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

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

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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 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

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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 scuff 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).

Immunosuppressive 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% NH4Cl. The SpC were then washed twice with HBSS and resuspended in HBSS without serum at a final concentration of 3 x 10^7 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-LT4), 3.168.8 (anti-Lyt-2), and J11 (anti-Thy-1.2). All hybridomas were grown as ascites in pristane-conditioned nude mice. Guinea pig serum served as the source of complement (C3). Thy-1.2, CD4+, and CD8-depleted SpC suspensions were generated by treating 3 x 10^7 SpC (at a concentration of 5 x 10^6 cells/ml) with the respective mAb plus C3. The concentration of the mAbs used was 0.1 ml of appropriately preluted ascites fluid/5 x 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). SJJ/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 x BALB/cByJ)F1 and (BALB/cByJ x BALB/cJ)F1 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 EAO and EAE is not linked to the mutant BALB/cJ Afr 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
in the colocalization of several such genes. Shared susceptibility
genome exclusion mapping identifying susceptibility loci involved
clearly establish a precedent for the former. Similarly, comparative
specific. MHC-linked immune response genes and
role in multiple autoimmune diseases and those that are disease
non-MHC-linked disease susceptibility loci exist: those that play a
phenotypic expression of two independent models of organ-spe-
tific. 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% concord-
cence between susceptibility to EAO and EAE among the differ-
ent groups of BC2 progeny in which the dominant, wild-type
BALB/cByJ allele was segregating (Table I). In addition, the over-
all incidence of orchitis and EAE in the BC2 population, as
determined histologically, was not significantly different (% > 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 indepen-
dent 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 Bphps (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 colocaliza-
ion phenomenon of susceptibility loci among independent linkage
studies. Examples include the following. Idd3, a diabetes suscep-
ibility 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 dys-
genesis (15), and Orch3 and Orch5, two susceptibility loci in au-
toimmune 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 cyclo-
phosphamide 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 inci-
dence 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 suscepti-
bility of BALB/cJ 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 ad-
juvant-immunized animals exhibited a decrease in both the inci-
dence and severity of the lesions seen in EAE (Table III). In

<table>
<thead>
<tr>
<th>Treatment of Donor Mice</th>
<th>No. Spc Transferred (× 10^6)</th>
<th>Clinical</th>
<th>Histologic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Without symptoms</td>
<td>Flaccid tail and/or weakness</td>
</tr>
<tr>
<td>CFA + Ptx</td>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>SCH + CFA + Ptx</td>
<td>3</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>SCH + CFA + Ptx (BALB/cByJ donor)</td>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>SCH + CFA + Ptx Anti-Thy-1.2 + C-</td>
<td>3</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>SCH + CFA + Ptx Anti-CD8 + C-</td>
<td>3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SCH + CFA + Ptx Anti-CD4 + C-</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

* 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.
* Recipients were inoculated 3 days following transfer of donor SpC as described in Materials and Methods.
contrast, recipients receiving SpC from BALB/cByJ mice immunized with SCH plus adjuvants developed disease similar to that of mice receiving adjuvant-primed BALB/c 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+ to 12 weeks of age at the time of inoculation. The stocks of MTH, SCH, CFA, and Ptx used are as described in the Materials and Methods.

### Materials and Methods

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 substrains 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

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

<table>
<thead>
<tr>
<th>Vivarium</th>
<th>Autoimmune Orchitis</th>
<th>EAE Clinical</th>
<th>Histologic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTH stock</td>
<td>PI ± SE</td>
<td>Incidence</td>
</tr>
<tr>
<td><strong>University of Pennsylvania School of Medicine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>John Morgan Building</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>A</td>
<td>3.7 ± 0.8</td>
<td>13/17</td>
</tr>
<tr>
<td>BALB/cJ (Table I)</td>
<td>A</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td>SJL/J</td>
<td>A</td>
<td>3.1 ± 0.6</td>
<td>9/10</td>
</tr>
<tr>
<td><strong>Richards Building</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>A</td>
<td>3.9 ± 0.7</td>
<td>18/19</td>
</tr>
<tr>
<td>SJL/J</td>
<td>A</td>
<td>3.5 ± 1.0</td>
<td>8/9</td>
</tr>
<tr>
<td><strong>Medical Education Building</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>A</td>
<td>0</td>
<td>0/21</td>
</tr>
<tr>
<td>SJL/J</td>
<td>A</td>
<td>3.8 ± 0.8</td>
<td>8/9</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>A</td>
<td>5.3</td>
<td>8/8</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>A</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td><strong>Brigham Young University</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Old Medical School Building</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>A</td>
<td>3.5 ± 0.5</td>
<td>11/11</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>B</td>
<td>1.9 ± 0.9</td>
<td>3/6</td>
</tr>
<tr>
<td>SJL/J</td>
<td>A</td>
<td>5.6 ± 1.2</td>
<td>9/12</td>
</tr>
<tr>
<td><strong>University of Virginia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>B</td>
<td>5.7 ± 0.4</td>
<td>8/11</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>B</td>
<td>4.2 ± 1.1</td>
<td>3/4</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>A</td>
<td>2.3 ± 0.5</td>
<td>3/5</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>B</td>
<td>2.1 ± 0.1</td>
<td>4/7</td>
</tr>
<tr>
<td><strong>University of New Mexico School of Medicine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>B</td>
<td>5.7 ± 1.1</td>
<td>10/13</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>B</td>
<td>0.2 ± 0.2</td>
<td>2/10</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>B</td>
<td>1.2 ± 0.6</td>
<td>8/19</td>
</tr>
</tbody>
</table>

* 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 inoculation. The stocks of MTH, SCH, CFA, and Ptx used are as described in the Materials and Methods.

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

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

* 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.

* Previously reported data (7).
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 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 EAO and EAE. 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 substrate 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.

Acknowledgments

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References


