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**IMMUNE RESPONSES IN AKR MICE: THEIR RELATIONSHIP
TO THE DEVELOPMENT OF SPONTANEOUS LEUKEMIA
IN THIS STRAIN**

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IMMUNE RESPONSES IN AKR MICE: THEIR RELATIONSHIP
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Abstract

Studies of immunologic capacity of the preleukemic AKR mouse have shown that this animal is capable of normal humoral immune responses. Certain aspects of cellular immunity (MMI, GVH reactivity of spleen cells) have been shown to be defective, whereas other cellular immune functions have been found to be intact (tumor and skin allograft rejection).

These mice have a life-long infection with GMuLV. They have a genetically determined impaired immune response to this virus and a defective but ongoing immunity to the cell surface antigens produced by GMuLV. These factors allow for a continued viral replication, resulting ultimately in levels of virus in the thymus which trigger the development of the characteristic thymic lymphoma of the AKR strain.

Abbreviations

ALS	- antilymphocytic serum	MMI	- macrophage migration inhibition
AMP	- adenosine monophosphate		
BCG	- Bacillus Calmette Guerin	SRBC	- sheep red blood cells
BUDR	- bromodeoxyuridine		
GCSA	- Gross cell surface antigen		
GMuLV	- Gross murine leukemia virus		
gs	- group specific		
GSA	- Gross soluble antigen		
GVH	- graft vs. host		

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Introduction

AKR mice have a unique susceptibility to development of a characteristic form of lymphoma. It begins in the thymus and disseminates to the lymphoid tissues. Nearly all of the animals of this inbred strain will die of this disease between the ages of 6 and 14 months---with a peak incidence at 9-10 months of age. The disease is etiologically related to a virus---the GMuLV, and the animals of the AKR strain are born with, and maintain a chronic infection with this agent.

The fact that this animal model represents one of a naturally occurring viral infection resulting in lymphoma makes it a relevant one for studies in neoplastic disease. The following questions arise when one considers the immune reactivity of this strain. Is this animal with a chronic viral infection immunosuppressed? Does the fact that the thymus is the primary target organ for this disease relate to immunologic alterations in this mouse strain? Does the AKR mouse demonstrate impaired immunologic responsiveness to virus and virus related cell surface antigens? Do factors that stimulate immune responses suppress lymphomagenesis in the AKR? In an attempt to answer these questions, the following review will discuss the general humoral and cellular immune responses of the AKR mouse, as well as the specific immune responses associated with GMuLV infection and attempts that have been made to enhance them.

Humoral Immunity - Metcalf and Moulds (26) have shown that preleukemic AKR mice have normal formation of hemolytic plaques by spleen cells, as well as normal hemolysin and hemagglutinin titers after stimulation with SRBC. In our laboratory we have also demonstrated normal antibody responses to SRBC in AKR mice, as well as immunoglobulin levels comparable to those found in normal and GMuLV infected C3H mice (8). Production of a humoral response to a normal cell surface antigen is evident by the fact that 5-12 week old AKR mice are used for the production of theta C3H antisera, a serum which detects thymus derived lymphocyte populations in mice (32).

Cellular Immunity - Several studies have indicated that the cellular immune response in AKR mice is impaired. AKR female mice have been shown to have prolonged rejection times of syngeneic male skin when compared to that of C57BL mice. The latter strain rejected all of the grafts within

40 days, whereas, only 2 of 16 grafts were rejected by the AKR animals at 55 and 77 days, respectively (6).

An impaired MMI response by tuberculin sensitized lymphocytes in AKR mice when compared to that of normal C3H mice has been demonstrated (8). This was found in animals of all age groups. A similar impairment of MMI was found in C3H mice inoculated with GMuLV, suggesting that viral infection, per se, may be related to this observation. A specific inability of AKR spleen cells to produce GVH reactions has been demonstrated in AKR x SJL F₁ hybrids (13). Using the Simonsen assay for splenomegaly, suckling hybrid mice were inoculated with parental spleen cells. Positive spleen indices were consistently found after SJL spleen cell inoculation, but were, for the most part, either negative or weakly positive after AKR cell inoculation. Immunizing the AKR donors with hybrid cells had no effect in enhancing this weak response. Similar findings were obtained with the reverse hybrid cross. Pre-inoculation of donors with GMuLV did not alter this response pattern. In contrast to the MMI study, inoculation of C3H donors with GMuLV did not alter their normal capacity to produce GVH reactions in C3H x SJL F₁ hybrids.

Evidence of normal cellular immune reactivity in the AKR is demonstrated by the fact that they are able to reject allografted lymphoma cells from H-2 compatible mice (12).

The capacity of AKR mice to reject allogeneic skin grafts was also found to be intact. AKR (H-2^k) mice were grafted with C3H/HeJ (H-2^k) and C57BL (H-2^b) skin from animals of the same sex. Both types of graft showed beginning rejection at 18 days and were completely rejected at 20 days (14). Other investigators (11) have also presented evidence for the immunocompetence of AKR lymphocytes in both immediate and delayed hypersensitivity reactions.

Some alteration of cellular immunity might be expected in the AKR mouse since thymic derived lymphocytes are those that mediate such responses, and could be impaired in their function by GMuLV. This degree of immunosuppression apparently does not have functional significance, for this animal is able to cope adequately with the numerous infectious agents which must always be present in the normal laboratory environment. The poor immune response to GMuLV is an exception.

Antigens Derived from and Related to GMuLV in the AKR Mouse - Evidence for Immune Responses to Them

1. Virion Antigens - GMuLV contains gs antigens. These antigens are internal in the virion and are shared by all C-type RNA tumor viruses. They are non-infectious and are prevalent in the murine population, including AKR (30). The gs-1 antigen is species specific, and the gs-3 is inter-species specific. Gs-1 is found in embryonic normal and tumor tissue. Natural antibodies to these antigens are not found in several mouse strains tested, thus suggesting that immunologic tolerance to them exists (18). Recent studies finding complement fixing antibodies immunologically related to the internal viral components in the renal glomeruli of AKR mice cast some doubt on this concept (31). The type-specific envelope antigens of GMuLV are weakly antigenic and antibodies to them are not found in the serum of AKR mice. They have been demonstrated in sera of mice from some other strains (3).

2. Cell Surface Antigens - Infection with GMuLV in the newborn AKR results in the development of antigens on the surface of the cell directed by GMuLV infection. They are not part of the virion. Such antigens are found on normal lymphoid cells, as well as on lymphoma cells. One example of this is the GCSA (29). Also, a second antigen, the GSA, is found in serum and tissue extracts of normal and leukemic AKR mice (2). It could represent exfoliated GCSA. Both of these antigens are present from birth, and since GMuLV infection is not cytodestructive in mouse lymphoid tissue, the cells with GCSA can be considered to be continuously productive of virus.

Such antigens on lymphoma cells of AKR mice will produce antisera in allogeneic mice which are cytotoxic for cells from lymphomas induced by GMuLV (12, 37). These antisera are also able to induce specific transplantation resistance to syngeneic lymphoma cells (12). Virus neutralizing antisera are not produced by the cell immunization experiments in mice (9, 12).

Conversely, when allogeneic lymphoma cells are given to AKR mice, absent or weak cytotoxicity for syngeneic cells is demonstrated and transplantation resistance cannot be achieved, indicating a defective immune response to cell surface antigens of Gross specificity (12, 38). Although AKR mice have GCSA positive cells present throughout life, natural antibody to GCSA has not been demonstrated in their

serum. Such antibody is present in animals of strains resistant to lymphoma development (1). It has been shown, however, that prolonged immunization of AKR mice with syngeneic tumor cells can produce cytotoxic antibodies against the immunizing cells; and, that inoculation of allogeneic Gross virus induced lymphoma cells can improve survival of AKR mice challenged with small doses of syngeneic lymphoma cells (7). Additional evidence for an immune response to the GCSA is shown by the finding of immune complexes in the glomeruli of AKR mice which contain immunoglobulin specific for the GCSA (31). Therefore, the AKR can be considered to have a definite, but defective immune response to GMuLV infection.

Another GMuLV related cell surface antigen, which is present on lymphoid cells of normal mice, has been described (35). This is called G_{IX} . GCSA is demonstrated in very small amounts on thymocytes of AKR mice. These mice, as mentioned above, have easily demonstrable GCSA on other normal lymphoid tissue cells, as well as on lymphoma cells. G_{IX} is demonstrable in AKR thymocytes, as well as all other lymphoid tissues and lymphoma cells. G_{IX} is related to GMuLV infection, i.e., it appears de novo in cells that become productively infected with GMuLV. It exhibits Mendelian inheritance (2 genes) but it is not clear, as yet, whether it belongs to the viral or cellular genome. The difficulty of demonstrating GCSA on thymocytes and presence of G_{IX} suggests that these cells are infected with MuLV, but that full expression of the virion in them has not been achieved. Perhaps, as suggested by Stockert, et al. (35), the G_{IX} is the chromosomal integration site of MuLV and in AKR productive viral infection of thymocytes with resulting GCSA occurs later than productive viral infection of other lymphoid cells.

3. Methods of Increasing Immune Response to these Antigens as they are Present on Normal and Lymphoma Cells - There are several experiments which show that AKR mice can be induced to mount a more effective immune response to lymphoma cells. Treatment of cells with an enzyme to enhance their antigenicity (neuraminidase) and administration of such cells to AKR mice decreases the incidence and prolongs the latent period to lymphoma development (15). Some AKR mice with diagnosed lymphoma, given neuraminidase treated leukemic thymocytes after a course of chemotherapy, have been shown to survive without evidence of disease (16). Pre-treatment of the AKR mice with BCG reduces the mortality

from lymphoma after low doses of cells from a long transplanted syngeneic line (7). BCG given to 2.5 month old AKR mice, followed in 12 days by an inoculation of 2000 syngeneic lymphoma cells, also lowers the incidence and prolongs the latent period to spontaneous lymphoma development (20). A chronic infection with a protozoan, Besnoitia jellisoni, has also recently been shown to delay the onset of spontaneous leukemia in AKR mice (23). These animals have a natural resistance to infection with this protozoan.

All of the above mentioned experimental maneuvers have the common feature of increasing immune reactivity, i.e., in the AKR factors which, perhaps nonspecifically, stimulate the immune response and can enhance the hosts ongoing, but defective, immune response to GMuLV and its induced cellular antigens. Inhibition of growth of syngeneic lymphoma cells, transplanted to young AKR mice, with a synthetic polynucleotide complex, as reported by Meier, et al. (25), may also be due to this same mechanism through the known effect of synthetic polynucleotides on increasing cyclic AMP levels. Increased immune responsiveness has been shown to result from the use of substances which increase cyclic AMP (5).

Studies utilizing antilymphocyte antibodies in AKR mice have shown conflicting results. An acceleration of onset of spontaneous disease with ALS suggests that there is an immune response in pre-lymphomatous mice which can be suppressed; and protection of mice with ALS after inoculation with syngeneic tumor cells indicates that the antilymphocyte antibody may have a direct toxic effect on the lymphoma cells themselves (19). Another study using a different dosage schedule has shown that antithymocyte serum decreased incidence and prolonged latent period of spontaneous lymphoma in AKR mice (36).

4. Cellular Immunity - No firm evidence exists for a cellular immune response to GMuLV or its induced antigens in AKR mice. Metcalf and Wahren (27) found lymphocyte mediated cytotoxicity for syngeneic embryo cells in the pre-leukemic period in AKR mice 3 weeks-9 months of age. This was demonstrated by placing concentrated numbers of AKR spleen and lymph node cells on AKR embryo monolayers and demonstrating lytic areas on the monolayers at the site of the concentrated lymphocytes. The authors suggested that this was due to a specific cytotoxic immune reaction of AKR cells to the GCSA on the embryo monolayer.

The possibility of adoptively transferring a cellular

immune response to syngeneic lymphomas is being investigated in this laboratory. Preliminary results showed that AKR mice can be protected from developing lymphoma after administration of small doses of syngeneic lymphoma cells. 8×10^7 syngeneic lymphoid cells pooled from AKR mice immunized with an allogeneic GMuLV induced tumor were inoculated intravenously. Control animals received a similar dose of normal AKR lymphoid cells. The next day all of the animals were challenged with lymphoma cells. Eight of 11 mice treated with the "immune" cells survived and were sacrificed in good health 2 months later. Three mice of the treated group and all of the 16 controls died of lymphoma 22-30 days after receiving the challenge of 10^3 lymphoma cells subcutaneously.

Comparison of Immune Responsiveness in the AKR with that in the GMuLV Resistant C57BL Strain

A comparison of immune responses to the Gross antigens in the AKR (H-2^k) with C57BL (H-2^b) strain is presented on Table 1. Such a comparison emphasizes the significance of the above mentioned data. First, there are different genetic factors in each strain which regulate host response to GMuLV. There are two genetic loci, Rgv-1 and Rgv-2. These are dominant alleles endowing the host with a capacity for resistance to Gross virus (21). The AKR can be considered to be homozygous for the recessive allele for susceptibility at each of these two loci, whereas, the C57BL is homozygous for the allele for resistance at each locus. This resistance is mediated through the host immune response and not a property of host cells since it has been shown that C57BL embryo fibroblasts in vitro do not differ in their susceptibility to GMuLV infection when compared with fibroblasts derived from H-2^k strain mice (24). These genetic factors probably account for the differences in immune response described in the following paragraphs.

The gs antigen for MuLV is found in the two strains beginning in embryonic life (17). The titer is higher in AKR. Intact virus is not found in AKR or C57BL embryo cells, but its release can be induced on culture (28) or by treatment of cultured cells with BUdR (22). GMuLV virions are found shortly after birth (33) and presumably, because of the above mentioned genetic factors, are present in greater amounts in the AKR mouse. However, expression of virion increases with age in both animal strains.

The cell surface antigens derived from GMuLV infection, GCSA, GSA, and G_{IX}, are present on AKR lymphoid cells

and increase in amount as the animal ages. They are not found on C57BL cells, whereas, natural antibody to cell surface antigens is present and increases with age in mice of this strain (1). As mentioned before, AKR mice do not have antibody to cell surface antigens in their serum. The continuing antibody response in the AKR is shown by the presence of immune complexes in the renal glomeruli which also increase with time (31). The C57BL mouse has not been studied from this point of view. Significant titers of antibody to viral envelope (virus neutralizing antibody) have not been demonstrated in either strain (30). It is of interest to note at this point, that mouse typing serum for GCSA is prepared by inoculating AKR lymphoma cells into C57BL mice. Such serum has little neutralizing activity and no precipitating activity against GMuLV (9). The increasing titer of virus in the AKR results from a defective host immune response to the cells persistently infected with MuLV and altered immunologically by virtue of their infection. The C57BL mouse is able to immunologically destroy such cells, presumably, shortly after they are formed, therefore, keeping viral replication at a low level. Experimental induction of lymphoma in this strain has been shown to involve the thymus and be morphologically identical to that occurring spontaneously in the AKR (10). Therefore, natural lymphomagenesis is prevented in the C57BL strain because of a genetically directed immune response to cells infected with GMuLV and bearing viral induced cell surface antigens and not by an immune response to the virus itself. Such a theory denies the existence of any specific susceptibility to lymphoma.

Relationship of Viral Replication and Immune Responses to Lymphoma Development in the AKR Mouse

The studies reviewed here allow one to attempt an explanation of lymphomagenesis in the AKR mouse. Its inheritance of gs antigen and virion expression at birth does not differ from other mouse strains. GMuLV infection of lymphoid cells proceeds and such cells develop new virus directed cell surface antigens. Here, probably because of inheritance of the recessive Rgv genes, the picture changes. The AKR host cannot immunologically eliminate these virus infected cells, as can other murine strains. The infected cells persist, replicate more virus, resulting in an increase in numbers of virus infected cells with age. The only evidence of a natural immune response is the finding of antigen-antibody complexes in the glomeruli, i.e., an

immune response in the presence of antigen excess resulting from continuing GMuLV infection. Lymphoma develops in the thymus when the virus reaches a certain critical concentration there. It can be said, then, that the latent period to lymphoma development is a result of the hosts ongoing, but ineffective immune response to GMuLV related cell surface antigens, slowing, but not preventing replication of virus.

There are also other factors which are relevant to the persistence of GMuLV infection in the AKR. It has been shown by Aoki, et al. (4), that the GMuLV of AKR cells has H-2 on its surface, which may make it particularly difficult for the host to recognize as a foreign antigen and explain the absence of antiviral antibodies. Also, evidence presented by Sjögren, et al. (34), points out the fact that the "blocking antibodies" of tumor bearing mice, shown to abrogate the antitumor cytotoxic response of immune lymphocytes, may be tumor specific antigen-antibody complexes. Such complexes, as mentioned above, are known to be present in AKR mice and could protect lymphoma cells from reactions involving cellular immunity.

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TABLE 1
Gross Murine Leukemia Virus
Related Antigens and Host Response

Genetic Factors	AKR		C57BL	
	Recessive Rgv		Dominant Rgv	
Age	<3 mos	>3 mos	<3 mos	>3 mos
gs Antigen	++	++	++	++
GMuLV	+++	++++	±	+
GCSA	Lymphocytes		+++	+++
	Thymocytes		0	0
GSA	++	+++	0	0
G _{IX}	Lymphocytes		++	++
	Thymocytes		0	0
G Natural Antibody	Serum		0	0
	Kidney		++	+++
Antiviral Antibody	Serum		+	+
	Kidney		?	?
Antiviral Antibody	Serum		0	0
	Kidney		0	0