The University of Manitoba

MURINE NATURAL ANTI-TUMOR ANTIBODIES

by

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To my husband, Sam, and my parents.

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ABSTRACT

The objectives of our work were to investigate whether natural antibodies (NAb) contributed to host resistance to tumors and to gain some understanding about the mechanisms that regulate the production of NAb. Our first study was designed to investigate whether natural antibodies bound *in vivo* to tumor cells. We found that several tumors rapidly acquired immunoglobulin after being inoculated intraperitoneally into syngeneic mice. We demonstrated that at least part of this immunoglobulin was anti-tumor antibody in experiments in which the Ig was eluted and the specificity of the rebinding was examined. Furthermore, the L5178Y-F9 tumor became susceptible *in vitro* to exogenous complement when examined within 18 hours of i.p. growth.

Our second project was a correlative study in which the tumor frequency of selected tumor variants (clones) was compared to their susceptibility to NAb plus complement, natural killer cells (NK) and activated macrophages, and the host resistance to the tumors was related to the levels of NAb and NK cell lysis. The tumor frequencies of variants of the L5178Y lymphoma correlated with their ability to bind syngeneic NAb while the tumorigenicity of the SL2 variants correlated with their susceptibility to NK cell lysis. However, studies of the ontogeny of the *in vivo* resistance and of NAb and NK cells revealed that more than one mechanism is probably involved in the host resistance to each individual tumor, since the host resistance did not correlate perfectly with NK or NAb activity. Further evidence in

favour of the hypothesis that NAb can participate in the rejection of incipient tumors was obtained in a Winn type assay. It was found that NAb coated P815-16 tumor cells were less tumorigenic than control cells.

In subsequent studies we attempted to elucidate some of the factors involved in the regulation of NAb production. We found that NAb levels were unaffected by the lack of a functional thymus and that microbial products could stimulate the production of these antibodies while a macrophage toxin, silica, strongly inhibited it. We suggested, therefore, that NAb production was thymus-independent and was probably non-specifically regulated by macrophages. Genetic studies showed that high levels of NAb were inherited recessively and the production of the antibodies reactive with the L5178Y-F9 was MHC-linked. The MHC restriction was probably due to self tolerance, since the allogeneic natural antibodies recognized MHC associated antigenic determinants. Another antigen was identified on the SL2 lymphoma, since this tumor cross-reacted more extensively with DBA/2 thymocytes than with the other tumors when syngeneic serum was examined. The CBA/N mutant was deficient in the antibodies that recognized the SL2-5 and YAC-1.3 but not in those reactive with the L5178Y-F9 lymphoma and IgG NAb were detected against the SL2-5 but not against the other tumors. It could be concluded that although NAb can be regulated non-specifically by macrophages, selective regulation of NAb specificities also occurs.

The regulation of another natural resistance mechanism, NK cells, was also studied and compared to natural antibodies. Some similarities in the two mechanisms were observed. In

particular, both NAb and NK activity increased in response to reticuloendothelial stimulants, but there were also some differences, notably in the ontogeny and in the genetics. Natural killer cell activity was short lived, while the NAb against the same target did not decrease with age. High levels of natural antibodies were inherited recessively, while NK activity was inherited dominantly. The specificities of both mechanisms, studied by comparing absorption of NAb and inhibition of NK cell function with several tumors were quite comparable, although there were some exceptions. These studies suggested that some of the antigenic determinants recognized by NK and NAb were concomitantly expressed, while others were independent.

PART 1

INTRODUCTION

PART 1 - INTRODUCTION

In 1959 Thomas, in an attempt to rationalize the existence of homograft rejection, proposed that the biological function of this reaction "has to do with the universal requirement of multicellular organisms to preserve uniformity of cell type and to prevent mutant cells from colonizing and flourishing" and he predicted that "the phenomenon of homograft rejection will turn but to represent a primary mechanism for natural defence against neoplasia....".

The concept of "immunological surveillance" was further developed by Burnet during the following decade.

Burnet restated the theory and proposed "that the immune system primarily and probably exclusively responsible for immunological surveillance and for its laboratory equivalent, homograft immunity, is the thymus-dependent system and that the plasma-cell, antibody system plays no part" (Burnet, 1970).

The theory made several predictions that when experimentally tested failed to provide supporting evidence (Möller and Möller, 1976).

The most damaging evidence against the thymus-dependent surveillance comes from the observation that congenitally athymic mice do not develop a higher number of spontaneous tumors than euthymic mice, although they are unable to reject skin allografts (Rygaard and Povlsen, 1976). In addition, the frequency and latency of chemically-induced tumors are the same in normal mice and athymic or ALS-suppressed mice (Stut-

man, 1974; Gillette and Fox, 1975).

However, athymic mice succumb to bacterial and viral infections if care is not taken to control their environment (Rygaard and Povlsen, 1976) and they have an increased incidence of virally induced tumors (Allison et al., 1974; Vandeputte et al., 1974). This high susceptibility of athymic mice to exogenous pathogens is indeed sufficient evidence for the "raison d'etre" of the thymus-dependent immune system.

The rejection of the thymus-dependent immune surveillance theory leads to three possibilities:

(1) Surveillance of neoplasia does not exist (Möller and Möller, 1979);

(2) Surveillance is mediated by mechanisms that are not immunological in nature (Apffel, 1976);

(3) Immune surveillance does exist but it is mediated by thymus-independent mechanisms.

Three thymus-independent mechanisms are currently being studied as possible contributors to immune surveillance: natural cell mediated cytotoxicity or natural killer (NK) cells, natural antibodies (NAb) and macrophage mediated natural cytotoxicity. These three mechanisms of natural surveillance are reviewed in this introduction.

A. NATURAL CELL MEDIATED CYTOTOXICITY

Natural cell mediated cytotoxicity (NCMC) had been observed for many years, but it has become only recently the subject of intense studies. The basic observation is that cells from normal individuals of a number of species can kill tumor

and other target cells without being intentionally sensitized or immunized against the target antigens.

Takasugi and co-workers (1973) and Oldham et al. (1973) first described the phenomenon that lymphocytes from normal, healthy human donors frequently had greater reactivity against tumor lines than "immune" cancer patient lymphocytes. At about the same time, NCMC was also observed in mice (Herberman et al., 1973; Greenberg and Playfair, 1974), and rats (Nunn et al., 1973), and has since been found in several other species including birds (Linna et al., 1977). Spontaneous cytotoxic leukocytes have been also described in some invertebrates (Boiledieu et al., 1977; Hostetter and Cooper, 1972). Although the phylogenetic relationship between invertebrate and vertebrate effector mechanisms is not known, the latter observation suggests that NCMC could be a primitive defence mechanism that has been conserved, probably with some changes, throughout evolution.

A.I Characteristics of NCMC, Organ Distribution and Ontogeny

In this section, the characteristics of non-macrophage cell mediated cytotoxicity will be discussed. Macrophage mediated tumor cell destruction will be described in section C.

The difficulty in summarizing the characteristics of NCMC is that the phenomenon is not uniform and depends on the assay system.

In mice a short term assay (4 to 6 hours) and several target cells, but in particular the YAC lymphoma, have been most extensively used. Using this system, it has been found that the effector cells, called natural killer cells or NK cells, are easily detectable in spleen and peripheral blood. They are

less abundant in bone marrow and lymph nodes and are undetectable in the thymus (Herberman et al., 1975a; Kiessling et al., 1975a).

The ontogeny of NK cell activity is different in mice and humans. NK cells in mice appear about 4 or 5 weeks of age, peak between 5 and 8 weeks and are almost undetectable by 12 weeks of age (Kiessling et al., 1975a; Herberman et al., 1975a). In contrast to the marked age dependence of NK reactivity in rodents, age has not been found to have a major effect on human NK reactivity (Takasugi et al., 1973; Oldham et al., 1975). An age independent NCMC has been described also in mice (Stutman et al., 1978). The cytotoxicity in this system is detected on a long term assay, 18 to 24 hours, in contrast to the classical NK cells that are usually studied in short term assays (4 to 6 hours). Other parameters also differ between the two cells, e.g. NK cell activity is not detected in the thymus, while the age independent NCMC is detected in this organ (Stutman et al., 1978).

A.II Organs Influencing NK Cell Activity

As previously mentioned, NCMC cells are thymus-independent inasmuch as the level of cytotoxicity is unaffected (Stutman et al., 1978) or increased in the absence of a functional thymus (Kiessling et al., 1975b; Herberman et al., 1975a).

Although in mice the spleen exhibits high levels of NK cell activity, the absence of this organ, either due to a genetic mutation (congenitally asplenic mice) or to neonatal splenectomy, does not affect NK cell levels in the individual (Haller et al., 1978; Herberman and Holden, 1978).

Evidence for the bone marrow origin of NK cells was obtained in two experimental systems: (i) treatment with the bone seeking isotope ^{89}Sr caused a marked decrease in splenic NK cell function (Haller and Wigzell, 1977) and (ii) adoptive transfers of bone marrow (an organ that in itself has low NK function) coferred to the irradiated recipients the NK cell status of the donor (Haller et al., 1977a).

A.III Cell Type

NK cells are nonadherent, nonphagocytic cells and are resistant to cytolysis when treated with anti-macrophage antibodies in the presence of complement (Kiessling et al., 1975a, b; Sendo et al., 1975, Herberman et al., 1975b; Ojo and Wigzell, 1978a). Therefore, they can be classified as non-macrophages. Furthermore, NK cells have the morphological appearance of small lymphocytes (Kiessling et al., 1975b).

Since they lack surface immunoglobulin (Kiessling et al., 1975b) and complement receptors (Herberman et al., 1975b), they are not mature B cells.

Receptors for the Fc of the IgG molecule are easily detectable on human NK cells (Peter et al., 1975; West et al., 1977). In contrast, the presence of Fc receptors on the surface of murine NK cells has been more difficult to detect. However, using a sensitive method, Herberman and co-workers (1977a) were able to demonstrate that low affinity Fc receptors are expressed on at least a portion of murine NK cells.

The age independent NCMC is also mediated by a cell that is not a mature T cell, nor a B cell, nor a macrophage (Paige

et al., 1978). This cell, called natural cytotoxic cell (NC), differs from the classical NK cell in several parameters including its presence in the thymus, strain distribution and its adherence to nylon wool (Stutman et al., 1978; Paige et al., 1978).

A.III.(a) The relationship between T cells and NK cells.

The relationship between T cells and NK cells is a point of contention. It is known that NK cells are not mature T cells, but it has been argued that they may be immature pre-T cells (Herberman and Holden, 1978). As in the case of the Fc receptors, T cell markers were not found on murine or human NK cells when traditional methodology was used (Kiessling et al., 1975b; Herberman et al., 1975b; Sendo et al., 1975). However, several more recent reports have described that human NK cells can form rosettes with sheep erythrocytes (West et al., 1977) and that murine NK cells are sensitive to repeated treatments with anti-Thy-1 serum plus complement (Herberman et al., 1978).

The observations that NK cells exhibit a low density of T cell markers and that athymic mice express higher NK activity than their euthymic controls has led to the hypothesis that NK cells are T cell precursors and that they accumulate in the athymic mice due to the absence of the thymus (Herberman and Holden, 1978). Opponents of this hypothesis, however, point out that the Thy-1 antigen is also expressed on a variety of nonlymphoid cells (Clarck and Harmon, 1980) and that the high levels of NK cell activity in athymic mice could be the result of several phenomena. For example, stimulation of NK cell

function due to pathogens that proliferate to a greater extent in athymic mice than in euthymic, normal mice.

A.III.(b) The relationship between NK cells and antibody-dependent cell mediated cytotoxicity (ADCC).

When comparing ADCC to NCMC, it is important to recognize that several cell types are able to mediate ADCC. Cytotoxicity of antibody coated chicken erythrocytes is mediated by a monocyte type effector cell that expresses high affinity Fc receptors and C3 receptors (Greenberg et al., 1975), three characteristics that distinguish it from the NK cell, and indeed the murine NK cell could not be shown to contribute to ADCC against IgG coated chicken erythrocytes (Kiessling et al., 1976a). The effector cell that mediates ADCC against nucleated cells is, however, of a different nature (Greenberg et al., 1975). Recent comparisons of NCMC and ADCC using nucleated target cells have revealed striking similarities between these cell types. They were found to have similar ontogeny, tissue distribution and strain distribution patterns (Ojo and Wigzell, 1978b; Santoni et al., 1979). Furthermore, when analyzing fractionated cell populations, it was found that ADCC and NK activity were present in the same fraction (Ojo and Wigzell, 1978b). Interestingly, a tumor (P815) that was insensitive to NK cell cytotoxicity became susceptible when coated with alloantibodies (Ojo and Wigzell, 1978b).

A.III.(c) Antigens that can be detected on NK cells.

Glimcher and co-workers (1977) found that NK activity could be eliminated with an anti-Ly 1.2 antisera plus complement. Further studies demonstrated that the antibodies reac-

tive with the NK cells were not the anti-Ly 1.2, but contaminant antibodies. Based on this information and using the appropriate strain combinations, Cantor and co-workers (1979) elicited an antiserum that reacted with NK cells but not with T cells. The antigen was denominated NK-1 and at least two allelic determinants were found. Another antiserum that is able to abrogate NK cell activity in the presence of complement, but that also inhibits NK cell function by itself, is the anti-Ly 5 antiserum (Komuro et al., 1975). The antigen is expressed in two allelic forms and although it was thought to be a T cell antigen, it is now known that it is also expressed on a small but significant number of non-T cells in the spleen and bone marrow (Cantor et al., 1979). Since the expression of the allelic forms in the NK cells follows those on T cells, it was concluded that the antigen on natural killer cells was the Ly 5 itself or the product of a gene closely linked to the Ly 5 gene (Cantor et al., 1979).

A.IV Genetic Control of NCMC

The study of the genetic control of NCMC is hampered by several factors, e.g. environmental stimulation can alter NK levels such as to make it difficult to classify strains with low or high NK function. Even so, several attempts have been made and some conclusions could be obtained. Kiessling and co-workers (1975a) were able to classify mouse strains according to their lytic capacity into low, intermediate and high. Genetic crosses revealed that high levels of NK cells were inherited dominantly (Petrányi et al., 1975). Occasionally, the crosses of intermediate strains gave rise to F_1 hybrids

of high NK cell activity, suggesting that gene complementation had occurred (Klein et al., 1978). When backcross studies were done, it was found that NK cell activity was weakly linked to the H-2. In experiments in which the YAC lymphoma was used as target cell, it was concluded that the responsible gene was probably some distance from the H-2 (Kiessling and Wigzell, 1979). In other studies in which the EL-4 lymphoma was used as target cell, genes within the H-2 complex seemed to control NK cell levels (Harmon et al., 1977).

Genes outside the H-2 can also control NK activity. For example, a mutation on chromosome 13 (bg/bg) dramatically affects the levels of NK (Roder and Duwe, 1979).

Because interferon (IF) activates NK cells (see section A.VI), it is possible that genes affecting IF production also influence NCMC. Some experimental evidence suggests that that is the case: when NK reactive CBA mice are injected with the interferon inducing agent Tilorone, they show a good IF response and an increase in NK activity. In contrast, after Tilorone treatment AKR mice do not develop IF and their NK remains low (Clark and Harmon, 1980). However, AKR mice do show good augmented NCMC if they are injected directly with IF (Gidlund et al., 1978). It could be concluded from these experiments that the low levels of NK cell activity in AKR mice are due to a defect in interferon production, and that in an indirect fashion genes regulating interferon production also control NK activity.

In summary, NK cell function is inherited codominantly and seems to be under polygenic control, one of the responsible

genes being an MHC linked gene.

A.V Specificity of NK Cells

Very little is known about how NK cells recognize and kill target cells.

NK cells can lyse syngeneic, allogeneic and xenogeneic tumor targets (Petrányi et al., 1974; Santoli et al., 1976; Haller et al., 1977b; Nunn and Herberman, 1979; Hansson et al., 1978).

Recently it has been reported that NK cells can lyse targets lacking MHC determinants (Stern et al., 1980), strongly suggesting that NK cells can recognize antigenic determinants not associated with the MHC. However, MHC associated determinants may also be targets for NCMC, since there is some evidence that NK cells can recognize the MHC associated hybrid histocompatibility 1 (Hh-1) antigen (Kumar et al., 1979).

Initial studies with murine lymphomas suggested that NCMC was directed against C-type virus-associated cell surface antigens, since a correlation was found between the presence of C-type particles and target cell susceptibility (Kiessling et al., 1975a; Herberman et al., 1975a; Sendo et al., 1975). In contrast, using other effector-target cell combinations, Becker and co-workers (1976) found no correlation between susceptibility to NK cell lysis and the presence on targets of type or group-specific antigens of murine C viral proteins. Furthermore, human cell lines superinfected with xenotropic mouse C-type viruses expressed surface MuLV antigens but showed no difference in their sensitivity to

NK cells compared to uninfected lines (Kiessling et al., 1978). These findings suggest that NK cells do not always recognize murine C-virus-associated antigens, but do not eliminate the possibility that NK cells can also recognize virus associated antigens.

Although transformed cells are, in general, the most susceptible targets to NK cell lysis, some normal tissues are also susceptible. NK mediated lysis of peritoneal and bone marrow cells has been shown (Nunn et al., 1977; Ono et al., 1977). Normal cells highly susceptible to NK lysis are fetal fibroblasts (Saksela et al., 1979) and thymocytes from young mice (Hansson et al., 1979). In addition, genetic resistance to bone marrow grafts may be mediated by a cell similar to the NK cell (Kiessling et al., 1977). Based on these observations, it has been suggested that NK cells contribute to the maintenance of homeostasis and to the regulation of hematopoiesis (Cudkowicz and Hochman, 1979).

Since NK cells can lyse a large number of targets but, at the same time, there are cells, normal and neoplastic, that are resistant to NK cell lysis, it is apparent that NCMC is at least selective if not specific. Selectivity could arise from two different mechanisms: (i) the effector cell may recognize a limited number of antigenic determinants that are present on a large number of cells, but not in all cells, and (ii) the effector cells express polyclonality of the type described for B or T cell populations. It is at present difficult to distinguish between these two possibilities, although from inhibition studies (Herberman et al., 1975a, Sendo et al.,