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Resistance of Mice Deficient in IL-4 to Retrovirus-Induced Immunodeficiency Syndrome (MAIDS)

Osami Kanagawa,* Barbara A. Vaupel, Shinyo Gayama, Georges Koehler, Manfred Kopf

The murine acquired immunodeficiency syndrome (MAIDS) is induced by a defective murine leukemia virus and has many symptoms similar to those found in patients infected with the human immunodeficiency virus. The presence of both B cells and CD4$^+$ T cells is critical for the development of the disease. Furthermore, a Th2 cytokine response dominates during the progression of the disease. When interleukin-4 (IL-4)–deficient mice that are defective in Th2 cytokine responses were infected, there was no lethality, and the development of the T cell abnormalities associated with MAIDS was delayed. These data suggest that IL-4 or a Th2 response is involved in the development of retrovirus-induced immunodeficiency in mice.

During MAIDS progression, abnormal functioning of CD4$^+$ T cells is observed, including loss of proliferative capacity and IL-2 production in response to mitogens and antigens as well as impaired help for the induction of cytolytic T lymphocyte responses (*1*). At later times after infection, T cells specifically produce IL-4 and IL-10 and low amounts of interferon-γ, which is characteristic of a dominant Th2 response (*2*). This suggests that Th2 cytokines may determine the fatal outcome of the disease.

To investigate the role of IL-4 and Th2 cytokines in MAIDS development, we used IL-4–deficient mice, which carry a targeted disruption of the gene encoding IL-4 as a result of homologous recombination in embryonic stem cells (*3*). The IL-4–deficient (IL-4$^{-/-}$) and control (IL-4$^{+/+}$) mice (F_2 interbreeding from 129/Sv × C57B1/6) were infected with LP-BM5 virus, as a mixture of replication-competent and replication-defective murine leukemia viruses produced by SC-1 cells (*4*). The development of lymphoadenopathy, T cell function, and mortality was monitored. From 6 to 20 weeks after infection, 28 out of 28 IL-4$^{+/+}$ mice progressively developed visible lymph node enlargement at the cervical and axillar regions (Table 1). In contrast, 1 out of 24 and 3 out of 24 IL-4$^{-/-}$ mice developed lymphoadenopathy at 12 and 20 weeks, respectively, after infection. The development of splenomegaly in infected IL-4$^{+/+}$ mice was observed as a 10- to 12-fold increase in the number of spleen cells as compared with that in uninfected controls. In contrast, the number of spleen cells in infected IL-4$^{-/-}$ mice increased less than twofold (Table 2). To further examine T cell function, we stimulated spleen cells with either monoclonal antibody to the T cell receptor (anti-TCR) or phorbol 12-myristate 13-acetate (PMA) plus ionomycin and assessed the cells for IL-2 production. By 8 weeks after infection, spleen cells from IL-4$^{+/+}$ mice were no longer able to produce IL-2 in response to both PMA plus ionomycin and anti-TCR stimulation (Table 2). In contrast, spleen cells of infected IL-4$^{-/-}$ mice remained functionally intact. However, T cells from one IL-4$^{-/-}$ mouse that developed lymphoadenopathy more than 20 weeks after infection also were unable to respond to anti-TCR and anti-CD3 in vitro (*5*). All IL-4$^{+/+}$ mice infected with LP-BM5 virus died within 25 weeks after infection, whereas none of the IL-4$^{-/-}$ mice died during this period of time (Fig. 1). These results demonstrate that IL-4–deficient mice are resistant to the development of MAIDS virus–induced disease.

We next determined the surface phenotype of lymph node T cells in mice infected with LP-BM5 virus. Although the percent-

Table 1. Resistance of IL-4–deficient mice to infection with LP-BM5. LP-BM5 virus produced by chronically infected SC-1 cells was inoculated intraperitoneally (0.5 ml) into young (4- to 5-week-old) IL-4$^{-/-}$ and IL-4$^{+/+}$ mice. Mice were monitored daily for the development of lymphoadenopathy at the inguinal, axillar, and cervical lymph nodes.

Mice	Mice with lymphoadenopathy/mice infected			
	6 weeks	8 weeks	12 weeks	20 weeks
		Experiment 1		
IL-4$^{-/-}$	0/12	0/12	0/12	2/12
IL-4$^{+/+}$	7/12	11/12	12/12	3/3*
		Experiment 2		
IL-4$^{-/-}$	0/12	0/12	1/12	1/12
IL-4$^{+/+}$	15/16	16/16	16/16	16/16

*Only three mice survived to this point.

O. Kanagawa, B. A. Vaupel, S. Gayama, Department of Pathology and Medicine, Washington University School of Medicine, St. Louis, MO 63110.
G. Koehler and M. Kopf, Max Planck Institute for Immunobiology, Stuebeweg 51, D-7800 Freiburg, Germany.
*To whom correspondence should be addressed.

age of T cells in spleen decreased from 40 to 20% in the IL-$4^{+/+}$ mice 6 weeks after infection, no difference was found for the expression of TCR, CD4, CD8, leukocyte function–associated antigen–1 (LFA-1), and IL-2 receptor in T cells from IL-$4^{-/-}$ and IL-$4^{+/+}$ mice (5). However, analysis of markers known to correlate with the state of T cell activation (6) revealed a consistent difference between T cell populations from two groups of mice. T cells from infected IL-$4^{+/+}$ mice had low amounts of lectin–cellular adhesion molecule–1 (LECAM-1) and high amounts of phagocyte glycoprotein (PGP)–1 (CD 44), whereas those from IL-$4^{-/-}$ mice were high in LECAM-1 and low in PGP-1 (Fig. 2). These results indicate that T cells from IL-$4^{+/+}$ mice are activated as a consequence of the virus infection in vivo (7), whereas no activation of T cells occurs in IL-$4^{-/-}$ mice at this stage. We do not know whether this lack of T cell activation in IL-4–deficient mice is due to a lack of IL-4 or to the secondary effect of resistance to the disease.

We next investigated the presence of the defective LP-BM5 virus mRNA in infected mouse lymph node cells. The RNA samples were prepared from mice 10 weeks after virus infection and used for reverse transcriptase–polymerase chain reaction (RT-PCR) amplification of the viral gene encoding the P12 gag protein. The defective virus gag P12 sequence differs from that of other murine leukemia viruses, including endogenous virus, and contains a Sma I site. Therefore, the presence of the Sma I sites in PCR products correlates with the presence of the pathogenic defective virus mRNA (8). Defective virus RNA was found in lymph nodes from both IL-$4^{-/-}$ and IL-$4^{+/+}$ mice (Fig. 3). Previous reports demonstrated that genetically determined MAIDS susceptibility and resistance phenotype correlated with the presence or absence of viral RNA in lymphocytes (9). The reduced expression of viral *gag* mRNA in IL-$4^{-/-}$ mice suggests that the number of virus-infected cells may increase more slowly in these mice as compared with that in IL-$4^{+/+}$ mice. Thus, mice are rendered resistant in the absence of IL-4, possibly

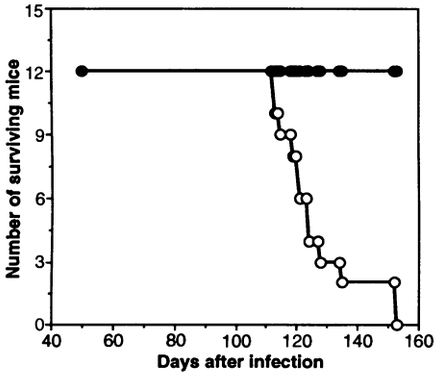

Fig. 1. Survival of IL-$4^{-/-}$ mice after infection with LP-BM5 virus. Mice [4- to 5-week-old IL-$4^{-/-}$ (filled circles) and IL-$4^{+/+}$ controls (open circles)] were infected intraperitoneally (0.5 ml) with LP-BM5 virus . Survival is shown for groups of 12 mice that were also monitored for the development of lymphoadenopathy (Table 1, experiment 1).

Table 2. Resistance of IL-4–deficient mice to the induction of immunodeficiency by LP-BM5 virus. Mice were infected with LP-BM5 virus as described in Table 1. At the indicated times after infection, mice were killed, and spleen cell number was determined. Spleen cells (5×10^5) were stimulated with PMA (10 ng/ml) plus ionomycin (1 μM) (P + I), plate-bound anti-TCR (H57-579, 10 μg/ml) (a-TCR), or medium alone (None) in a final volume of 200 μl containing 5% fetal calf serum, Dulbecco's minimum essential medium in flat-bottom microtiter plates. After 24 hours of incubation, supernatants were collected and tested for IL-2 activity with the use of the IL-2–dependent cell line, CTLL-2.

Mice	Infection	Spleen cell number	Response to		
			P + I	a-TCR	None
		Experiment 1			
−/−	−	5.8×10^7	>1458*	>1458	<3
+/+	−	7.8×10^7	>1458	1216	<3
−/−	+ (6 weeks)	9.8×10^7	>1458	1322	<3
−/−	+ (6 weeks)	7.6×10^7	>1458	1208	<3
+/+	+ (6 weeks)	9.4×10^8	>1458	<3	<3
+/+	+ (6 weeks)	8.6×10^8	1113	<3	<3
		Experiment 2			
−/−	−	7.3×10^7	>1458	921	<3
−/−	+ (8 weeks)	16.2×10^7	>1458	468	<3
−/−	+ (8 weeks)	14.2×10^7	1320	388	<3
+/+	+ (8 weeks)	8.5×10^8	207	<3	<3
+/+	+ (8 weeks)	7.2×10^8	58	<3	<3

*Units of IL-2 activity per milliliter (*16*).

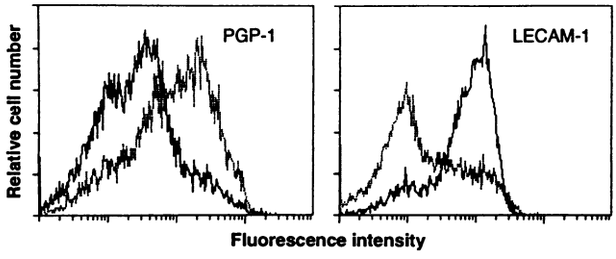

Fig. 2. Surface phenotype of T cells from mice infected with LP-BM5 virus. Lymph node cells from mice infected with LP-BM5 virus (10 weeks after infection) were stained with antibody to Thy 1 (AT83) followed by fluorescein isothiocyanate–coupled goat antibody to rat immunoglobulin G and counterstained with biotinylated antibody to PGP-1 (IM7) or antibody to LECAM-1 (MEL 14) followed by avidin-coupled phycoerythrin. Samples were analyzed by FACScan (Becton Dickinson) using the C30 program (*16*). Solid line, T cells from IL-$4^{-/-}$ mice; dotted line, T cells from IL-$4^{+/+}$ mice.

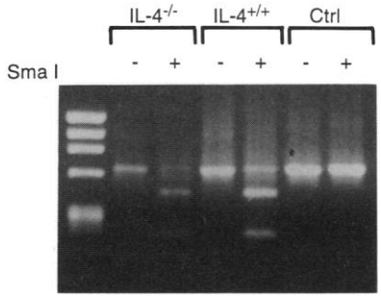

Fig. 3. Detection of *gag* mRNA in lymph nodes of virus-infected mice by PCR. Lymph nodes were removed from IL-$4^{-/-}$ mice (5×10^7 cells per mouse) or IL-$4^{+/+}$ mice (4×10^8 cells per mouse) 10 weeks after infection with LP-BM5 virus. Total cellular RNA was isolated by a standard guanidine hydrochloride lysis and CsCl gradient protocol (*17*). Complementary DNA was synthesized from cellular RNA (20 μg) with Abelson murine leukemia virus transcriptase (20 U), oligo(dT) primer (1.5 μg), dithiothreitol (10 mM), and 0.5 mM deoxynucleotide triphosphates (1 hour at 42°C). Polymerase chain reaction was done with a primer containing the P12 portion of the *gag* gene (GTGG-GTCGACCATCGTTGCCCCGGACTGCC and GATCAGGTACCATATATCGTCACCTGGGAG) for 30 cycles (denaturation at 94°C, annealing at 55°C, and extension at 72°C, each for 1 min). The PCR products were analyzed by agarose electrophoresis with (+) or without (−) treatment with Sma I restriction enzyme. The control sample was obtained from normal B6 thymocytes, which express endogenous murine leukemia virus lacking the Sma I site in the P12 region. DNA size markers (ϕX 174RF DNA digested with Hae III, Boehringer) are shown in the left lane. Polymerase chain reaction and integrity of cDNA were monitored with actin-specific primers (data not shown).

because the onset and progression of the disease is markedly delayed.

Taken together, these results demonstrate that IL-4 is critical to the development of MAIDS. The mechanism by which IL-4 mediates its effects in the development of the disease is not clear. Infection with the LP-BM5 virus causes a switch from a Th1 to a Th2 response with progression of the disease (*2*). This may be due to superantigen activity expressed by virus-infected B cells (*10*) that stimulate aberrant IL-4 production in T cells. A mouse line that expresses high amounts of IL-4 derived from a transgene shows a variety of immunological abnormalities, including a deficient proliferative response of T cells to the mitogen concanavalin A (*11*). Furthermore, Th2 cytokine responses are dependent on IL-4 production (*12*). Thus, other Th2 cytokines that have been shown to be significantly reduced in IL-$4^{-/-}$ mice, such as IL-5, IL-9, IL-10, and IL-6 (*13*), may also be involved in the development of MAIDS. This could be tested by reconstituting infected IL-$4^{-/-}$ mice or by infecting mice deficient in other cytokines. Although human immunodeficiency virus (HIV) and the murine leukemia virus causing MAIDS represent different types of retroviruses, some of the features of the diseases are similar, such as lymphoadenopathy, B cell hyperactivity, T cell immunodeficiency, and development of certain tumor cells at the late stage of the disease (*1*, *14*). Furthermore, a shift from a Th1 to a Th2 response and a high serum immunoglobulin E level have been reported for HIV-infected patients (*15*). These findings suggest that IL-4 as well as other Th2-related cytokines may be crucial to the development of T cell dysfunction induced by the two different viruses. Thus, understanding the role of IL-4 in the development of MAIDS may provide insight into the mechanisms causing dysregulated cytokine production and generalized T cell immunodeficiency found in human acquired immunodeficiency syndrome.

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HLA-DPB1 Glutamate 69: A Genetic Marker of Beryllium Disease

Luca Richeldi, Rosa Sorrentino, Cesare Saltini*

Chronic beryllium disease (CBD) is a lung disorder related to beryllium exposure and is characterized by the accumulation in the lung of beryllium-specific $CD4^+$ major histocompatibility complex (MHC) class II–restricted T lymphocytes. Evaluation of MHC class II genes in 33 CBD cases and 44 controls has shown a negative association with HLA-DPB1*0401 ($P < 0.001$) and a positive association with HLA-DPB1*0201 ($P < 0.05$) alleles, which differ at residues 36, 55 to 56, and 69 of the β_1 chain. Among CBD cases, 97 percent expressed the HLA-DPB1*0201–associated glutamic acid (unaffected population, 30 percent; $P < 0.001$) at residue 69, a position involved in susceptibility to autoimmune disorders. This suggests that HLA-DP has a role in conferring susceptibility and that residue 69 of HLA-DPB1 could be used in risk assessment for CBD.

Exposure to metals such as Co, Al, Ti, Zr, and Be is associated with a variety of chronic disorders of the lung (*1*). CBD is a hypersensitivity lung disorder caused by exposure to Be that is not strictly dependent on Be concentration (*2*). As predicted by its immunopathologic features, CBD is maintained by an accumulation of large numbers of Be-specific $CD4^+$ T cells in patient lungs (*3*). In this context, CBD could be used as a model to test the relation between environmental and immunogenetic factors in occupation-related diseases. T cell clones from CBD patient lungs are MHC class II–restricted, that is, they only respond to Be in association with MHC class II molecules on the surface of the antigen-presenting cell (*3*). Genes of the MHC class II (HLA-DR, -DQ, and -DP) that are associated with susceptibility to autoimmune disorders (*4*) are likely candidates as susceptibility genes to CBD. This hypothesis is supported by the observations that MHC class II genes are involved in T cell responses to metals such as Ni and Au (*5*), and they play a key role in susceptibility to experimental hypersensitivity to Be and other metals (*6*).

We tested this concept, after a preliminary analysis of a small sample of patients that did not show strong associations with HLA-DR or HLA-DQ genes, by evaluating the association of the HLA-DP gene with CBD in a group of 33 CBD individuals and a group of 44 Be-exposed unaffected individuals (*7*). Frequencies of the HLA-DPB1 alleles in the Be-exposed unaffected group were similar to those of the normal population (*8*), regardless of the race composition of the group. The HLA-DPB1*0201 and *0401 allele frequencies in the total population sample were similar to those in Caucasians alone [percent of total population sample unaffected, *0201 (10%), *0401 (48%); Caucasians unaffected, *0201 (11%), *0401 (52%); $P > 0.2$, compared with published populations (8)] (9). In contrast, allelic frequencies in the CBD case group were biased. The frequency of the DPB1*0201 allele was increased [CBD, 30%; unaffected, 10%; $\chi^2 = 9.94$, $P = 0.0016$, Bonferroni corrected $P < 0.05$ (19 alleles)]. Conversely, the frequency of the DPB1*0401 allele was decreased (CBD, 14%; unaffected, 48%; $\chi^2 = 19.79$, $P = 0.00001$, corrected $P < 0.001$). When we evaluated the phenotypic frequencies, we observed a similar bias (DPB1*0201: CBD, 52%; unaffected, 18%; corrected $P < 0.05$; DPB1*0401: CBD, 27%; unaffected, 68%; corrected $P < 0.01$), indicating that, as in

L. Richeldi, Postgraduate School of Cardiorespiratory Physiopathology, Universities of Roma and Modena, Italy.
R. Sorrentino, Department of Experimental Medicine, University of L'Aquila, and Department of Cellular and Developmental Biology, University La Sapienza, Roma, Italy.
C. Saltini, Institute of Tuberculosis and Respiratory Diseases, University of Modena, USL 16 Modena and Postgraduate School of Cardiorespiratory Physiopathology, Universities of Roma and Modena, Italy.

*To whom correspondence should be addressed.