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TECHNICAL COMMENTS

Resistance to Murine Acquired Immunodeficiency Syndrome (MAIDS)

I have performed genetic studies on murine acquired immunodeficiency syndrome (MAIDS) susceptibility that suggest an alternative explanation for the results reported by O. Kanagawa *et al.* (*1*). My results raise the possibility that immune suppression caused by the murine retrovirus (LP-BM5 MuLV) may not result from a change in the pattern of cytokine expression of helper T cells from T_H1 to T_H2 phenotype, as Kanagawa *et al.* suggest. The IL-4–deficient mice appear resistant to disease, while their IL-4–sufficient littermates are sensitive. Kanagawa *et al.* conclude that IL-4 deficiency interferes with disease progression by preventing the T_H1 to T_H2 conversion, which implies that the immune response to the virus is pathogenic, but there are genetic factors that could be at work in this system. The embryonic stem (ES) cell line used to generate the "knock-out" mice is derived from a 129 strain mouse (*2*). Therefore, all IL-$4^{-/-}$ progeny must be homozygous for the 129-derived chromosome 11, and conversely, all IL-$4^{+/+}$ progeny must carry both copies of the C57Bl/6-derived homolog.

Inbred mouse strains vary in their sensitivity to LP-BM5 MuLV disease; C57Bl/6 is a sensitive strain, but I have found that 129/J mice are resistant to LP-BM5 disease. There are three documented bases of resistance in mice: MHC ($H2D^d$), Fv-1^n, and R_{mcf}^R, but there is evidence that other loci may be involved (*3*). In F1 crosses between resistant and sensitive strains, *sensitivity* to disease is dominant, rather than resistance. The precise reason for resistance in 129 mice is not known, but IL-$4^{-/-}$ mice may be resistant to MAIDS because they are homozygous for the 129-derived chromosome 11, rather than the defective IL-4 gene.

The 129-derived chromosome 11 could confer resistance by any of a variety of mechanisms. For example, 129 mice carry an endogenous ecotropic retrovirus on chromosome 11 (*4*) that can be expressed as infectious virus. Prior infection of sensitive C57Bl/6 mice with a nonpathogenic ecotropic virus renders them resistant to LP-BM5 disease (*5*). If the 129-derived virus is expressed in the IL-4–deficient mice, then these mice could be resistant as a result of an endogenous infection, not of IL-4 deficiency.

A second possibility is that 129 mice carry another retrovirus resistance locus on chromosome 11. In selecting IL-$4^{-/-}$ mice, Kanagawa *et al.* may have selected mice that were resistant because they were homozygous for a potential resistance locus, not because they were IL-4–deficient.

The absence of resistance in the IL-$4^{+/+}$ littermates should also be addressed. The F1 parents of these mice are Fv-$1^{n/b}$. Fv-1 should segregate independently of IL-4, because these loci are on different chromosomes. One-quarter of the F2 IL-$4^{+/+}$ littermates should be Fv-$1^{n/n}$, and therefore be resistant to LP-BM5 disease, yet 100% (28/28) of the +/+ mice were sensitive.

IL-4 deficiency could indeed be the reason for LP-BM5 MuLV resistance in these mice; in fact, there are many reasons to expect that interfering with immune function may inhibit disease (*6*). However, the genetic factors of viral resistance, or endogenous retroviruses, or both, need to be excluded before making statements as to the effect of cytokine expression, Th phenotype, or both, on the mechanism of retrovirus-induced immune suppression.

Gary L. Gilmore
Western Pennsylvania Cancer Institute, 720 Gross Street, Pittsburgh, PA 15224

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MAIDS is a retrovirus-induced disease of mice with many similarities to HIV-induced disease in humans (*1*, *2*). Studies of both infectious processes have shown that the progression of disease can be associated with a switch from a cytokine profile of a Type 1 T helper cell (T_H1) (high IL-2, low IL-4, and IL-10) to one in which the cytokines of Type 2 T helper cells (T_H2) IL-4 and IL-10 dominate (*3*). The hypothesis that the balance between T_H1 and T_H2 cytokines may be critical to the pathogenesis of these immunodeficiency syndromes (*3*) appears to be supported by several recent findings. First, IL-12, an agent that stimulates T_H1 differentiation and the production of T_H1 cytokines, has been found to inhibit the development of MAIDS (*4*) and to restore in vitro cell-mediated immune responses in individuals that are positive for HIV (*5*). Second, studies of IL-4–deficient (129/Sv × B6)F_2 mice showed them to be resistant to MAIDS (*6*).

Susceptibility and resistance to MAIDS are genetically determined with prominent

influences made by genes of the major histocompatibility complex (MHC) and by non-MHC loci (*7*). The prototype susceptible strain is C57BL/6 (B6). The 129 strain of mice is highly resistant and shows no signs of disease 38 weeks after infection (Table 1) (*1*). To extend our understanding of the contributions of IL-4 to MAIDS while avoiding potential contributions of 129 loci that might influence disease resistance, we studied an IL-4–deficient mouse. This mutant strain was generated by gene targeting in 129/Ola embryonic stem cells (*8*), breeding with C57BL/6 (B6) mice for seven or eight generations, and then mating heterozygotes to generate IL-4–deficient ($IL\text{-}4^{-/-}$) and control ($IL\text{-}4^{+/-}$ and $IL\text{-}4^{+/+}$) animals. Mice were followed for the development of lymphoproiferation and immunodeficiency through the ninth week after infection. Spleen weights at the third through the ninth weeks showed comparable progression of lymphoproliferation for mice of all three genotypes (Fig. 1A). Spleen cells from the same mice were tested for their proliferative responsiveness to T cell [concanavalin A (ConA)] and B cell [lipopolysaccharide (LPS)] mitogens. No differences were observed in the rate or extent to which these responses were lost in $IL\text{-}4^{-/-}$ or $IL\text{-}4^{+/-}$ mice (Fig. 1B). Semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) techniques were used to examine expression of BM5def and different cytokines. After infection, transcripts for the defective virus were present in comparable amounts in mice of each genotype (data not shown). In agreement with earlier studies of B6 mice infected for the same period of time (*3*), transcripts for IL-2 were slightly decreased and those for IL-10 and IFN-γ were increased as a consequence of infection. IL-4 transcripts were also increased for $IL\text{-}4^{-/-}$ mice and for the controls, although the transcripts derived from the mutated gene do not yield biologically active IL-4 (*8*). Finally, spleen cells from these mice were tested by flow cytometry to detect the development of alterations characteristic of MAIDS, and fixed, stained sections of tissues obtained at autopsy were examined for histopathologic features of this syndrome. These studies revealed no significant differences between cells or tissues from infected control or $IL\text{-}4^{-/-}$ mice (data not shown). These results demonstrate that IL-4–deficient mice are indistinguishable from controls in the development of several prominent manifestations of MAIDS, at least through the ninth week after infection.

To examine this question from another perspective, we studied the 129/Sv strain of mice, which carries an IL-4 transgene expressed in B cells (*10*). Expression of the transgene renders this normally resistant strain susceptible to infection with *Leishmania major* by converting a strongly polarized T_H1 response to one in which T_H2 cytokines are more strongly represented (*11*). Normal 129 mice are also highly resistant to MAIDS; they showed no signs of disease for as long as 38 weeks after infection (Table 1). We reasoned that if IL-4 expression were important for determining susceptibility to MAIDS, infection of the transgenic strain would result in disease. Analyses of infected 129 IL-4 transgenic mice showed that they remained resistant to MAIDS, showing no evidence of significant lymphadenopathy or splenomegaly, and no detectable features of MAIDS, as revealed by flow cytometric or histopathologic studies (Table 1). This contrasts with the rapid induction of disease in C57L mice infected with the same virus preparation (Table 1). In addition, RT-PCR analyses of BM5def expression showed that the transgenic mice cleared the defective virus at least as rapidly as their normal counterparts, even though helper virus expression was greater than that for normal infected mice (Table 1).

Analyses of cytokine gene expression by RT-PCR showed that transcripts for IL-2, IL-10, and IFN-γ were increased in spleens of 129 mice infected for 3 or 6 weeks, a pattern similar to that of MAIDS-resistant BALB/c mice (*2*), except that amounts of IL-4 were not increased in 129 mice. Aside from slight increases in IL-4, only IL-10 transcripts were increased in the transgenics, although other studies have shown that IFN-γ transcripts may be increased in uninfected mice of this strain (*13*).

It was possible that the T_H1 cytokine component of the 129 response to LP-BM5 virus infection was too strong to be overcome by the additional IL-4 generated from the transgene. To test this, the transgenic mice were treated at the time of infection with a neutralizing anti-IFN-γ monoclonal antibody in doses that have been shown to block the highly polarized T_H1 response that develops in 129 mice infected with *L. major* (*14*). In vivo neutralization of IFN-γ had no effect on the resistance of 129 IL-4 transgenic mice to induction of MAIDS through the twelfth week after infection (data not shown), indicating that even in the absence of IFN-γ, enhanced expression of IL-4 was not sufficient to induce susceptibility to MAIDS. Taken together, the studies of the IL-4–deficient mice and the IL-4 transgenics suggest that modulation of IL-4 alone is insufficient to alter the phenotypes of resistance or sensitivity to MAIDS exhibited by 129 and B6 mice, respectively.

As a final approach to understanding the role of IL-4 in MAIDS, we treated B6 mice with anti–IL-4 monoclonal antibody from the time of infection to determine effects on disease induction, or beginning 4 weeks after infection to determine effects on dis-

Table 1. Responses to virus infection of 129, 129-IL-4 transgenic, and C57L mice. Because 129, 129-IL-4 transgenic (both $Fv\text{-}1^{nr}$), and C57L ($Fv\text{-}1^{n}$) mice restrict replication of the B-tropic helper viruses in the LP-BM5 mixture, the N-tropic amphotropic (4070A) pseudotype of 1/27/A1 molecular clone of BM5def (*9*) was utilized to facilitate spread of the defective virus. Mice were infected at 4 to 6 weeks of age with virus stocks generated from supernatants of chronically infected *Mus dunni* cells cotransfected with 1/27/A1 and 4070A DNAs by calcium precipitation. Mice were killed at the indicated times after infection, spleen weights determined, and spleen cells tested by flow cytometry (FACS) for abnormalities characteristic of MAIDS. Selected tissues obtained at autopsy were fixed, sectioned, and stained for histopathologic studies. FACS and histologic studies were used to stage the progression of disease (*11*). Briefly, N indicates indistinguishable from normal; R indicates reactive changes indicative of response to infection but insufficient to warrant a diagnosis of MAIDS; 1, 2, and 3 indicate changes clearly consistent with the diagnosis of MAIDS and of increasing severity. The frequencies of spleen cells producing infectious amphotropic virus were determined by infectious center tests of cells treated with mitomycin C with the use of PG4 feline S^+L^- cells (*12*). Although PG4 cells are not exclusively sensitive to amphotropic MuLV but develop foci when infected by xenotropic and MCF viruses, neither C57L nor 129 spontaneously produce MuLVs detectable by this assay (unpublished data). NA, not applicable; ND, not done. Numbers indicate mean values for two to three mice per time point.

Strain	Weeks after infection	IL-4TG	Spleen weight (mg)	Stage of disease		Amphotropic virus recovery [FFU $(\log_{10})/10^7$ cells]
				FACS	Histopathology	
129	0	−	60	R	R	NA
		+	110	R	R	NA
	3	−	85	R	R	4.3
		+	127	R	R	5.5
	6	−	82	R	R	4.0
		+	138	R	R	5.1
	12	−	100	R	R	2.8
		+	237	R	R	4.5
C57L	0	−	120	N	N	NA
	4	−	414	2	1	4.7
	12	−	671	2	1	ND

ease progression; selective effects of IL-4 deficiency on these stages of disease development could not be determined from studies of IL-4 mutant animals. Analyses of mice treated for 8 weeks from the time of infection showed that antibody to IL-4 had no effect on any parameter of disease or virus expression examined 9 weeks after inoculation (data not shown). Delayed treatment with antibody to IL-4 had only a modest influence on progression of disease. In three experiments examining the effects of delayed treatment, the weights of spleens from mice treated with antibody to IL-4 averaged 73% of those from mice treated with control antibody ($P < 0.05$). This suggests that expression of IL-4 has some limited role in disease progression, but not in induction of MAIDS.

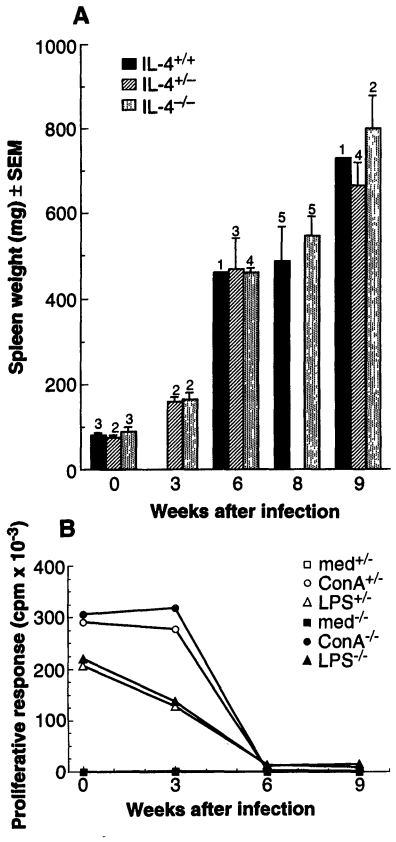

Fig. 1. Responses of B6 mice to infection with LP-BM5 MuLV. Mice from intercrosses of backcross generations 7 and 8 to B6 of a disrupted IL-4 gene of 129/Ola mice were typed by analyses of tail biopsy DNA as wild-type (IL-$4^{+/+}$), heterozygous (IL-$4^{+/-}$), or homozygous (IL-$4^{-/-}$) for the mutation as described previously (*8*). Mice 4 to 6 weeks of age were inoculated intraperitoneally with the mixture of B-tropic, ecotropic, and mink cell focus-inducing MuLV and disease-causing defective virus produced by the G6 clone of SC-1 cells (*9*). (**A**) Spleen weights were measured at autopsy at the indicated times after infection. Bars indicate the mean ± 1 SE for the number of mice indicated above each bar. Both control genotypes were not tested at each time point. (**B**) Proliferative responses of spleen cells from IL-$4^{+/-}$ and IL-$4^{-/-}$ mice. Suspensions of spleen cells obtained from uninfected mice or mice infected with virus for the indicated times were cultured in medium alone (med), or with ConA (5 μg/ml), or with LPS (10 μg/ml). Cultures were harvested for counting at 72 hours after a 6 hour pulse with (^{3}H)-thymidine. Points indicate the mean for two to four mice tested at each time.

The conclusion that modulation of IL-4 expression alone does not have a prominent effect on induction or progression of MAIDS contradicts that reached by others studying IL-$4^{-/-}$ and control (129/Sv × C57BL/6)F_2 mice (6). The reasons for the results obtained by Kanagawa *et al.* (6) are unclear, but may relate to the fact that 129 mice are highly resistant to development of MAIDS and may contribute genes that can lead to disease resistance. Because the IL-$4^{-/-}$ F_2 mice in their study (6) would be homozygous for 129 genes that flank the IL-4 locus, and F_2 IL-$4^{+/+}$ control mice would always be homozygous for B6 genes near IL-4, loci in this region of chromosome 11 may prove to be of crucial importance. It is also possible that other genes from 129 mice, segregating in the F_2 mice, could affect MAIDS resistance. Alleles of dominant genes carried by 129 mice but not by B6 mice [for example, the *nr* allele of *Fv-1* (*14*)] could act to limit spread of helper viruses present in the LP-BM5 virus mixture, thereby affecting cell-to-cell transmission of BM5def virus. Finally, genes from 129 mice, such as those that direct a T_H1 response after infection with *L. major*, could influence the cytokine response to BM5def virus toward a T_H1 profile.

Regardless of the mechanisms involved, it is clear that IL-4 is not required for MAIDS, and the hypothesis that sensitivity and resistance to disease can be understood solely in terms of a T_H1 and T_H2 dichotomy in cytokine expression is not tenable. This does not mean that IL-4 has no contribution to MAIDS sensitivity. Indeed, the observation of a limited effect of delayed treatment with antibody to IL-4 is consistent with a role for this cytokine in the sensitivity of B6 mice. The demonstrations that development of MAIDS is prolonged by concurrent infections with agents that induce strong T_H1 responses [*Toxoplasma gondii* (*15*) or *L. major* (*16*)] and that treatment with IL-12 can retard disease (*4*) indicate that the contributions of cytokines to MAIDS pathogenesis are probably multifactorial. The finding that B6 IL-$4^{-/-}$ and (129 × B6)F_2 IL-$4^{-/-}$ mice differ in their responses to infection suggests that some of these factors are genetically determined and may provide an opportunity to determine the nature of the relevant loci.

Renate A. Morawetz
Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

T. Mark Doherty
DNAX Research Institute, Palo Alto, CA 94304-1104, USA
Nathalia A. Giese
Janet W. Hartley
Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases
Werner Müller
Ralf Kühn
Klaus Rajewsky
Institute for Genetics, University of Cologne, Cologne, D50931, Germany
Robert Coffman
DNAX Research Institute
Herbert C. Morse III
Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases

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Response: We have also investigated the susceptibility of the 129Sv strain of mice to MAIDS. In 129Sv-B6 aggregation chimera mice infected with MAIDS (LP-BM5) virus, lymphocytes derived from MAIDS-resistant 129Sv and -susceptible B6 strains are equally susceptible to MAIDS virus-induced pathology, which includes the expression of the defective MAIDS virus RNA, uncontrolled proliferation, and loss of reactivity to antigen receptor–mediated stimulations (*1*). However, the 129Sv strain is resistant to MAIDS, and we detected no defective MAIDS virus in lymphocytes from 129Sv mice infected with LP-BM5 virus. Morawets *et al.* also found that the defective virus was rapidly cleared by 129Sv mice. Thus, it seems that resistance in 129Sv mice to MAIDS could be a result of the inability of this defective virus to infect this particular mouse strain. One would expect, therefore, that the expression of an IL-4 transgene would not make 129Sv mice susceptible to MAIDS, as IL-4 is unlikely to change the infectivity of the virus.

We have reported that, in contrast to 129Sv mice (129Sv × B6), IL-$4^{-/-}$ mice could be infected with the defective virus, but the development of the disease was significantly delayed. Six out of 18 mice in one experiment ultimately developed the MAIDS symptoms 50 weeks after infection, which indicates that infection and the development of MAIDS were not completely dependent on IL-4 expression (*1*). These results suggest that the nature of resistance to MAIDS differs significantly between 129Sv mice and (129Sv × B6) IL-$4^{-/-}$ mice.

We are aware of the possibility that homozygosity of chromosome 11 that is derived from the 129 strain in IL-$4^{-/-}$ mice may account for the observed resistance to MAIDS. We have tested the susceptibility of the F1 offspring of IL-$4^{-/-}$ (129Sv × B6) mated with IL-$4^{+/+}$ 129Sv mice, which should all be homozyous for chromosome 11 and heterozygous (KO/WT) at the IL-4 locus. Four out of 10 mice developed lymphoadenopathy and T cell anergy with kinetics similar to those of the IL-$4^{+/+}$ (129Sv × B6) mice 8 weeks after infection. However, the onset of disease was significantly delayed in other F1 mice. The heterogeneity observed in these mice may be a result of the mixed genetic background of the IL-$4^{-/-}$ (129Sv × B6) mice. From these results, we conclude that homozygosity of chromosome 11 from the 129Sv strain does not itself alone confer resistance to MAIDS.

Mice used for the experiments in our report (*1*) were offsprings of F2 × F2 mice. We used four IL-$4^{+/+}$ mice and eight IL-$4^{-/-}$ mice to obtain experimental mice. Therefore, these mice were genetically mixed, but contained similar background except for the IL-4–deficient phenotype. We observed a clear difference between IL-$4^{+/+}$ and IL-$4^{-/-}$ mice in their susceptibility to MAIDS in three independent experiments, with a total of 40 mice for each group. We therefore believe that this resistance is a result of the IL-4 deficiency in these mice. However, because the background of these mice is genetically mixed, it is difficult to investigate other possible factors that may be involved in the IL-4–mediated pathology.

In contrast to our results, Morawetz *et al.* tested IL-$4^{-/-}$ mice, which were bred to B6 mice for seven to eight generations, and found no effect of IL-4 deficiency in the susceptibility to MAIDS. There are many results indicating that resistance and susceptibility to MAIDS is influenced by multiple factors, which include MHC (*2*) and nonMHC genes (*3*–*5*) and the strains of virus used for the infection (*4*, *6*). Recently it was shown that B6 mice carrying Xid deficiency (Bruton's kinase deficiency) were resistant to MAIDS, as compared with the normal B6 strain (*5*). However, the same genetic defect had no effect on the susceptibility of CBA × B6 F1 mice to MAIDS (*7*). These results demonstrate that mutation affecting B cell physiology and resistance to the MAIDS virus depends on the genetic background of the mouse strains. An interaction between a background gene and a D^d class I MHC molecule that may result in resistance to MAIDS has also been reported (*2*). It is possible that similar complexity may exist for the effect of IL-4 in the development of MAIDS. Namely, a IL-4–deficient phenotype confers resistance to MAIDS in mice with a certain genetic background (129Sv × B6), but not to mice of another strain (B6).

We have established IL-4–deficient mice from embryonic stem cells derived from 129Sv and B6 strains. We hope that use of these mice will allow us to determine the genetic factors influencing the function of IL-4 in the development of MAIDS. Identification of the genetic loci and the elucidation of the function of the gene product controlling the susceptibility to MAIDS will be an important step to understanding retrovirus pathogenesis.

Osami Kanagawa
Barbara A. Vaupel
Shinyo Gayama
Department of Pathology and Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
Georges Koehler
Manfred Kipf
Max-Planck-Institute for Immunobiology, Stuebeweg 51, D-7800 Freibourg, Germany

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