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J Immunol 2001; 166:4237-4243; doi: 10.4049/jimmunol.166.6.4237
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Environmental Modulation of Autoimmune Arthritis Involves the Spontaneous Microbial Induction of T Cell Responses to Regulatory Determinants Within Heat Shock Protein 65

Kamal D. Moudgil, Eugene Kim, Oliver J. Yun, Howard H. Chi, Ernest Brahn, and Eli E. Sercarz

Both genetic and environmental factors are believed to be involved in the induction of autoimmune diseases. Adjuvant arthritis (AA) is inducible in susceptible rat strains by injection of Mycobacterium tuberculosis, and arthritic rats raise T cell responses to the 65-kDa mycobacterial heat-shock protein (Bhs6p5). We observed that Fischer 344 (F344) rats raised in a barrier facility (BF-F344) are susceptible to AA, whereas F344 rats maintained in a conventional facility (CV-F344) show significantly reduced incidence and severity of AA, despite responding well to the arthritogenic determinant within Bhs6p5. The acquisition of protection from AA can be circumvented if rats are maintained on neomycin/acidified water. Strikingly, naive unimmunized CV-F344 rats but not BF-F344 rats raised T cell responses to Bhs6p5 C-terminal determinants (BCTD) (we have previously shown that BCTD are involved in regulation of acute AA in the Lewis rat); however, T cells of naive CV-F344 and BF-F344 gave a comparable level of proliferative response to a mitogen, but no response at all to an irrelevant Ag. Furthermore, adoptive transfer into naive BF-F344 rats of splenic cells of naive CV-F344 rats (restimulated with BCTD in vitro) before induction of AA resulted in a considerably reduced severity of AA. These results suggest that spontaneous (inadvertent) priming of BCTD-reactive T cells, owing to determinant mimicry between Bhs6p5 and its homologues in microbial agents in the conventional environment, is involved in modulating the severity of AA in CV-F344 rats. These results have important implications in broadening understanding of the host-microbe interaction in human autoimmune diseases. The Journal of Immunology, 2001, 166: 4237–4243.

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This work was supported by Grants from the National Institutes of Health (AR-45799, AI-11183, and AI-47790); Arthritis Foundation (AF/011634; Southern California Chapter, Los Angeles, CA; and IR-3–43698; National office, Atlanta, GA; and the Bertram A. Maltz Laboratory of Molecular Rheumatology, University of California School of Medicine. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. Address correspondence and reprint requests to Dr. Kamal D. Moudgil, Department of Microbiology and Immunology, University of Maryland School of Medicine, BRB 13-019, 655 West Baltimore Street, Baltimore, MD, 21201-1509. E-mail address: kmoudg001@umaryland.edu

Abbreviations used in this paper: AA, adjuvant arthritis; Bhs6p5, the 65-kDa mycobacterial heat-shock protein; BCTD, Bhs6p5 C-terminal determinants; BF, barrier facility; CV, conventional facility; HELL, hen egg white lysozyme; SPC, spleen cells; F344, Fischer 344; BF-F344, F344 rats raised in a BF; CV-F344, F344 rats maintained in a CV; AI, arthritic index; PAS, peak arthritic score; S.I., stimulation index; BF→CV F344, BF-F344 rats transferred at the age of 4–5 wk into, and kept there-after, in a CV.
In other experimental animal models of autoimmunity, the initiation and/or propagation of autoimmunity has been attributed to the conventional environment; for example, joint and intestinal inflammatory disease in HLA-B27 transgenic rats (12), iodine-induced autoimmune thyroiditis in NOD-H2b mice (13), experimental autoimmune thyroiditis in rats (14), hemolytic anemia (15), experimental autoimmune encephalomyelitis (16), and Pristane-induced arthritis (17). In contrast, the current study has revealed that exposure of a susceptible rat strain to the conventional environment rather leads to a significant reduction in the incidence and severity of an autoimmune disease, AA, through spontaneous induction of T cells against regulatory determinants of Bhs65. These results add a new dimension to the host-microbes relationship in autoimmunity, and are of significance in understanding of the pathogenesis of human autoimmune diseases.

Materials and Methods

Animals

BF-bred F344 rats (RT.1l) were purchased from Charles River Laboratories (Wilmington, MA). The CV-F344 rats were derived from breeding BF-F344 rats in the institutional CV (initially at University of California, Los Angeles, CA, and recently at University of Maryland School of Medicine, Baltimore, MD), and then housing the newborns in the same environment. For some experiments, BF-F344 rats were obtained from Harlant Sprague-Dawley (San Diego, CA) (F344/Nsdh). The major observations of experiments performed using BF-F344 rats from one source could be reproduced in BF-F344 rats from the other source, and the same was applicable to CV-F344 rats raised under two different conventional vivarium conditions. Male rats, 4–16 wk old, were used in almost all the experiments. A BF system (virus-free or specific-pathogen-free system) uses triple air locks and contains a Hepa filtration device. In addition, the holding cages, bedding, and food are sterilized by autoclaving, and water is filtered, chlorinated, and acidified. In contrast, the conventional animal (rat) facility consists of a single door that leads into the unfiltered housing facility. The caretakers or other workers are not required to take a shower or wear sterile clothes before entering the facility. The rats are housed in cages without filtertops, and the cages and bedding are not autoclaved. Furthermore, rats received filtered water.

Ags/peptides/mitogens

The peptides containing amino acid sequences of Bhs65 (18) were either synthesized and purified in the University of California Peptide Core Laboratory as described earlier (19) or obtained from Macromolecular Resources (Fort Collins, CO)/Chiron Mimotopes (San Diego, CA). Hen egg-white lysozyme (HEL), three-times recrystallized, was purchased from Sigma (St. Louis, MO), and then further purified by chromatography as described (20). Con A was obtained from Sigma.

Induction of AA

Rats were immunized s.c. with 200 μl of M. tuberculosis H37Ra (Difco, Detroit, MI) (10 mg/ml) suspended in IFA (Difco) or in mineral oil (Sigma) (5). Beginning day 7 after immunization, the rats were scored daily for clinical signs of arthritis. The severity of arthritis in each paw was evaluated on the basis of erythema, swelling, and deformity of the joint (21, 22), and graded on a scale of 0 to 4 as follows: 0, no erythema or swelling; 1, slight erythema or swelling of the ankle or wrist; 2, moderate erythema and swelling of the entire forepaw or hindpaw; 4, severe erythema and swelling of the entire forepaw or hindpaw (5, 23). The evaluation of the disease score in different groups of rats was performed by a blinded fashion; each day the arthritis scores of rats were recorded on a separate new sheet so that the observer had no information about the scores of the same rats on the preceding days. The sum of the arthritic score of the paws graded yielded the total daily arthritic score for each rat. This information was used to derive the arthritic index (AI) or mean peak arthritic score (PAS). AI indicates the sum of daily arthritic scores of individual rats during the entire course of AA, and the mean PAS was derived by averaging the AI of the entire group. Mean PAS of a group of rats was derived by finding the average daily score of the group, and then identifying the highest score reached during the course of AA.

Splenic T cell proliferation assay

Rat spleens were removed and a single-cell suspension was prepared (5). Stomonal debris was removed, and the cells were washed twice with HBSS (Life Technologies, Rockville, MD). These SPC were cultured in flat-bottom 96-well plates at 6 × 10^5 cells/well in HL-1 serum-free medium (Ventrex Laboratories, Portland, ME) supplemented with 2 nM 1,2-glutamine, 100 μM NaHCO_3, and 100 μg/ml streptomycin sulfate, with or without Ag (added at different concentrations). Con A or Tuberculin purified protein derivative (PPD) was used as a positive control. A total of 1 μCi of [3H]thymidine (International Chemical and Nuclear, Irvine, CA) was added per well for the last 18 h of a 5-day culture. The cells were then harvested on a Printed Filtermat A glass fiber filter (Wallac, Turku, Finland) using a Micro Cell Harvester (Skatron, Sterling, VA), and the incorporation of radioactivity was assayed by a liquid scintillation LKB 1205 Betaplate counter. The results were expressed as either cpm or stimulation index (S.I. = cpm with Ag/cpm with cells in medium alone). For some repeat experiments, supplemented culture medium was used: HL-1 medium with 1% (v/v) heat-inactivated FCS (Gemini Biological Products, Los Angeles, CA, and recently at University of Maryland School of Medicine, Baltimore, MD) supplemented with 2% FCS and/or 5 × 10^-3 M 2-ME (Sigma). Although generally 6 × 10^5 SPC were cultured per well, in some assays 5 × 10^6 cells/well were used.

Lymph node T cell proliferation assay

The draining lymph nodes of rats immunized s.c. with M. tuberculosis were removed and a single-cell suspension prepared (5, 19). Lymph node proliferation assay was performed as described for SPC, except for plating lymph node cells (LNC) at a concentration of 5 × 10^5 cells/well.

Adaptive transfer experiments

Naive CV-F344 rats (5–16 wk old, male) were used as donors. BF-F344 rats (5–6 wk old, male) were used as recipients or controls. Spleens of CV-F344 rats were removed, a single-cell suspension prepared, and the RBC in the sample were lysed. Thereafter, SPC were washed thoroughly, and cultured in vitro in flasks (2–3 × 10^6 cells/ml) in RPMI 1640 medium (supplemented with 1% 1-glutamine, 1% penicillin-streptomycin, 2.5 × 10^-3 M 2-ME, and 5% FCS) in an atmosphere of 5% CO_2 and 95% air for 3 days in the presence of an equimolar mixture of five peptides comprising BCTD (peptides 417–431, 441–455, 465–479, 513–527, and 521–535 of Bhs65) or an irrelevant Ag, HEL. Following restimulation in vitro, SPC were harvested, pooled, and washed with HBSS, live cells were counted and then injected at a concentration of 2 × 10^6 cells i.v. or i.p. into naive BF-F344 recipients. Within 18 h, the recipient F344 rats and a group of age- and sex-matched naive BF-F344 rats (controls) were challenged with M. tuberculosis s.c. for the purpose of induction of AA. Thereafter, all rats were observed regularly for signs of arthritis, and the severity of the disease was scored as described (5).

Results

BF-F344 rats are susceptible to AA, whereas CV-F344 rats reveal considerably reduced incidence and severity of AA

F344 rats raised under different environmental conditions (BF-F344, CV-F344, and BF-F344 rats transferred at the age of 4–5 wk into, and kept thereafter, in a CV (BF→CV F344)) were tested for their susceptibility to AA. Cohorts each of BF-F344, BF→CV F344, and CV-F344 rats were injected with M. tuberculosis s.c., and from day 7 after immunization, were examined regularly for signs of AA. The results are summarized in Table I. Strikingly, 20/28 (71.4%) of BF-F344 rats revealed a typical course of AA in comparison to 16.7% of BF→CV F344 rats, and 17.2% of CV-F344 rats. This difference between BF-F344 and each of the remaining two groups of rats was statistically significant (p < 0.005). The suppressive effect of the conventional environment on AA is further highlighted by the differences in the quantitative parameters including the AI and the PAS of the respective groups (Table I). The differences between BF-F344 vs BF→CV F344 as well as those between BF-F344 vs CV-F344 groups were statistically significant. Thus, the susceptibility to AA of BF-F344 rats can be modulated significantly in a conventional environment.
The level of gut microbial load significantly modulates susceptibility to AA of F344 rats

On the basis of the above results, we reasoned that environmental agents (e.g., gut microbial flora) in the CV might be contributing to the reduced incidence and severity of AA in CV-F344 rats. To determine the relationship between gut microbial load and susceptibility to AA of F344 rats, we studied BF-F344 rats that were transferred (at the age of 4–5 wk) into the conventional environment but maintained thereafter by feeding either regular filtered water (group 1) that was also used for CV-F344 rats, neomycin (2 mg/ml) added to the drinking water (group 2), or acidified water (pH 2–3) (group 3). The objective of feeding neomycin or acidified water was to reduce the existing gut microbial load, and to prevent/reduce further colonization of the gut with bacteria from the environment. All rats received the same food. After 4 wk, all rats were immunized with M. tuberculosis s.c., and then followed for signs of arthritis. Interestingly, F344 rats in group 1 showed a significant reduction in the incidence of AA; only 2 of 12 (16.6%) rats developed arthritis. On the contrary, 14 of 14 (100%) F344 rats in groups 2 and 3 combined maintained their susceptibility to AA, and developed arthritis. This difference in relative susceptibility to AA (16.6 vs 100%) was statistically significant (p < 0.005).

CV-F344 rats are not deficient in mounting responses to the potentially arthritogenic determinant of Bshp65

AA-susceptible Lewis rats develop potent responses to peptide 177–191 of Bshp65 (p177–191) (which contains the minimal arthritogenic determinant 180–188 for the Lewis rat and is cross-reactive with it) upon challenge with M. tuberculosis (5). We tested whether AA-susceptible BF-F344 and relatively less susceptible CV-F344 rats differ significantly in their ability to respond to p177–191 after immunization with M. tuberculosis. The results shown in Fig. 1 demonstrate that both BF-F344 and CV-F344 rats have strong and comparable levels of proliferative T cell responses to p177–191. Thus, the reduction in the incidence of AA of CV-F344 rats cannot be attributed to a lack of response to the potentially arthritogenic determinant of Bshp65. In this regard, a more likely explanation is the induction of Bshp65-reactive T cells, potentially capable of modulating the course of AA, in CV-F344 rats in the conventional environment.

CV-F344 rats but not BF-F344 rats develop spontaneous proliferative T cell responses to BCTD

The results of our previous study suggest that T cell responses to BCTD (represented by peptides 417–431, 441–455, 465–479, 513–527, and 521–535 of Bshp65) are involved in natural remission from AA in the Lewis rat (5). Based on these results, we reasoned that the reduced incidence and severity of AA in CV-F344 rats might be due to inadvertent induction of T cell responses to BCTD, possibly following their exposure to environmental agents, such as bacteria, in the CV. Because hsp are highly conserved, the environmental agent(s) might possess a homologue of Bshp65, which could fortuitously prime T cell responses to BCTD through determinant mimicry. To examine the above proposition, we tested SPC of three groups of naive unimmunized F344 rats (BF-F344, BF→CV F344, and CV-F344 rats, none of which had been exposed to M. tuberculosis in any form) in a proliferation assay using peptides comprising BCTD and other regions of Bshp65. The results are given in Fig. 2. Clearly, CV-F344 rats raised relatively much higher proliferative responses to BCTD and to peptides comprising two of the amino-terminal determinants of Bshp65 (namely, 1–15 and 13–27) compared with BF-F344 rats. Of different Bshp65 peptides, the lowest responses were observed with peptides 33–48, 121–135, and 177–191 of Bshp65 in CV-F344. The difference in the response of BF-F344 and CV-F344 rats to three of the BCTD peptides (417–431, 513–527, and 521–535) was statistically significant (p < 0.05–0.025). Interestingly, there was a gradual increase in the level of proliferative response to Bshp65 peptides in the three groups of rats (BF-F344, BF→CV F344, and CV-F344, in increasing order of response) in direct relation to the duration, and thereby, extent of exposure of these peptides.
rats to the conventional environment. Importantly, CV-F344 and BF-F344 but not BF-F344 show reduction in the incidence and severity of AA (Table I). According to the results of our previous study, the amino-terminal determinants of Bhsp65 did not play any role in attenuation of AA under the conditions of the test (5). In this regard, BCTD appear to be the most relevant candidate in modulating the course of AA in CV-F344 and BF-F344 rats. Considering that the proliferative T cell responses shown in Fig. 2 are of naive unimmunized rats, the cut off level to determine a positive response was set at a S.I. of 1.5 to account for relatively lower responses of naive rats (compared with rats traditionally immunized with Ag in adjuvant, whose responses are expectedly much higher). In a recently reported study, the change in the pattern/trend of proliferative T cell response of patients with multiple sclerosis was similarly deemed to be of physiologic significance despite the relatively low levels of S.I. (24, 25).

Adoptive transfer of SPC of naive CV-F344 rats into naive BF-F344 rats leads to reduced severity of AA in recipients following injection of M. tuberculosis

To further define the immunologic basis of relatively reduced susceptibility to AA of CV-F344 rats, we determined whether splenic T cells (SPC) of CV-F344 rats could down-modulate the course of AA in naive BF-F344 rats. For this purpose, SPC of naive CV-F344 rats were cultured for 3 days in vitro with an equimolar pool of peptides comprising defined determinants within Bhsp65, including BCTD (represented by peptides 417–431, 441–455, 465–479, 513–527, and 521–535 of Bhsp65) were used for testing in vitro recall response. The response to each peptide of a particular group of rats is shown as a S.I. (mean ± SEM). The difference in the response of BF-F344 and CV-F344 rats to three of the BCTD peptides (417–431, 513–527, and 521–535) was statistically significant (p < 0.05–0.025).
of five peptides comprising BCTD (peptides 417–431, 441–455, 465–479, 513–527, and 521–535 of Bhsp65), and then injected i.p. into naive BF-F344 rats (experimental group). Age- and sex-matched naive BF-F344 rats, which had either not received any SPC at all or received HEL-restimulated SPC, served as controls. All experimental and control rats were immunized with M. tuberculosis s.c. to induce AA. The results of a representative experiment are shown in Fig. 4, and in another format, in Table II (experiment no. 1). Also included in Table II are the results of several repeat adoptive transfer experiments. Collectively, these results demonstrate that transfer into BF-F344 rats of BCTD-restimulated SPC of CV-F344 rats led to a significant reduction in the severity of subsequent AA in the recipients, whereas the control rats revealed the usual course of AA. Collectively, the incidence of AA with a typical disease course in the three groups of rats (Table II) was as follows: BF-F344, (26/27 (96.3%)); BCTD group, (4/24 (16.7%)); and HEL group, (13/13 (100%)). These results underscore the extent of down-modulation of AA effected by adoptive transfer into naive BF-F344 rats of BCTD-restimulated SPC of CV-F344 rats in comparison to recipients of HEL-restimulated SPC or no cells at all. In most experiments, the incidence as well as overall course of disease in BF-F344 and HEL group of rats was comparable. In one experiment (no. 3, Table II), apparently there was a slight suppression of disease activity in HEL group; however, the difference between the two groups was not statistically significant. These results show that the presence of potentially BCTD-reactive T cells in the donor CV-F344 SPC restimulated in vitro with an irrelevant Ag (HEL) was not sufficient for effecting reduction in the severity of subsequent AA in recipients to a significant level unlike that in experimental groups given BCTD-restimulated SPC. These results suggest that re-activation and expansion of BCTD-reactive T cells above a certain threshold was essential for modulating the severity of AA. We have described above adoptive transfer experiments using whole SPC. Such an approach has successfully been used in a recent study in animal model of type I diabetes (26).

**Discussion**

In this study we have observed that naive unimmunized, conventionally raised F344 rats, but not barrier-raised F344 rats, spontaneously develop T cell responses to BCTD, and that CV-F344 rats reveal a significantly reduced incidence and severity of AA despite their ability to raise T cell responses to the potentially arthritogenic determinant of Bhsp65. The results of our earlier study have shown that BCTD but not peptides representing the amino-terminal determinants of Bhsp65 were capable of modulating the course of AA in Lewis rats (5). In this regard, spontaneously induced BCTD-reactive T cells in CV-F344 and BF→CV F344 rats are most likely to be involved in modulating the course of AA in these rats. This proposition is further validated by the results showing that T cells of naive BF-F344 and CV-F344 rats give a comparable level of proliferative response to Con A but no recall response at all to an irrelevant Ag like HEL; and adoptive transfer of BCTD-restimulated but not HEL-restimulated SPC derived from naive CV-F344 rats can lead to reduced severity of AA in recipient BF-F344 rats. The approach using whole SPC in adoptive transfer experiments in this study is similar to that used in a recently reported study in the murine model of autoimmune diabetes (26). Despite the relatively low S.I. of T cell response to BCTD, the trend revealed by the pattern of response of CV-F344/BF→CV F344 rats compared with BF-F344 rats underscores a phenomenon of physiologic significance. Such interpretation of results based on low S.I. values has also been emphasized by other investigators studying T cell response of multiple sclerosis patients (24, 25). Thus, the results of this study provide an immunologic explanation underlying the reduced incidence and severity of AA observed in CV-F344 rats: priming of BCTD-reactive T cells. We suggest that this T cell priming is owing to molecular mimicry (10, 11, 27, 28, 33–35 (18–23), 20, 23, 25 (p < 0.05–0.01)), and lower S.I. values has also been emphasized by other investigators studying T cell response of multiple sclerosis patients (24, 25).

### Table II. Adoptive transfer of BCTD-restimulated spleen cells of CV-F344 rats into BF-F344 rats leads to reduced severity of AA in recipients

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Group</th>
<th>No. of Rats per Group</th>
<th>Rats with a Typical Unattenuated Disease</th>
<th>Mean Day of Onset of AA</th>
<th>Mean AA</th>
<th>Mean PAS of the Control or Affected Group (mean ± SEM)</th>
<th>Days of Significant Protection from AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BF-F344</td>
<td>3</td>
<td>0/3 (100)</td>
<td>12.0</td>
<td>30.83</td>
<td>4.83 ± 1.01</td>
<td>18–23 (p &lt; 0.05–0.01)</td>
</tr>
<tr>
<td></td>
<td>BCTD</td>
<td>4</td>
<td>0/4 (0)</td>
<td>13.0</td>
<td>12.75</td>
<td>2.00 ± 0.71</td>
<td>11–18 (p &lt; 0.05–0.01)</td>
</tr>
<tr>
<td>2</td>
<td>BF-F344</td>
<td>3</td>
<td>0/3 (100)</td>
<td>11.0</td>
<td>70.38</td>
<td>8.33 ± 1.33</td>
<td>5.62 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>BCTD</td>
<td>3</td>
<td>0/3 (0)</td>
<td>16.0</td>
<td>28.31</td>
<td>3.66 ± 0.66</td>
<td>12, 20, 23, 25 (p &lt; 0.05–0.01)</td>
</tr>
<tr>
<td>3</td>
<td>BF-F344</td>
<td>4</td>
<td>0/4 (100)</td>
<td>12.0</td>
<td>73.87</td>
<td>5.62 ± 1.53</td>
<td>14, 20–22, 33–35 (p &lt; 0.05)</td>
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<tr>
<td></td>
<td>HEL</td>
<td>4</td>
<td>0/4 (100)</td>
<td>10.0</td>
<td>42.53</td>
<td>4.75 ± 0.63</td>
<td>1.50 ± 0.38</td>
</tr>
<tr>
<td>4</td>
<td>BF-F344</td>
<td>3</td>
<td>0/3 (100)</td>
<td>13.7</td>
<td>17.75</td>
<td>0.63 ± 0.63</td>
<td>11–14, 16 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>BCTD</td>
<td>4</td>
<td>2/4 (50)</td>
<td>14.0</td>
<td>4.75</td>
<td>2.67 ± 1.18</td>
<td>5.63 ± 3.63</td>
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<tr>
<td>5</td>
<td>BF-F344</td>
<td>3</td>
<td>0/3 (100)</td>
<td>14.0</td>
<td>24.58</td>
<td>1.53 ± 0.33</td>
<td>3.06 ± 0.58</td>
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<tr>
<td></td>
<td>HEL</td>
<td>4</td>
<td>0/4 (100)</td>
<td>13.75</td>
<td>23.93</td>
<td>1.83 ± 0.33</td>
<td>8.71 ± 0.71</td>
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<td>6</td>
<td>BF-F344</td>
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<td>0/3 (100)</td>
<td>13.0</td>
<td>16.08</td>
<td>0.63 ± 0.13</td>
<td>14, 20–22, 33–35 (p &lt; 0.05)</td>
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<tr>
<td></td>
<td>BCTD</td>
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<td>0/2 (0)</td>
<td>15.5</td>
<td>4.37</td>
<td>1.50 ± 0.38</td>
<td>1.50 ± 0.38</td>
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<tr>
<td>7</td>
<td>BF-F344</td>
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<td>0/3 (100)</td>
<td>15.7</td>
<td>10.67</td>
<td>2.81 ± 0.43</td>
<td>11–14, 16 (p &lt; 0.05)</td>
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<tr>
<td></td>
<td>HEL</td>
<td>4</td>
<td>0/4 (100)</td>
<td>12.5</td>
<td>24.50</td>
<td>2.00 ± 0.50</td>
<td>15–28 (p &lt; 0.05–0.01)</td>
</tr>
<tr>
<td>8</td>
<td>BF-F344</td>
<td>4</td>
<td>0/4 (100)</td>
<td>16.0</td>
<td>4.75</td>
<td>1.06 ± 0.37</td>
<td>15–28 (p &lt; 0.05–0.01)</td>
</tr>
</tbody>
</table>

*The adoptive transfer experiment was performed as described in Materials and Methods, and also in Fig. 4. Groups: BF-F344, naive BF-F344 rats immunized with M. tuberculosis s.c.; BCTD, naive BF-F344 rats injected i.p. with BCTD-restimulated SPC of CV-F344 rats followed by injection of M. tuberculosis s.c.; and HEL, naive BF-F344 rat recipients of HEL-restimulated SPC of CV-F344 rats followed by M. tuberculosis injection.

*AI represents the sum of daily scores of individual rats during the entire course of AA. The mean AI depicts the average AI of the entire group.

*Mean PAS of a group of rats was derived by finding the average daily score of the group, and then identifying the highest score reached during the course of AA.

*The statistical analysis of the data was performed using two different methods, Student’s t test and Wilcoxon rank sum test. The results of Student’s t test are shown in the Table; the days of significant protection indicate those corresponding days during the course of AA at which the difference of the arthritic score (mean ± SEM) of the control and experimental groups was statistically significant. The Wilcoxon test compared the difference in the arthritic scores during the entire course of AA of the control and experimental groups, and this test was significant for results of BCTD vs BF-F344 groups in experiments 1, 2, 7, and 8 but not for any of the HEL vs BF-F344 groups; BCTD groups in remaining two experiments could not be analyzed by this test because of sample size restriction.
BCTD bring about these effects is currently under investigation. However, because the nature of microbial flora in different conventional facilities might be different, it is likely that T cell responses to certain Bhsp65 determinant regions (6, 38, 39) other than, or in addition to, BCTD might be crucial in inducing suppressive effect on AA in F344 rats. In addition, microbial agents can also influence susceptibility to autoimmunity by mechanisms other than determinant mimicry, e.g., bystander activation of potentially autoreactive T cells (34, 40), and by the action of superantigen (41). Interestingly, it has been shown that infection with Mycoplasma pulmonis can modulate the course of AA in Lewis rats (42). An additional factor that might contribute to the reduced incidence and severity of AA in CV-F344 rats might be the level of activity of the hypothalamic-pituitary-adrenal axis (43). However, in one study, no significant difference in the levels of plasma corticosterone in response to IL-1α between germ-free F344 and CV-F344 rats was observed (37). The outcome of the influence of the above factors on AA is further compounded by the genetic susceptibility/resistance to the disease (44).

The results of this study using F344 rats have important implications for the susceptibility to autoimmunity of heterogeneous human populations living under different environmental conditions. It is conceivable that exposure to various agents (such as bacteria and viruses) is one of the instrumental causes in modulating the incidence, severity, or even the type of autoimmune process acquired by an individual (45–51). Likewise, this type of adventitious microbial stimulation occurring throughout the life of an individual could play an important role in establishing expanded repertoires of pathogenic/regulatory memory T cells (52–54). These factors, for example may contribute to the lack of concordance of susceptibility to certain autoimmune diseases between siblings, including identical human twins.

Acknowledgments

We thank Jeffrey Canceko for excellent technical assistance, and David B. Stevens and Geraldine Werk for helpful discussion.

References


