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A Common Immunoregulatory Locus Controls Susceptibility to Actively Induced Experimental Allergic Encephalomyelitis and Experimental Allergic Orchitis in BALB/c Mice[1]

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Previous studies have shown that differential susceptibility to actively induced experimental allergic encephalomyelitis (EAE) and experimental allergic orchitis (EAO) exists among various BALB/c substrains. Of eight substrains studied for EAE and 13 for EAO, BALB/cJ mice are phenotypically the most resistant to disease induction. Resistance to both diseases is controlled by single recessive mutations unlinked to any of the known alleles distinguishing BALB/cJ mice. In this study, segregation analysis employing a second generation backcross population shows that resistance to both EAE and EAO is due to a mutation in a common immunoregulatory gene. The role of immunoregulatory cells in controlling EAE resistance was examined using adoptive transfer protocols. BALB/cJ mice immunized with spinal cord homogenate plus adjuvants generate immunoregulatory spleen cells (SpC) that, when transferred to naive BALB/cByJ recipients, reduce the incidence and severity of EAE. Treatment of such cells with either cytotoxic monoclonal anti-Thy1.2 or anti-CD4 plus C′ before transfer abrogates the ability of BALB/cJ SpC to inhibit disease. In contrast, neither SpC from adjuvant-immunized BALB/cJ nor spinal cord homogenate- plus adjuvant-primed BALB/cByJ donors influences the incidence or severity of disease observed in recipients. In addition, the role of environment in influencing susceptibility to EAE and EAO in BALB/c mice is documented. Taken together, these results support the existence of a common disease susceptibility locus in the pathways leading to two autoantigenically distinct CD4$^+$ T cell-mediated, organ-specific, autoimmune diseases. ***The Journal of Immunology,*** **1998, 160: 2751–2756.**

Experimental allergic encephalomyelitis (EAE)[3] is an organ-specific autoimmune disease that can be readily induced in genetically susceptible strains of mice by injecting either whole spinal cord homogenate (SCH) or encephalitogenic proteins or peptides in adjuvants. CD4$^+$ T cells initiate disease by infiltrating the central nervous system. Subsequently, they recruit additional lymphocytes and mononuclear cells to cross the blood-brain barrier, resulting in inflammation and demyelination leading to varying degrees of paraparesis and paralysis (1). Significant differences in susceptibility to actively induced EAE have been shown to exist among various sublines of BALB/c mice (2–5). A similar diversity in BALB/c subline susceptibility is also seen in actively induced experimental allergic orchitis (EAO), a model of testicular autoimmunity (6, 7). Of the various sublines studied, BALB/cJ mice are consistently the most resistant to the induction of both diseases. It was shown that disease resistance is neither a reflection of a nonspecific generalized impairment of cellular immunity nor an alteration in the phenotypic expression of *Bordetella pertussis*-induced histamine sensitization (6), a phenotype associated with susceptibility to both diseases (8, 9). However, it has been shown that the EAO-resistant phenotype in BALB/cJ mice is mediated by CD4$^+$ T cells (10, 11).

Genetic analysis revealed that the phenotypic expression of susceptibility among different sublines was inherited as a dominant trait and that resistance to both EAE and EAO in BALB/cJ mice is due to single recessive mutations (3, 4). Linkage analysis failed to show an association of disease resistance with any of the known allelic differences that distinguish BALB/cJ mice (3, 4). In this study, a genetic approach was used to determine whether resistance to EAE and EAO in BALB/cJ mice is due to a mutation in a common immunoregulatory locus affecting both diseases or is the result of mutations in two independent, disease-specific loci. In addition, the nature of the genetically controlled mechanism(s) operating in the prevention of EAE in BALB/cJ mice was investigated using adoptive transfer protocols, and the role of the environment in disease susceptibility was documented.

Materials and Methods

Animals

BALB/cJ, BALB/cByJ, and SJL/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). (BALB/cJ × BALB/cByJ) and (BALB/cByJ × BALB/cJ)F_1 hybrids and (BALB/cByJ × BALB/cJ) × BALB/cJ backcross 1 (BC1) and BC1 × BALB/cJ backcross 2 (BC2) generation mice were generated and maintained in the John Morgan Building vivarium at the University of Pennsylvania School of Medicine (Philadelphia, PA). In addition, animals were maintained in the vivariums of the Medical Education Building and Richards Building at the University of Pennsylvania, the Widtsoe Building at Brigham Young University (Salt Lake City, UT), and the Old Medical School at the University of Virginia (Charlottesville, VA). BC2 mice were derived by breeding one male BC1 parental mouse with three BALB/cJ females. Animals were fed mouse pellets and acidified water ad libitum.

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[3] Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; SCH, spinal cord homogenate; EAO, experimental allergic orchitis; J, BALB/cJ; ByJ, BALB/cByJ; MTH, mouse testicular homogenate; Ptx, pertussis toxin; SpC, spleen cells; PCT, plasmacytomagenesis.

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Induction and evaluation of EAO and EAE

For the induction of EAO, ether-anesthetized mice were immunized with 10.0 mg of dry weight pooled allogeneic mouse testicular homogenate (MTH). MTH-adjuvant emulsions were prepared as follows: 10.0 mg of dry weight MTH in 0.05 ml of PBS was emulsified with an equal volume of CFA containing 0.45 mg of *Mycobacterium tuberculosis* (H37Ra). Each animal received 0.1 ml of the emulsion equally distributed in both hind footpads. In addition, each animal received 10.0 μg of crude pertussis toxin (Ptx; provided by Dr. John J. Munoz, Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, MT) dissolved in 0.1 ml of 0.015 M Tris-HCl buffer, containing 0.5 M NaCl and 0.017% Triton X-100, pH 7.6, and an additional 5.0 μg 24 h later by i.p. injection. All animals were killed 30 days after immunization unless otherwise noted. The testes were fixed in Bouin's fixative and embedded in paraffin, and 5-μm thick sections were cut and stained with hematoxylin-eosin for histologic examination. Orchitis was quantified as previously described (12). A pathology index was determined for each testis by examining two to four sections taken at four different levels, and the average for each animal and/or group was calculated.

For the induction of EAE, female mice received 0.1 ml of encephalitogenic emulsion in each hind footpad and scruff of the neck, distributed equally among these three sites. The emulsion consisted of equal volumes of 0.9 N saline containing 40.0 mg/ml of a lyophilized, homogenized pool of allogeneic mouse SCH prepared from SWR mice and CFA supplemented with 4.0 mg/ml *M. tuberculosis* (H37Ra). Thus, each animal received 2.0 mg of SCH in adjuvant. On the day of immunization or 3 days thereafter mice were given 10.0 μg of Ptx i.v. and 5.0 μg i.p. as detailed above. The mice were observed daily for clinical symptoms of EAE from day 10 through day 30 postinjection (3). Animals were killed at either the peak of disease or 30 days postimmunization and processed for histopathologic analysis. Individual animals were assigned values as follows: 0, no evidence of central nervous system inflammation; 1, small, primarily meningeal, scattered inflammatory infiltrates; 2, large but scattered infiltrates in meninges and parenchyma; and 3, large, numerous, occasionally confluent, inflammatory infiltrates (2).

Immunosuppresisve treatments

Groups of BALB/cJ mice were treated with either low doses of cyclophosphamide (20 mg/kg) or sublethal irradiation (350 rad) 2 days before inoculation for the induction of EAE.

Adoptive transfer of disease resistance (10)

Spleens from BALB/cJ mice immunized with SCH plus adjuvants 20 days earlier were excised under aseptic conditions and placed in HBSS containing either 5% FCS or γ-globulin-free horse serum. Spleen cell (SpC) suspensions were prepared using a tissue homogenizer. Subsequently, they were filtered through nylon gauze, and the erythrocytes were lysed with 0.83% NH_4Cl. The SpC were then washed twice with HBSS and resuspended in HBSS without serum at a final concentration of 3×10^8 cells/ml. One milliliter of the SpC suspension was injected i.p. into BALB/cByJ mice 3 days before inoculation for the induction of EAE.

Depletion of Thy-1.2, CD4$^+$, and CD8$^+$ cells (10)

The following mAbs were used: RL172.4 (anti-L3T4), 3.168.8 (anti-Lyt-2), and J1j (anti-Thy-1.2). All hybridomas were grown as ascites in pristane-conditioned nude mice. Guinea pig serum served as the source of complement (C′). Thy-1.2, CD4$^-$, and CD8-depleted SpC suspensions were generated by treating 3×10^8 SpC (at a concentration of 5×10^7 cells/ml) with the respective mAb plus C′. The concentration of the mAbs used was 0.1 ml of appropriately prediluted ascites fluid/5×10^7 cells. Cells were incubated for 60 min at 37°C, following which they were washed twice in HBSS and resuspended in 1 ml of HBSS without serum for i.p. injection into BALB/cByJ recipients.

Results and Discussion

BALB/cJ and BALB/cByJ mice were studied for susceptibility and resistance to the induction of EAO and EAE. BALB/cJ mice were resistant to the induction of both EAO and EAE, whereas BALB/cByJ mice exhibited a non-fully penetrant, susceptible phenotype (Table I). SJL/J mice, which served as positive controls for EAE induction, exhibited 90 and 100% clinical and histologic disease, respectively. Susceptibility to both diseases was inherited as the dominant phenotype in reciprocal (BALB/cJ $\times$ BALB/cByJ)F_1 and (BALB/cByJ $\times$ BALB/cJ)F_1 hybrids, suggesting the lack of a sex-linked influence. These results are consistent with previously reported BALB/c substrain differences in susceptibility to EAE and EAO (2–7, 10, 11). It was suggested that a mutation in a single pleiotropic regulatory locus may be responsible for most of the differential phenotypes observed in BALB/cJ mice (13). However, it has been shown that resistance to EAE and EAO is not linked to the mutant BALB/cJ *Afr1*^{b} allele, suggesting that multiple mutational events have occurred in BALB/cJ mice (3, 4). Genetic analyses of resistance to EAO and EAE in BALB/cJ mice revealed that both phenotypes are controlled by single recessive mutations, suggesting that a mutation may have occurred in a common immunoregulatory locus in the pathways leading to both diseases (3, 4). To test this hypothesis we established a panel of BC2 mice

Table I. *Susceptibility to EAO and EAE in BALB/cJ, BALB/cByJ, F_1 hybrids, and BC2 populations*

Substrain/Cross	Autoimmune Orchitis[a]	EAE[b]	
		Clinical	Histologic
SJL/J	8/10	9/10	10/10
BALB/cByJ	6/6	7/14	8/14
BALB/cJ	0/10	0/8	1/8
(BALB/cByJ x BALB/cJ)F_1	4/5	3/9	4/9
(BALB/cJ x BALB/cByJ)F_1	10/11	3/7	3/7
BALB/cJ x BC1 #1[c]			
Male	6/10	—	—
Female	—	4/8	5/8
BALB/cJ x BC1 #2			
Male	0/6	—	—
Female	—	0/6	0/6
BALB/cJ x BC1 #3			
Male	8/8	—	—
Female	—	4/6	6/6
BALB/cJ x BC1 #4			
Male	4/8	—	—
Female	—	3/6	5/6
BALB/cJ x BC1 #5			
Male	6/6	—	—
Female	—	4/7	5/7
BALB/cJ x BC1 #6			
Male	0/5	—	—
Female	—	0/7	0/7
BALB/cJ x BC1 #7			
Male	0/5	—	—
Female	—	0/9	1/9
BALB/cJ x BC1 #8			
Male	6/9	—	—
Female	—	5/8	6/8
BALB/cJ x BC1 #9			
Male	0/6	—	—
Female	—	0/7	0/7
BALB/cJ x BC1 #10			
Male	1/4	—	—
Female	—	2/5	3/5
BALB/cJ x BC1 #11			
Male	0/7	—	—
Female	—	0/10	0/10
Total	31/74	22/79	31/79
	$p > 0.05$	$p < 0.05$	$p > 0.05$

[a] Animals were immunized with 10.0 mg dry weight MTH as described in *Materials and Methods.* All animals were killed at day 30 postimmunization and studied histologically for autoimmune orchitis.

[b] Animals were immunized with 2.0 mg dry weight SCH in CFA with Ptx as an ancillary adjuvant as described in *Materials and Methods.* Mice were observed daily for clinical signs of EAE from day 10 to day 30 postinjection. Clinical symptoms included flaccid tail and weakness, hind limb paralysis, and/or moribund state. All animals were killed either at the peak of illness or on day 30, and their CNSs were studied histologically for evidence of EAE.

[c] Multiple liters of BC2 mice were generated for each parental BC1 animal by breeding the BC1 male with three female BALB/cJ mice. All animals were between 8 and 12 weeks of age at the time of inoculation.

Table II. *Effects of cyclophosphamide pretreatment and sublethal irradiation of EAE susceptibility in BALB/cJ mice*

Treatment	No.	Immunogen	Clinical			Histologic	
			Without symptoms	Flaccid tail and/or weakness	Hind limb paralysis and/or moribund state	Infiltrate detected	Inflammatory PI
Cyclophosphamide[a]	5	CFA + Ptx	5	0	0	0	0
	10	SCH + CFA + Ptx	5	4	1	5	3, 2, 2, 2, 1
Low dose irradiation[b]	5	CFA + Ptx	0	0	0	0	0
	11	SCH + CFA + Ptx	5	5	1	7	3, 2, 2, 2, 1, 1, 1

[a] Animals received cyclophosphamide injections i.p. (20 mg/kg) 2 days prior to inoculation. Immunizations were carried out as detailed in *Materials and Methods.*
[b] Animals were irradiated (350 rads) 2 days prior to inoculation. Immunizations were carried out as detailed in *Materials and Methods.*

(BC1 × BALB/cJ) and studied the female offspring for susceptibility to EAE and the male offspring for susceptibility to EAO. If the mutation in BALB/cJ mice occurred in a single gene involved in both diseases, then there would be a high degree of concordance for the resistant phenotype in progeny derived from the same BC1 parents. In contrast, if independent mutations in separate loci occurred, then little concordance between the two diseases in the BC2 populations would be seen. In 153 BC2 progeny derived from 11 BC1 animals, six sets of progeny clearly exhibited offspring susceptible to both EAO and EAE, while five were essentially resistant to both diseases. The data reveal essentially 100% concordance between susceptibility to EAO and EAE among the different groups of BC2 progeny in which the dominant, wild-type BALB/cByJ allele was segregating (Table I). In addition, the overall incidence of orchitis and EAE in the BC2 population, as determined histologically, was not significantly different ($p > 0.05$) from that expected for a single gene. These data are therefore consistent with the idea that a single mutation has occurred in a common immunoregulatory gene involved in the pathways leading to both EAE and EAO rather than two mutations in independent loci.

The finding that a mutation in a common gene affecting the phenotypic expression of two independent models of organ-specific autoimmune disease supports the idea that two classes of non-MHC-linked disease susceptibility loci exist: those that play a role in multiple autoimmune diseases and those that are disease specific. MHC-linked immune response genes and *Bphs* (8, 9, 14) clearly establish a precedent for the former. Similarly, comparative genome exclusion mapping identifying susceptibility loci involved in different models of organ-specific autoimmunity have resulted in the colocalization of several such genes. Shared susceptibility loci or gene complexes will be detected as nonrandom colocalization phenomenon of susceptibility loci among independent linkage studies. Examples include the following. *Idd3*, a diabetes susceptibility locus in the NOD mouse colocalizes to the same region of chromosome 3 as *Aod2*, the locus controlling the development of atrophy in day 3 thymectomy-induced autoimmune ovarian dysgenesis (15), and *Orch3* and *Orch5*, two susceptibility loci in autoimmune orchitis colocalize to the same regions of chromosomes 11 and 1 as *Idd4* and *Idd5*, respectively (16).

Immunosuppressive treatments, such as low dose irradiation and low dose cyclophosphamide, can convert BALB/cJ mice from an EAO-resistant phenotype to a susceptible one (10). Groups of BALB/cJ mice were therefore pretreated with either low dose cyclophosphamide or sublethal irradiation 2 days before inoculation for the induction of EAE. As shown in Table II, pretreating BALB/cJ mice with either agent leads to an increase in the incidence of both clinical and histologic disease. These results are consistent with prior reports concerning the effects of pretreatment with low dose cyclophosphamide and irradiation on the susceptibility of BALB/c mice to EAE (17, 18).

Previously, it was shown that resistance to autoimmune orchitis could be transferred to naive BALB/cByJ mice with $CD4^+$ T cells from MTH- plus adjuvant-primed BALB/cJ donors (10, 11). To address the possibility that resistance to EAE in BALB/cJ mice is also due to $CD4^+$ T cells, SpC from immunized BALB/cJ mice were adoptively transferred to naive BALB/cByJ recipients 3 days before inoculation for the induction of EAE. BALB/cByJ mice that received SpC from BALB/cJ mice primed with adjuvants served as controls. BALB/cByJ mice that received SpC from SCH- plus adjuvant-immunized animals exhibited a decrease in both the incidence and severity of the lesions seen in EAE (Table III). In

Table III. *Adoptive transfer of BALB/cJ disease resistance to normal BALB/cByJ recipients*

Treatment of Donor Mice[a]	No. Spc Transferred[b] ($\times 10^8$)	No.	Clinical[c]			Histologic	
			Without symptoms	Flaccid tail and/or weakness	Hind limb paralysis and/or moribund state	Infiltrate detected	Scores
CFA + Ptx	3	8	4	2	2	4	3, 3, 2, 2
SCH + CFA + Ptx	3	7	6	1	0	1	1
SCH + CFA + Ptx (BALB/cByJ donor)	3	8	4	2	2	5	3, 3, 2, 1, 1
SCH + CFA + Ptx Anti-Thy-1.2 + C′	3	9	5	3	1	4	3, 2, 2, 2
SCH + CFA + Ptx Anti-CD8 + C′	3	8	8	0	0	1	1
SCH + CFA + Ptx Anti-CD4 + C′	3	7	3	3	1	5	3, 2, 2, 1, 1

[a] BALB/cJ and BALB/cByJ donor mice received 2.0 mg SCH-CFA emulsion as described in *Materials and Methods* accompanied by Ptx as an ancillary adjuvant.
[b] The cells remaining following mAb + C′ treatment of 3×10^8 total spleen cells.
[c] Recipients were inoculated 3 days following transfer of donor SpC as described in *Materials and Methods.*

Table IV. *Susceptibility of BALB/cByJ mice generated and maintained in different vivariums to the induction of EAO and EAE*[a]

Vivarium	Autoimmune Orchitis			EAE Clinical			Histologic	
	MTH stock	PI ± SE	Incidence	Without symptoms	Flaccid tail and/or weakness	Hind limb paralysis and/or moribund state	Infiltrate detected	Scores
University of Pennsylvania School of Medicine								
John Morgan Building								
BALB/cByJ	A	3.7 ± 0.8	13/17	7	7	0	7	2, 2, 2, 1, 1, 1, 1
BALB/cJ (Table I)	A	0	0/10	—	—	—	—	—
SJL/J	A	3.1 ± 0.6	9/10	1	3	4	7	3, 3, 3, 2, 2, 2, 2
Richards Building								
BALB/cByJ	A	3.9 ± 0.7	18/19	8	4	1	6	2, 2, 2, 2, 1, 1
SJL/J	A	3.5 ± 1.0	8/9	2	2	3	6	3, 3, 2, 2, 2, 1
Medical Education Building								
BALB/cByJ	A	0	0/21	16	0	0	1	1
SJL/J	A	3.8 ± 0.8	8/9	2	2	4	6	3, 3, 3, 2, 1, 1
Unknown[b]								
BALB/cByJ	A	5.3	8/8	12	3	14	21	1.5
BALB/cJ	A	0	0/10	15	0	0	3	0.2
Brigham Young University								
Widtsoe Building[c]								
BALB/cByJ	A	3.5 ± 0.5	11/11	5	7	1	8	3, 2, 2, 1, 1, 1, 1, 1
BALB/cJ	B	1.9 ± 0.9	3/6	8[d]	2	0	2	1, 1
SJL/J	A	5.6 ± 1.2	9/12	2	5	2	7	3, 3, 2, 2, 1, 1, 1
University of Virginia								
Old Medical School Building[c]								
BALB/cByJ	A	5.7 ± 0.4	8/11	—	—	—	—	—
	B	4.2 ± 1.1	3/4	—	—	—	—	—
BALB/cJ	A	2.3 ± 0.5	3/5	—	—	—	—	—
	B	2.1 ± 0.1	4/7	—	—	—	—	—
University of New Mexico School of Medicine[e]								
BALB/cByJ	B	5.7 ± 1.1	10/13	—	—	—	—	—
BALB/cJ	B	0.2 ± 0.2	2/10	—	—	—	—	—
BALB/cJ	B	1.2 ± 0.6	8/19	—	—	—	—	—

[a] BALB/cByJ and SJL/J colonies were established in each of the three vivariums using male and female mice obtained from The Jackson Laboratory (Bar Harbor, ME). Breeder and holding cages were maintained under standard operating conditions at each location. Pregnant females were removed from breeder cages and housed individually, and pups were weaned at 20 days of age. Male and female mice used in the experiment were all third generation offspring generated by random inter-cage mating and between 8 to 12 weeks of age at the time of incoulation. The stocks of MTH, SCH, CFA, and Ptx used are as described in the *Materials and Methods.*

[b] Previously reported data (3). The exact vivarium in which these studies were carried out is not known.

[c] Mice were purchased from Jackson Laboratory, shipped to the vivarium in the Widtsoe Building at Brigham Young University and the Old Medical School Building vivarium at the University of Virginia, and studied for susceptiblity and resistance to EAE and/or EAO at 3 weeks following their arrival.

[d] The EAE results presented were obtained from animals studied at a different time from the BALB/cByJ and SJL/J mice. The immunization protocol was, however, identical.

[e] Previously reported data (7).

contrast, recipients receiving SpC from BALB/cByJ mice immunized with SCH plus adjuvants developed disease similar to that of mice receiving adjuvant-primed BALB/cJ SpC. To characterize the phenotype of the BALB/cJ cells mediating the reduction in susceptibility to EAE, SpC were pretreated with cytotoxic mAbs specific for Thy-1.2, CD4, or CD8 plus C′. SpC depleted of Thy-1.2$^+$ and CD4$^+$ cells failed to reduce either the incidence of clinical disease or lesion severity, whereas SpC treated with anti-CD8 plus C′ were as effective at reducing the incidence of EAE as intact SpC. These results suggest that the immunoregulatory SpC population associated with disease resistance in BALB/cJ mice comprises Ag-specific CD4$^+$ T cells.

Karpus and co-workers (19) have shown that postrecovery suppressor cells harvested following recovery from acute EAE are CD4$^+$ and that failure to reinduce active EAE in animals that have recovered from a monophasic bout of EAE is due to CD4$^+$ T cells secreting IL-4 but not IL2 (20). Ellerman and co-workers (21) established a CD4$^+$ cell line that can suppress EAE effector cell functions in vitro and in vivo. CD4$^+$ suppressor cells have also been shown to play a role in mediating thyroid-stimulating hormone-induced suppression of experimental autoimmune thyroiditis (22), in partial or complete suppression of allograft rejection (23, 24), and in protecting mice from lethal graft-vs-host disease elicited by CD8$^+$ cells in class I-different hosts (25). At present the mechanisms of resistance mediated by such cells are unclear, but they may be related to differential inhibition of lymphokine production.

Two distinct subsets of CD4$^+$ T cells (Th1 and Th2) have been defined by their patterns of lymphokine production and functional activities. Th1 cells typically secrete IFN-γ and are involved in inflammatory or cell-mediated immune responses, whereas Th2 cells are most often characterized by IL-4 secretion and are important in the development of humoral immunity (26, 27). Resistance to EAE and EAO in BALB/cJ substrain mice may be due to the result of a mutation in an immunoregulatory locus that governs the ratio of Th1:Th2 cells produced in response to the relevant autoantigens. Polarized T cell responses have been implicated in both systemic and organ-specific autoimmune diseases (28). In BALB/cByJ mice and in other disease-susceptible substrains, the autoimmune response may normally be biased toward a Th1 response that would be consistent with the development and role of delayed-type hypersensitivity in disease pathogenesis. In contrast, the mutant allele expressed in BALB/cJ mice may predispose the animals toward a Th2 response and the lack of delayed-type hypersensitivity effector mechanisms required to elicit disease. A

preliminary study suggests that lymph node cells from MTH-immunized BALB/cJ mice proliferate in vitro as well as or better than lymph node cells from MTH-immunized BALB/cByJ mice in response to testicular cell Ags (data not shown). Whether an associated polarized cytokine production by T cells of the two substrains in response to testicular Ags exists is under investigation.

A genetically predisposed imbalance in $CD4^+$ T cell subsets does not necessarily have to be confined to a locus that directly controls the ratio of such populations, particularly considering the role that infectious agents play in biasing the Th1:Th2 response (27). The mutation in BALB/cJ mice controlling resistance to EAE and EAO may be in a locus that can shift the ratio of Th1:Th2 cells lacking any overt clinical symptoms. To address the role of environmental factors in the susceptibility of BALB/cByJ mice to EAE and EAO, small breeding colonies were established in three different vivariums at University of Pennsylvania School of Medicine. The results were then compared with disease susceptibility in mice purchased from The Jackson Laboratory and housed at the vivariums at Brigham Young University and University of Virginia, along with the results obtained at University of New Mexico (Albuquerque, NM). SJL/J mice served as controls. The colonies were maintained under standard operating conditions for each location. Pregnant females were removed from breeder cages and housed individually, and pups were weaned at 20 days of age. Male and female mice used in the experiment were all third generation offspring generated by random intercage mating and were between 10 and 12 wk of age at the time of inoculation. The same stocks of MTH, SCH, CFA, and Ptx were used for disease induction in all University of Pennsylvania School of Medicine and Brigham Young University groups. EAO susceptibility was also studied at University of Virginia using either the same MTH stock as that described above (designated MTH stock A in Table IV) or MTH generated independently and designated MTH stock B.

BALB/cByJ mice generated and maintained under the three different environmental conditions exhibit varying degrees of susceptibility to autoimmune orchitis and EAE, ranging from fully susceptible to completely resistant (Table IV). BALB/cByJ mice housed in the vivarium of the Widtsoe Building at Brigham Young University and University of Virginia exhibited the susceptible phenotype, but BALB/cByJ mice housed in the Medical Education Building at University of Pennsylvania School of Medicine were completely resistant to EAE and EAO. In contrast, SJL/J mice generated and maintained under the same conditions did not exhibit a significant deviation in the range of susceptible phenotypes. Interestingly, while the BALB/cJ mice housed in the John Morgan Building at University of Pennsylvania School of Medicine were highly resistant to EAO induction, BALB/cJ mice housed at University of Virginia were susceptible to EAO induction within 15 to 21 days following immunization, and the difference was not dependent on the Ag used as immunogen (29).

Concerning EAE, marked differences in the susceptibility of BALB/c wild-type mice have been observed with animals obtained from different colonies (2). Similarly, susceptibility to pristane-induced plasmacytomagenesis (PCT) is dependent on both T cells (30) and the environment in which the PCT-susceptible BALB/c substrain is generated. Susceptible mice generated in specific pathogen-free conditions are refractory to PCT induction (31). It should be noted that both environmental influences and possible regulatory T cells have been reported to play a role in the induction and prevention of EAE in SJL mice expressing transgenic TCRs specific for an encephalitogenic peptide (32, 33). In summary, the results presented suggest that the use of BALB/c substrains offer a unique opportunity to study both the genetic and environmental factors that play a role in the development of $CD4^+$ effector and $CD4^+$ regulatory T cells in organ-specific autoimmune diseases such as EAE and EAO.

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