within this volume.

The antigenicity of plasmacytomas is highly variable and this variability can easily be realized by the current literature available. Although in some investigations, antigenicity has been difficult to demonstrate (15), many studies indicate that most plasmacytomas are immunogenic, even if it is difficult to see (11, 14, 37, 44, 47, 48). It is quite accepted in modern tumor immunology that most, if not all, tumors are indeed immunogenic, but the degree of immunogenicity may vary and the ability to demonstrate immunogenicity may vary.

In the plasmacytomas used in our investigations (MOPC104E, J606, HOPC-1, S104), we, as well, experienced varying degrees of success in terms of illustrating antigenicity. A method used to demonstrate antigenicity in all four plasmacytomas involved pretreating a group of mice with X-irradiated (3000 R) tumor cells, followed by a viable tumor cell challenge of the appropriate line 21 days later, similar to the method used by Williams and Krueger (14). MOPC104E, although showing no difference in the pretreated group with respect to the control group in terms of tumor growth rate, showed significant increases in survival time (See fig. V-1). Also, a major indication of antigenicity was the fact the 25% of the pretreated group rejected the tumor completely and a further 25% grew the tumor initially and later (day 81) regressed it completely. This is considered as good evidence for the antigenic nature of tumors. MOPC104E was also investigated by Rollinghoff and his associates (39) and it was determined by this group that MOPC104E was not immunogenic. However, since it is well known that antigenicities may drastically change or even disappear upon multiple transplantation, the present antigenicity of the MOPC104E which we investigated may not be

antigenically identical to that of the Rollinghoff group (26,27).

Using the same method to induce immunity in J606, however, resulted in a poor indication of the antigenic nature of this myeloma (see Fig. V-12). Although there was, at one point (day 18), a significant difference in tumor diameters, the remaining points were not significantly different so, on this basis, one must conclude that the antigenicity of J606 is not easily illustrated. Clearly, this does not mean that J606 has no antigens upon its cell surface which evoke an immune response, but rather simply that this method was inadequate to detect antigenicity. In separation studies, discussed later, it cannot be refuted that antigenicity is indeed present, since some alteration in the tumor-host relationship becomes apparent.

The antigenicity of HOPC-1 is less controversial. Although, no antigenicity could be demonstrated in the animals immunized with X-irradiated tumor cells (See fig. V-15), it was possible to indicate immunogenicity using another method. Using X-irradiated tumor cell pre-treatment together with 0.1mg BCG, followed by challenge 21 days later resulted in a dramatic detection of tumor antigenicity. As can be seen in Fig. V-16, one animal in the control group (i.e. untreated except for the HOPC-1 challenge dose) totally regressed the tumor after approximately 42 days, and regrew a tumor at 108 days. This "normal" animal obviously had the ability to reject the tumor because of either, (a) a state of immunity as a result of pre-exposure to the same or similar antigens of the challenging tumor cell or, (b) an immediate immune response to the antigenic structures of the tumor cell followed by a successful remission 42 days later. It is a well known fact that, indeed, normal animals have antibodies in their serum against the PC-1 antigen and the xVEA (viral envelope antigen) (11). Furthermore, normal lymphoid cells have been shown to be able to respond <u>in vitro</u> to tumor cells in a mixed lymphocyte-tumor assay (51). Also, the pretreated group (which received X-irradiated tumor cells and BCG) experienced an immunological attack on the challenge tumor dose. 25% of this group did not grow the tumor until very late in the experiment (day 84) and 25% of the group grew the tumor but maintained it at a relatively small size for about 72 days until it totally regressed. However, upon total regression, it immediately grew again at a normal rate until the animal died. The remaining 50% of the group grew the tumor very similar to the control group.

These results indicate very nicely the fine balance between maintaining tumor cells under control and losing control. It's quite possible that the degree of antigenicity of the particular tumor plays a paramount role in establishing this balance.

In another experiment, we attempted to grow HOPC-1 in the subcutaneous tissues of the tail. However, because of technical difficulties, this was impossible. Nevertheless, we subsequently challenged these "pretreated" mice with viable tumor cells and compared the results to a control group which received only the challenge dose of tumor cells. However, because of technical difficulties, only 50% of the control animals took the tumor. On the other hand, 100% of the mice in the pretreated group accepted the tumor and, in fact, although no significant difference in tumor growth rate was noted the pretreated group lived significantly shorter lives (see Fig. V-17). These results indicate a classical example of immunological enhancement, discussed earlier. Therefore, although no protection was afforded by the pretreatment and hence, in this sense no antigenicity was demonstrated, immunological enhancement, in indirect terms, represents antigenicity. This idea becomes clear when one considers the reason (and theory) of enhancement: prior exposure to a tumor resulting in an increase in tumor growth instead of the expected decrease after challenge with the same tumor. The increase in tumor growth comes about only because of prior communication of the tumor with the host, and therefore we can postulate that the tumor cell effected the host or the host effected the tumor cell, potentially as a result of a difference from "self". We can, on the basis of the experiments above conclude that HOPC-1 is, indeed immunogenic.

Attempts to induce a state of immunity to S104 using the same methods as for HOPC-1 were generally unsuccessful, (see Fig. V-19,20,21).

However, in the tail injection and challenge experiments (see Fig. V-21), in terms of tumor growth, enhancement was found to be statistically significant at days 35 and 52. It is quite possible that, since similar results were obtained with HOPC-1, immunological enhancement can easily be manifested by pretreating the animals with a low dose of viable tumor cells, too low for tumor induction, but possibly high enough to expose the host to the antigenic properties of the tumor cell e.g., virus particles, PC-1, x VEA etc. Then upon challenge, instead of immunity,

enhancement results.

Adoptive transfer experiments performed by Rollinghoff, and other groups utilizing the Winn Assay were, in terms of conferring protection, successful (36,39,40). However, these investigators used only whole spleen cells from normal and immunized mice for their studies.

In our experiments, we also used whole spleen cells extensively for preliminary examination of the phonomenon of cell-mediated protection described by many other groups. In our experiments with MOPC-104E, using whole spleen cells of tumor-immune and normal animals in the Winn Assay, the results were encouraging. As can be seen in Fig. V-2, significant protection was afforded in the spleen cell pretreated group at days 27 and 36. However, Fig.V-3, indicated no protection at all with either whole spleen cells from tumor-bearing animals or normal animals. These experiments vividly indicated again the fine balance between protection and no protection. The reason for the discrepancies, however, are unknown. It is quite possible, though, that, because of the large numbers of various cell types within the spleen, antagonism between them in terms of manifesting each cell type's purpose results in masking any beneficial effects certain spleen cell types may be attempting. Indeed, suppressor cells in the spleen and else where have been described which suppress the normal immune response to antigens for unknown reasons (96). If these cell types are in large enough numbers, the tumor may grow unhindered even when injected with immune spleen cells, since the protective cells' efforts are masked. However, to partially overcome this problem, separation studies using nylon wool were used to separate T-cells from non T-cells. (For simplicity the non T-cells are collectively referred to as B-cells. However, it must be kept in mind that within the "B-cell" population, other cell types may be present).

In fact, the variations due to "masking" of cell functions is graphically demonstrated in Fig. V-4. This figure shows that T-cells from tumor-bearing animals significantly protect the group in terms of tumor growth. Whole spleen cells, however, do not. B-cells, as well, do not protect. In another experiment, similar findings were made, but this time both T-cell and whole spleen cell treated groups were afforded pro-

tection in terms of survival increase and tumor growth rate (see Fig. V-5). In fact, all cell fractions (T,B, T+B, and whole spleen) significantly protected with respect to the control group in terms of tumor growth at days 36 and 44 and all groups except the B-cell treated group protected in terms of survival time increases. Protection by T-cells, B-cells and whole spleen cells have been documented many times before, as mentioned (15,95,101,102).

Besides the "masking" effect described above, the discrepancies in Fig. V-2 and Fig. V-3 and between Fig. V-4 and Fig. V-5 can be explained in other terms. It is possible that, because the spleens from tumor-bearing animals were taken using the tumor size as an indicator (i.e. spleens were taken when tumor was 1.5-2.0 cm. in diameter), these discrepancies occurred. The alternative would have been to remove the spleens a fixed time period after the tumor injection. If this procedure was adapted, the results may or may not have proven to be consistent. However, the former method was adapted but it is understood that variations in the spleen cell populations may occur as a function of time after tumor injection (experiments in progress) or as a function of tumor size.

It is also possible that, in the spleens removed from tumor-bearing animals, tumor cells may have been present due to metastasis. However, based on microscopic examination and based on the fact that the spleens were not enlarged, it is not likely to be the case at the stage in time when the spleens were removed in these experiments. It is acknowledged that this, indeed, may be the case as the tumor progresses further since metastases was noted with J606 a short time before the animal succumbed to the tumor. Furthermore, in preliminary experiments, the surgical removal of MOPC104E followed by a lethal dose tumor challenge 21 days later resulted in rejection of the tumor, not only indicating immunity to MOPC104E but also that no metastasis to the spleen had developed.

The persistent presence of viruses, potentially pathogenic under certain conditions, may in some animals confer a state of natural immunity. This fact has been well documented and may be very important as a natural defence mechanism to potentially carcinogenic

viruses (11). With this in mind, it is possible that the natural immunity in some animals and not in others will alter experimental results by inducing a higher degree of protection (or possibly enhancement as well) upon subsequent exposure to the tumor. This holds true for both normal spleen cells and spleen cells from tumor-bearing mice since their cell populations may have been altered as a result of the pre-exposure to the virus.

Lastly, it is necessary to regard variations in local temperature, humidity, cage location, feeding and watering amongst the many animals as a factor contributing to the discrepancies noted above in some cases.

In another experiment, closely following the method of Rollinghoff by X-irradiating the mice first which was followed by bone marrow reconstitution (Rollinghoff. however, sublethally irradiated the animals in all his experiments with 450 R), negative results were found. Fig. V-8 shows that there were no significant differences in survival time or tumor growth rate induced by T-cells, B-cells, T + B cells or whole spleen cells with respect to a control group which received only MOPC104E.

By lethal X-irradiation (850 R), the animals should be reduced immunologically to almost nil, but, of course, bone marrow reconstitution, necessary for survival, also reconstitutes to some extent the immunological armament. However, since the tumor was injected only 24 hours after bone marrow reconstitution, one can expect that at that time, and for at least 10-14 days thereafter, immune capabilities would be suppressed. Therefore, the only cells capable of causing rejection or effecting the tumor growth in any way would be the cells which are injected (T,B, T+B, whole spleen) together with the tumor. However, it must be kept in mind that radioresistant cells such as macrophages would also exert an effect and these cells do play an important role (106). The fact that the results indicate no effect by these injected fractions indicates that either not enough effector cells were administered or other cells temporarily effected by X-irradiation normally cooperate and were not available for the cooperation to take place.

However, definite tendencies appear in the figure and if larger groups of animals had been used these tendencies may have proven to be statistically significant. From the figure, whole spleen cells protect, like the experiments of Rollinghoff (15). Furthermore, T-cells and B-cells indicate a tendency to enhance tumor growth or suppress the response to the tumor cells. These actions of T and B-cells are well documented but poorly understood (96,115).

Normal fractionated spleen cells and whole spleen cells had varying effects, Figure V-7 indicates no effect whatsoever. Figure V-6, however, indicates that normal B-cells protect and normal whole spleen cell enhances in terms of tumor growth. Enhancement with normal spleen cells has been documented before (50). Since antibodies have been found in the sera of normal mice which afford protection to a viable tumor cell challenge (11), there is no reason why normal B-cells cannot afford protection, since, indeed, B-cells are antibody producers. It is possible, then, that normal B-cells represent "natural killer cells."

It has been postulated that "normal" animals never previously exposed to tumor cells per se may have been exposed to virus particles, suspected etiological agents of at least some cancers (11). Since these virus particles are antigenic in themselves, and since the virus quite often represents itself directly or indirectly on the surface of the transformed cells, immunity to the virus may mean immunity to the tumor which under certain conditions may result from this virus. Therefore, through this mechanism, normal animals can be immune to some cancers because of this pre-exposure.

Adoptive transfer experiments using nylon wool separated spleen cells and J606 as seen in Figure V-13 had no effect on tumor growth or survival. However, the group treated with whole spleen cells from tumor-bearing mice had 20% of the group reject the tumor completely. This indicates that, despite earlier futile antigenicity studies, J606 can be shown to be immunogenic, since a non-immunogenic tumor could not be rejected. However, despite no significant statistical differences between the groups, again, tendencies seem to be indicated. T-cells show that they have a tendency to enhance, but because of the variation of tumor appearances in the control group, larger standard errors result, and hence statistical significance becomes increasingly more difficult to establish between the groups. For the same reason, whole spleen cells do not statistically protect although the general tendency seems to indicate protection if a larger group was used. Normal B-cells as can be seen in Fig.V-14, were found to enhance with respect to survival time whereas normal T-cells and normal spleen cells had no effect. The enhancement seen with normal spleen cells has already been mentioned and therefore, it is not difficult to accept that individual cell types from

normal spleens may as well enhance. It is quite conceivable, as briefly mentioned earlier, that suppressor cells, whether from normal or primed tissues, represent one mode of inducing enhancement. This could be achieved because suppressor cells may abrogate normal immunological reactivity which would normally kill, inactivate or slow down the activity of antigenic tumor cells. Because of suppressor cell activity then, and because of their possible preponderance due to primary immunization, for example, upon re-exposure to the same antigens, suppressor cells could mask beneficial effects of cytotoxic lymphocytes and or antibody. It may be expected, then, from this model, that cells from animals preexposed to the tumor, would induce enhancement more easily than normal cells (96).

Although it was not possible to fractionate the spleens of HOPC-1-bearing mice, the fractionation of normal spleens into component T-cells and B-cells confirmed the conclusion made earlier that this tumor is relatively antigenic. As can be seen from Fig. V-18, although whole spleen cells again are ineffective at causing an alteration in tumor growth or survival, component T and B-cells are both very effective. 20% of the group treated with normal T-cells rejected the tumor completely and a further 20% of the group didn't develop a tumor until very late (day 115). Similarly, 40% of the group treated with normal B-cells totally rejected the tumor and 40% of the group treated with T-cells mixed with B-cells totally reject the tumor. T-cell fractions when mixed with B-cell fractions from the nylon wool column <u>do not</u> constitute the equivalent of a "whole spleen" fraction. It is obvious from the above experiments that this is the case, since the T+B-cell treated group rejects 40% of the tumors and the whole spleen cell treated group is totally ineffective. This may mean that nylon wool retains a select population of cells, possibly the cell type involved in abrogating normal immune reactivity, if indeed such a cell exists. This (potential) drawback in any separation method must always be kept in mind.

In the adoptive transfer of fractionated spleen cells from S104-bearing mice, it was found that the T-cell treated group lived significantly shorter lives (i.e. immunological enhancement) than the control group. However, in terms of tumor growth patterns, no significant difference was caused by either T-cells, B-cells, T+B-cells or whole spleen cells as seen in Figure V-22. However, the normal counterparts exerted profound

effects as seen in Fig. V-23. 20% of the normal T-cell treated group regressed the tumor after 34 days. Similarly, 20% of the B-cell treated group regressed the tumor after 85 days. A further 20% of this group totally rejected the tumor. This indicates that S104 can be indeed, made to show antigenicity and that T- and B-cells are active in ridding the animal of the tumor. Again, whole spleen had absolutely no effect. It is sometimes difficult to understand why normal cells protect the animal against tumor induction and why cells from tumor-bearers do not, in light of the fact thatprior exposure to the tumor cell should trigger a better immune response. However, the same argument can explain the lack of any protection afforded by "activated" lymphoid cells since the pre-exposure to the tumor may result in the induction of suppressor mechanisms or otherwise gear the cell machinery in such a way as to allow the escape of the tumor. Therefore, with some tumors, and under certain conditions, prior exposure may result in nothing but enhanced growth upon subsequent encounter and yet in other tumors result in immediate protection.

Table VI-1 below attempts to summarize and compare the antigenic properties and the protective capacities of spleen cells and subpopulations thereof of MOPC104E, J606, HOPC-1 and S104. The scoring is based on a +/- system with each individual + or - representing protection or no effect, respectively, the number of each depending on the amount of criteria available. Please refer to the legend for details. The Table indicates that, using all criteria available as shown in the tables and graphs, MOPC104E and HOPC-1 are comparatively very antigenic and J606 and S104 are only weakly antigenic. In separation studies, whole spleen plays a protective role generally in tumor-bearing animals, but normal spleen cells have little protective effect but do possibly enhance. Separated T-cells and B-cells protect to varying degrees depending on the particular tumor.

TABLE VI-1 SUMMARY OF ANTIGENICITY AND SEPARATION STUDIES

INVESTIGATION		MOPC104E	J606	HOPC-1	S104
Antigenicity		+ + +	-	- + + + + (enhancement re survival)	- - - - - + (enhancement re diameter)
Separation studies with spleens from tumor-bearers	T	+ + +	-	N.D.	+ (enhancement re diameter)
	B	- - +	-	N.D.	- -
	T+B	+ +	-	N.D.	- -
	whole spleen	+ (enhancement re survival) - + + +	+	N.D.	-
Separation studies with normal spleens	T	- - -	- -	+ + -	- +
	B	+ + - -	-,+(enhancement re survival)	+ + +	+ + -
	T+B	N.D.	N.D.	+ + -	-
	whole spleen	+ + (enhancements re diameter + survival)	-	- -	-

Scoring:

+ awarded with protection by: - prolonging survival time significantly (p=0.05)
- reducing tumor growth at 2 points in time significantly.
- 20-30% of group showing total or prolonged rejection of tumor.

+ + awarded with protection by: - reducing tumor growth at 3 points in time significantly.
- 31-50% of group showing total or prolonged rejection of tumor.

+ (enhancement re {survival / diameter}) awarded for immunological enhancement by {survival time criteria / tumor growth criteria}

- awarded when no effect is seen in terms of survival times or tumor growth.

N.D. - Not Done.

In pilot experiments where the possible role of thymocytes was investigated (see F g. V-9, 10, 11), indicative results were obtained. As can be seen in Fig. V-9, no significant difference is noted between groups which received thymocytes + MOPC104E or thymocytes + spleen cells + MOPC104E. However, at one point (day 42), there was a significant difference between the group which received just spleen cells + MOPC104E and the group which received thymocytes + spleen cells + MOPC104E. At this one point, the thymocyte-treated group had larger tumors than the group given no thymocytes and just spleen cells from tumor-bearing mice. In separation experiments using T-cells and B-cells from the spleens of tumor-bearing animals and combining them with normal thymocytes and thymocytes from tumor-bearing animals, further substantiation of thymocyte involvement was made. In Figure V-11, it can be seen that no significant difference noted between the various cell combinations when compared to the control group which received only MOPC104E. However, upon further analyzing the data, interesting results were found. It was found that the group treated with thymocytes from tumor-bearers lived significantly shorter lives (41 ± 8) when compared to the group treated with normal thymocytes (59 ± 9, $p=.05$). Also, tumors were significantly larger in the former group at two points (days 23 and 31). Furthermore, when the group treated with thymocytes from tumor-bearers + B-cells from spleens of tumor-bearers was compared with the group treated with normal thymocytes + B-cells from tumor-bearers, the former group, again, had tumors significantly ($p=0.05$ and 0.01, respectively) larger at days 31 and 41. When the group treated with thymocytes from tumor-bearers + B-cells from tumor-bearers was compared to the group treated with just B-cells from tumor-bearers (+MOPC104E, obviously), again, at one point (day 23) the tumors were significantly ($p=0.05$) larger in the former group.

When similar combinations of cells were mixed with T-cells from tumor-bearing animals, no differences were found whatsoever, as can be seen in Fig. V-10, even upon inter-group comparisons.

These results indicate the potentially intimate association of thymocytes in controlling the immune response. The regulation of the immune response has been a most perplexing problem until recently when much has been discovered. The role of suppressor cells in the regulation of the immune response is rapidly becoming a cornerstone in modern immunobiology. Suppressor cells have, indeed, been found to be specific or non-

specific in their suppressive function and have been though to originate primarily in the thymus (96). Spleen cell subpopulations have also been cited as suppressor cell habitats. The results of these experiments corroborate the thymocyte suppressor cell, since larger tumors and shorter survival times have been noticed in our experiments in the groups treated with thymocytes from tumor-bearing animals + MOPC104E when compared to groups treated with normal thymocytes. This further indicates that, in tumor-bearers, thymocytes suppress the immune response more than normal thymocytes so a type of "priming" may play a role to maximize suppression to the tumor cells. Studies have indicated soluble mediators as the real suppressive agent produced by the suppressor cells which can be thymocytes or subpopulations of spleen cells (96). The results of the T-cell involvement with thymocytes are interesting, but somewhat perplexing, since the thymocytes seem to have no effect on T-cell populations. This may be because their effect is masked by all the cytolytic T-cells in the fraction, and hence suppression is not manifested (and neither is cytolysis). In this experiment, in any case, T-cells did not cause destruction of tumor cells when compared to the control which received only MOPC104E.

These experiments confer upon the reader the fact that thymocytes are indeed very important and further study in this area is certainly warranted. However, much technical progress is necessary to efficiently separate and isolate the many subpopulations of cells in the spleen and in the thymus to intelligently study these phenomena.

The study described in this volume can be considered to be of an introductory nature designed to probe into the basic immunobiology of the plasmacytomas studied. However, it must be recognized that in order to be a totally complete study, more experiments would be necessary. One important experiment which requires investigation to complete this fundamental study is a probe into the immunological specificity of the reactions observed, whether protective or not, afforded by the various treatments. With this study, one could then also make general conclusions about the antigenic similarities of the plasmacytomas investigated. Other studies which could follow include a concerted effort to specifically determine the cell type(s) involved in either protecting or

threatening the individual. This study has shown that many cell types are involved and that T-cells, B-cells and whole spleen cells can protect but do not always do so. The reason for this is unknown, but some discussion has been made concerning this paradox. Further experimentation with specific agents (e.g. anti-Ly antisera) could conceivably pin-point the cell types specifically involved and in this way make contradictory results more amenable to explanation. Determining the exact cell type(s) involved would also assist greatly in explaining the dynamic nature of the immune response, which is undoubtedly intimately associated with the various cell types involved in shaping an immunological attack.

CHAPTER VII

VII CONCLUSIONS

The following conclusions can be drawn from the experimental results of this study.

(1) Antigenicity of plasmacytomas is variable. MOPC104E and HOPC-1 are relatively strongly antigenic. On the other hand, the antigenicity of J606 and S104 is comparatively weak.

(2) Whole spleen cells play a protective role, in some cases, from tumor-bearing animals, and normal spleens do not exert much protective capacity when injected together with tumor cells. Separated T-cells and B-cells protect to varying degrees regardless whether they are from normal or tumor-bearing animals, but their action depends on the tumor investigated. Immunological enhancement could occur with any subpopulation or whole spleen cells.

(3) Thymocytes play a very important role in the regulation of the anti-tumor response. The thymocytes from a primed (i.e. tumor-bearing) animal may be more suppressive to the immune response.

(4) Adherent cells removed by nylon wool columns may also suppress the destructive effect of T and B-cells on the tumor.

(5) Effector and suppressor (enhancing) immune reactions co-exist in tumor bearing hosts. This indicates that the various suppressing mechanisms that may exist in tumor bearing hosts do not eliminate or inactivate completely the effector arm of the anti-tumor response. However, a radiosensitive host component, which was indicated to amplify the anti-tumor effect of such effector cells in normal recipients, may be deficient in tumor-bearing hosts.

(6) The balance between various effector and suppressor lymphoid cells is very dynamic in tumor-bearing hosts and easily tilted towards tumor destruction or enhancement. Opposite effects (i.e. destruction vs. enhancement) may occur in individual animals of an identically treated group.

CLAIMS TO ORIGINALITY

1. The investigation of the antigenicity of J606, HOPC-1 and S104 has never been attempted before, although MOPC104E has been studied previously. In contrast with other investigations, MOPC104E was shown to be immunogenic.
2. The use of nylon wool columns to study the effects of various spleen cell subpopulations on the growth of plasmacytomas has not been studied previously.
3. The *in vivo* demonstration that effector and suppressor cells co-exist in tumor-bearing hosts is new and original. This finding should facilitate further analysis of the immunobiology of tumor-host interactions.

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