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Diversification of T Cell Responses to Carboxy-terminal Determinants within the 65-kD Heat-shock Protein Is Involved in Regulation of Autoimmune Arthritis

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Summary

The T cell response to the 65-kD mycobacterial heat-shock protein (Bhsp65) has been implicated in the pathogenesis of autoimmune arthritis. Adjuvant arthritis (AA) induced in the Lewis rat (RT-11) by injection of Mycobacterium tuberculosis serves as an experimental model for human rheumatoid arthritis (RA). However, the immunological basis of regulation of acute AA, or of susceptibility/resistance to AA is not known. We have defined the specificity of the proliferative T cell responses to Bhsp65 during the course of AA in the Lewis rat. During the early phase of the disease (6–9 d after onset of AA), Lewis rats raised T cell responses to many determinants within Bhsp65, spread throughout the molecule. Importantly, in the late phase of the disease (8–10 wk after onset of AA), there was evidence for diversification of the T cell responses toward Bhsp65 carboxy-terminal determinants (BCTD) (namely, 417–431, 441–455, 465–479, 513–527, and 521–535). Moreover, arthritic rats in the late phase of AA also raised vigorous T cell responses to those carboxy-terminal determinants within self(rat) hsp65 (Rhsp65) that correspond in position to the above BCTD. These results suggest that the observed diversification is possibly triggered in vivo by induction of self(Rhsp65)-reactive T cells. Interestingly, another strain of rat, the Wistar Kyoto (WKY/NHsd) rat (RT-11), with the same major histocompatibility complex class II molecules as the Lewis rat, was found to be resistant to AA. In WKY rats, vigorous responses to the BCTD, to which the Lewis rat responded only in the late phase of AA, were observed very early, 10 d after injection of M. tuberculosis. Strikingly, pretreatment with the peptides comprising the set of BCTD, but not its amino-terminal determinants, provided significant protection to naive Lewis rats from subsequent induction of AA. Thus, T cell responses to the BCTD are involved in regulating inflammatory arthritis in the Lewis rat and in conferring resistance to AA in the WKY rat. These results have important implications in understanding the pathogenesis of RA and in devising new immunotherapeutic strategies for this disease.

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology (1, 2). In the past several years, considerable interest has been generated in the role of the 65-kD mycobacterial heat-shock protein (Bhsp65) in the pathogenesis of autoimmune arthritis both in experimental animals (3, 4) as well as in humans (5–7). In RA patients, an association between T cell responses to Bhsp65 and early stages of joint inflammation has been observed (8–10), suggesting that Bhsp65-reactive T cell responses are involved in the pathogenesis of this disease. Adjuvant arthritis (AA) can be induced in the inbred Lewis rat after immunization with Mycobacterium tuberculosis in oil (11–13). The disease can also be transferred to naive Lewis rats by T cell lines reactive to peptide 180–188 of Bhsp65 (3, 14). Although arthritic Lewis rats develop vigorous T cell responses to native Bhsp65 and to peptide 180–188 of Bhsp65, neither of these is arthritogenic when injected in protein or peptide form, respectively (15, 16). Interestingly, pretreatment with Bhsp65 can protect naive Lewis rats from development of arthritis upon subsequent immunization with
M. tuberculosis (4, 17), suggesting that Bhsp65 contains protective as well as disease-inducing determinants. T cell lines specific for Bhsp65 have also been shown to protect against AA (14, 18). Similarly, Lewis rats afflicted with AA are resistant to reinduction of AA (13). Neither the mechanism of natural regulation of the acute inflammatory phase of AA nor the mechanism of protection from subsequent induction of AA is known. Likewise, the immunological basis of susceptibility or resistance to AA of different rat strains has not been revealed.

In the present study, we have defined the changing pattern of specificity of the T cell responses of Lewis rats to determinants within Bhsp65 during the course of AA. Arthritic Lewis rats in the early and late phase of the disease revealed distinct patterns of T cell responses toward Bhsp65 carboxy-terminal determinants (BCTD). (The phenomenon of spreading of the T cell responses to new determinants within an antigen, after priming with a single determinant of the same antigen, has been previously described as determinant spreading [19].) On the other hand, we have termed the induction of T cell responses to new determinants after priming with the whole, multidentate antigen as diversification [20, 21]. Moreover, arthritic Lewis rats in the late phase of AA also raised significant responses to certain carboxy-terminal determinants within self(rat) hsp65 (R hsp65). (R at hsp60 [22] has been referred to as rat hsp60 in this study to emphasize its relationship with Bhsp65.) These self-determinants correspond in position precisely to that of the BCTD, suggesting that diversification of response to Bhsp65 observed in vivo, might be triggered in vivo by self-hsp65. In contrast with AA-susceptible Lewis rats, MHC class II-identical, Wistar Kyoto (WKY/NIHsd = WKY) rats (23, 24) were found to be resistant to induction of AA after immunization with M. tuberculosis. In fact, M. tuberculosis-immunized WKY rats raised early and vigorous responses to the BCTD to which the Lewis rats only respond during the late phase of the disease. Pertinently, pre-treatment of naïve Lewis rats with peptides comprising the BCTD, but not its amino-terminal determinants (BNTD), induced significant protection from AA. These results suggest that T cell responses to the BCTD are involved in regulation of acute inflammatory arthritis. Our study suggests one of the immunological bases for natural regulation of acute AA, and of protection (resistance) to development of AA.

Materials and Methods

Animals. Inbred Lewis (RT-1) and Wistar Kyoto (WKY/NIHsd = WKY) rats (23, 24) were obtained from Harlan Sprague-Dawley (San Diego, CA). Male rats, 5-12-wk-old, were used for the experiments. The handling of animals and all procedures performed on them were done in compliance with the guidelines of the UCLA Chancellor's Animal Research Committee.

Antigens-Peptides. Bhsp65 from Mycobacterium bovis (which is identical to hsp65 of M. tuberculosis) was obtained from the World Health Organization through Dr. R. van der Zee. The peptides containing amino acid sequences of the Bhsp65 or R hsp65 (22, 25, 26; the National Biomedical Research Foundation data base) were prepared by three methods: (a) A complete series of overlapping peptides (15-mers with an overlap of 11 amino acid residues) spanning the entire sequence of Bhsp65 was obtained from Chiron M. tuberculosis (Clayton, Australia). The peptides were synthesized using the multi-pin peptide synthesis technique using repeated cycles of Fmoc deprotection and amino acid couplings (27). The procedure had been modified so that the peptides could be cleaved from the pins. The terminal amino group of each peptide was acetylated, whereas diketopiperazine was attached at the carboxy terminus. (b) Several peptides of Bhsp65 and R hsp65 were synthesized in the UCLA Peptide Core Laboratory directed by Dr. J.R. Reeves, Jr., using a Multi-Pin Peptide Synthesizer (Advanced Chem Tech, 396 M PS, Louisville, KY) as described elsewhere (28). The identity and purity of these peptides were determined by Fast Atom Bombardment Mass Spectrometry at the UCLA Center for Molecular and Medical Sciences as Mass Spectrometry facility. (c) Some peptides were obtained from Macromolecular Resources (Colorado State University, Fort Collins, CO). These peptides were synthesized according to the method described elsewhere (29). Hen egg white lysozyme (HEL) peptide was synthesized according to the method previously described (29).

Induction of Adjuvant Arthritis. Inbred male Lewis rats were anesthetized using Halothane (Halocarbon Laboratories, River Edge, N.J.), and then immunized subcutaneously in a hind footpad with 200 μl of M. tuberculosis H37Ra (Difco Laboratories, Detroit, Ml) (10 mg/ml) suspended in IFA (Difco) or in mineral oil (Sigma Chemical Co., St Louis, MO). The bacteria were powdered in a mortar and pestle before suspension in oil. Beginning on day 7 after immunization, the rats were observed daily for clinical signs of arthritis in three of their limbs, excluding the limb in which immunization was performed. The severity of arthritis was evaluated on the basis of erythema, swelling, and deformity of the joint (30, 31), 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 at the wrist or ankle, 3 = moderate erythema and swelling at the wrist/metacarpals or ankle/metatarsals, 4 = severe erythema and swelling of the forepaw or hindpaw (32, 33). Because only the three uninjected limbs were evaluated, the maximum arithmetic score for any rat was 12.

Histopathological Examination of Arthritic Joints. The experimental or age- and sex-matched control rats were sacrificed under anesthesia. A hind or front leg was cut above the ankle or the wrist, respectively, with the help of a bone cutter. For histopathological examination, the skin from the entire limb was removed and the limb was immersed in 10% buffered formalin phosphate (Fisher Scientific, Fair Lawn, NJ) for fixation for at least 2 d before decalcification. After decalcification, the sections were cut with a cryotome and then stained with hematoxylin and eosin. The stained sections were studied under the microscope for histopathological changes in the joints as previously described (32, 33).

Lymph Node and Splenic T Cell Proliferation Assay. The draining lymph nodes of rats immunized subcutaneously with M. tuberculosis as described above, were removed and a single cell suspension prepared (29). The debris was allowed to settle, and the cells in the supernatant were washed twice with HBS (GIBCO BR L, Gaithersburg, MD). These lymph node cells (LNC) were cultured in flat-bottomed 96-well plates at 5 × 10^6 cells/well in HL-1 serum-free medium (Ventrex Laboratories, Inc., Portland, ME) supplemented with 2 mM L-glutamine, 100 U/ml penicillin G.
sodium, and 100 μg/ml streptomycin sulphate, with or without antigen. (Splenic T cell proliferation assays were performed as described for LNC, except for plating SPC at a concentration of 6 × 10^6 cells/well). For the pin peptides, one or two wells were tested per peptide. Tuberculin purified protein derivative (PPD) (Evans Medical Limited, Horsham, England) was used at a final concentration of 2,000 U/well as a positive control. 1 μCi of [3H]thymidine (International Chemical and Nuclear, Irvine, CA) was added per well for the last 18 h of a 5 d culture. The cells were then harvested on a Printed Filtermat A glass fiber filter (Wallac Oy, Turku, Finland) using a Mircro Cell Harvester (Skatron Instruments, Inc., Sterling, VA), and the incorporation of radioactivity was assayed by liquid scintillation counting, using the LKB 1205 Betaplate counter. The results were expressed as either cpm or stimulation index (S.I. = cpm with antigen/cpm with cells in medium alone). For some repeat experiments, HL-1 medium supplemented with 1% (vol/vol) heat-inactivated FCS (Gemini Bio-Products, Inc., Calabasas, CA) or X-Vivo 10 serum-free medium (Bio-Whittaker, Walkersville, MD) supplemented with 2% FCS and/or 5 × 10^{-5} M 2-mercaptoethanol (Sigma) was used in place of HL-1 medium. In some of these assays, 2.5–4.5 × 10^5 cells/well were used instead of 5–6 × 10^5 cells/well.

**Results**

Early and Late Arthritic Responses of Lewis Rats. Lewis rats were immunized subcutaneously in a hind footpad with M. tuberculosis in oil, and from 7 d after immunization onwards, were observed daily for clinical signs of arthritis. The induction of arthritis was further confirmed by histopathological examination of joints as described in Materials and Methods.

Two timepoints were chosen for study of proliferative T cell responses to Bhs65 of LNC of arthritic rats: 6–9 d (early phase; A) or 8–10 wk (late phase; B) after the appearance of clinical AA, rats were killed and their popliteal, inguinal, and axillary LNC pooled together were tested in a proliferation assay. Each group, LNC from four rats were pooled for testing with peptides. Overlapping pin peptides spanning the entire length of the Bhs65 were used for the in vitro recall response in the assay. Each peptide is identified by its first amino acid residue. The results are expressed as cpm. The results of a representative experiment using pooled LNC from four rats are shown in each section of the figure. Similar results were obtained in repeat experiments with pooled LNC or upon testing cells from individual rats with selected Bhs65 peptides (data not shown). In B, the T cell responses to new, unique Bhs65 carboxy-terminal determinants (BCTD) are indicated by arrows.

**Figure 1.** Response of arthritic Lewis rats to Bhs65 in the early (A) and late (B) phases of AA. Inbred Lewis rats were immunized subcutaneously with M. tuberculosis in a hind footpad, and then observed for signs of arthritis. 6–9 d (early phase; A) or 8–10 wk (late phase; B) after the appearance of clinical AA, rats were killed and their popliteal, inguinal, and axillary LNC pooled together were tested in a proliferation assay. Each group, LNC from four rats were pooled for testing with peptides. Overlapping pin peptides spanning the entire length of the Bhs65 were used for the in vitro recall response in the assay. Each peptide is identified by its first amino acid residue. The results are expressed as cpm. The results of a representative experiment using pooled LNC from four rats are shown in each section of the figure. Similar results were obtained in repeat experiments with pooled LNC or upon testing cells from individual rats with selected Bhs65 peptides (data not shown). In B, the T cell responses to new, unique Bhs65 carboxy-terminal determinants (BCTD) are indicated by arrows.
Responses to Carboxy-terminal Determinants within Self (rat)

Table 1. T Cell Responses of Arthritic Lewis Rat to the COOH-terminal Determinants of Bhsp65

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Early phase of AA</th>
<th>Late phase of AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm \times 10^{-3} (S.I.)</td>
<td>Relative strength</td>
</tr>
<tr>
<td>417–431</td>
<td>6.4 (2.2)</td>
<td>11.9</td>
</tr>
<tr>
<td>441–455</td>
<td>4.6 (1.6)</td>
<td>8.6</td>
</tr>
<tr>
<td>465–479</td>
<td>8.0 (2.7)</td>
<td>14.8</td>
</tr>
<tr>
<td>513–527</td>
<td>3.9 (1.3)</td>
<td>7.3</td>
</tr>
<tr>
<td>521–535</td>
<td>12.1 (4.1)</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean value</td>
<td>7.0 (2.4)</td>
<td>13.0</td>
</tr>
</tbody>
</table>

The T cell responses of arthritic Lewis rats to certain selected peptides of Bhsp65 shown in Fig. 1 are expressed above in three ways: cpm (in a \(\beta\)-scintillation counter), stimulation index (S.I. = cpm with antigen/cpm without antigen [cells in medium alone]) and relative strength (%). A common pool of the particular peptide relative to the mean cpm of the other four peaks in the pepscan of both the early and the late phase of AA; the value was expressed as a percentage. To assure comparable comparisons, the same four peaks were chosen as reference [100%] in the early and late phases of AA. To attempt to express an overall index, the average RS value for BCTD in early and late AA were calculated.

Table 2. The Amino Acid Sequences of the Peptides Comprising the COOH-terminal Determinants of Bhsp65 and Rhsp65

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhsp 417–431</td>
<td>LLQAAAPLDELKLEG</td>
</tr>
<tr>
<td>R hsp 418–432</td>
<td>--RCI-A--S--PAN</td>
</tr>
<tr>
<td>Bhsp 441–455</td>
<td>KVALEAPLQIAFNS</td>
</tr>
<tr>
<td>R hsp 441–455</td>
<td>II-R--KI-AMT--K</td>
</tr>
<tr>
<td>Bhsp 465–479</td>
<td>KVRLPAGHCLNQAG</td>
</tr>
<tr>
<td>R hsp 465–479</td>
<td>VE--ILQSSEV--YD--</td>
</tr>
<tr>
<td>Bhsp 513–527</td>
<td>TT*EAVVADKPEKEKA</td>
</tr>
<tr>
<td>R hsp 512–526</td>
<td>LL--A----TEI--*--</td>
</tr>
<tr>
<td>Bhsp 521–535</td>
<td>KPEKEKASV*PGGDM</td>
</tr>
<tr>
<td>R hsp 521–535</td>
<td>T--IPE--EKEK--M--A--</td>
</tr>
</tbody>
</table>

-- identical residue; * = gap introduced for best alignment.

Responses to the carboxy-terminal determinants of both Bhsp65 and R hsp65. These results suggest that there is induction of R hsp65-reactive T cells in vivo during the course of AA.

WKY rats, with the same MHC class II haplotype as the Lewis rat, are resistant to AA. To determine the role of MHC versus non-MHC genes in determining susceptibility or resistance to AA, we tested the WKY rats, which are of the same MHC class II haplotype as the Lewis rat (23, 24). Strikingly, under the same conditions used for induction of AA in the Lewis rat, age- and sex-matched WKY rats were found to be resistant to AA. Only 3 out of 51
Teh majority of WKY rats were protected against AA after pretreatment with the BCTD, whereas no protection was observed in control rats. Notably, in another series of experiments using the same protocol except for injection of M. tuberculosis, the protective effect of BCTD was confirmed in a separate group of WKY rats (data not shown). The results indicate that the BCTD can prevent the development of AA.

In conclusion, our findings suggest that the BCTD plays a crucial role in the prevention of AA. The BCTD contains determinants that induce regulatory T cells and inhibit the development of arthritis, leading to protection against AA. Further studies are needed to understand the mechanisms underlying the protective effect of the BCTD and to identify potential therapeutic targets for the treatment of AA.
In summary, the above results demonstrate that induction of T cell responses to the BCTD are involved in providing protection from subsequent induction of AA and, thereby, are also capable of inducing natural regression of acute inflammatory arthritis in vivo.

Discussion

The phenomenon of broadening of the T cell response to other determinants within a particular native antigen, after induction of disease with only a single determinant of the same antigen has previously been described as determinant spreading (19). In this study, we observed a shift in the specificity of T cell responses to new determinants within Bhs65 after priming with the whole multideterminant antigen (native Bhs65 as a component of M. tuberculosis), and have termed this diversification of the T cell response (20, 21). Determinant spreading has previously been reported in the diseases murine experimental autoimmune encephalomyelitis (EAE) and insulin-dependent diabetes mellitus (IDDM) (19, 34). In the case of EAE, the disease was induced by a self-peptide, AcL-11, of myelin basic protein (MBP), but during the course of the disease, the T cell response spread to other determinants within MBP, and in other studies (35, 36), to determinants on proteolipid protein (PLP) by intermolecular spreading. Likewise, in the case of spontaneously developed IDDM, there was evidence for both intramolecular (within glutamic acid decarboxylase; GAD65) and intermolecular (to determinants of hsp65, carboxypeptidase H, and insulin) determinant spreading (34). The novel features of our study on diversification of the T cell response in AA compared with the above two examples are the following: first, the observed diversification involved determinants within a foreign (mycobacterial) antigen, Bhs65; second, both in EAE and IDDM, determinant spreading was implicated in perpetuation of the autoimmune response, whereas in our study we demonstrate that diversification of the T cell response to the carboxy-terminal determinants of Bhs65 is involved in inducing regulation of inflammatory arthritis or protection from arthritis (regulatory diversification) in the AA-susceptible Lewis or the AA-resistant WKY rat strains, respectively. Furthermore, our results suggest that this diversification might be accentuated by determinants within self(rat) hsp65. In this regard, our study extends a novel dimension to the functional significance of diversification of T cell response in autoimmunity. Here, we describe diversification of T cell responses during the course of an autoimmune disease induced by a foreign antigen. In addition, this model offers us a unique opportunity to analyze relationships between the T cell repertoires directed against pathogenic foreign antigen and the homologous self-antigen. We believe that the principles elucidated in this study are widely applicable to several autoimmune situations in which autoreactivity is induced by molecular mimicry (29, 37, 37a) between a foreign and a self-antigen. Immediate and direct application of this knowledge would be in those autoimmune diseases (e.g., diabetes, Beh-
In this study, we observed that in the late phase of arthritis in the Lewis rat there was diversification of the T cell responses to include new determinants within the Bhsp65. T cell responses to the BCTD were evident even at 4 wk after onset of AA. Moreover, results of the experiments employing pretreatment with peptides comprising the BCTD clearly demonstrate that BCTD-reactive T cells are indeed responsible for inducing significant regression of acute inflammatory arthritis in the Lewis rat, presumably by down-regulating the activity of disease-inducing effector T cells. Furthermore, the protective effect against AA is not simply a property of peptides derived from any region of Bhsp65; in this study, pretreatment with BCTD but not BNTD brought about protection from subsequent induction of AA. However, the timing of pretreatment with BCTD is a critical factor in determining the final outcome; significant protection from AA in Lewis rats is only achieved if pretreatment is done 5–6 wk before injection of M. tuberculosis; pretreatment 2 wk before induction of AA has no effect on the course of the disease. These results suggest that the appearance of, and manifestation of the effect of, regulatory T cells either during the course of AA or after pretreatment of naive Lewis rats with the BCTD apparently requires 4–6 wk of time. Interestingly, it had been reported earlier that pretreatment of naive Lewis rats with native Bhsp65 or with peptide 180–188 of Bhsp65 induced protection from subsequent induction of AA; again, the protective effect of Bhsp65 or of peptide 180–188 was observed when these antigens were administered 5 wk before induction of AA (4, 16). At this time, we do not know how pretreatment with the BCTD affords protection from AA. It is conceivable that the activity of arthriticogenic T cells can be controlled either by cytokines (e.g., by induction of Th2 cells) or through a TCR-centered idiotypic circuit (38). Considering that peptide 180–188 (16) or peptide 256–270 (39) of Bhsp65 also afford protection from AA, we suggest that protection against AA induced by pretreatment with peptide 180–188 or 256–270, or by the BCTD might be mediated through parallel or, conceivably, a final common pathway of regulation. The precise mechanisms underlying this regulation are currently under investigation. Considering the size of Bhsp65, it is feasible that different regions of the same molecule are protective, and that this degeneracy in regulation (or multilayered regulation) might be of evolutionary advantage. Additional support for similar redundant regulatory pathways derives from studies using the nonobese diabetic (NOD) mouse: diabetes in this mouse strain can be modulated by administration of any one of several defined antigens (reviewed in reference 40).

The diversification of T cell responses to the carboxy-terminal determinants of hsp65 during the course of AA could be attributed to enhanced processing and presentation of Bhsp65 and/or Rhsps65 under the inflammatory conditions prevalent during acute AA (Fig. 5). Our results shown in Figs. 2 and 4 suggest that the T cell recept
these results is that because immunization with Bhsp65 (mixed in the adjuvant, DDA) did not cause AA, the requisite conditions for diversification of T cell responses to Bhsp65 simply did not exist.

We observed that coupled with the diversification of T cell response during the course of AA in the Lewis rat, the proliferative T cell responses to certain BNTD were significantly downmodulated in the late phase of the disease. At this time, we do not know whether downmodulation of responses to BNTD has any physiologic significance. One possibility is that besides the arthritogenic determinant 180–188, the BNTD also might be contributing to induction/perpetuation of arthritis in the Lewis rat and, therefore, to contain the autoimmune reactivity, the responses to these determinants must be downregulated.

We have observed that the WKY rat, with the same MHC class II molecules as the Lewis rat, is resistant to AA. In WKY rats, vigorous responses to the BCTD, to which the Lewis rat responded only in the late phase of AA, were observed very early, 10 d after injection of M. tuberculosis. These results suggest that T cell responses to the BCTD are involved in the downregulation of the acute inflammatory reaction in the late phase of the disease in AA-resistant Lewis rats; similarly, T cell responses to the same determinants initiated soon after injection of M. tuberculosis induce effective protection against arthritis in WKY rats. The early and efficient display of the BCTD by M. tuberculosis-immunized WKY rats but not by Lewis rats could be owing to subtle differences in the processing and presentation of Bhsp65 by the APC of WKY and Lewis rats, which in turn could be attributable to the difference in the non-MHC genes of these two rat strains. However, our results do not rule out other factors that also might contribute to the AA resistance of WKY rats, e.g., (a) hormonal and other neurophysiological parameters (45, 46), (b) difference in the RT.6 locus of WKY and Lewis rats (23, 47, 48), and (c) a change in the balance of Th1/Th2 induction (49). In most of the earlier reports on AA, the Fisher F344 rat (RT.11s), which is resistant to AA, has been extensively studied and used as a control strain for the Lewis rat (14). It has been reported that the Fisher rat is deficient in generating T cell responses to the arthritogenic determinant, 180–188, of Bhsp65 after injection of M. tuberculosis, and thereby is protected from AA (14). On the contrary, our results show that AA-resistant WKY rats raise vigorous responses to determinant 180–188 (within peptide 177–191) of Bhsp65 upon challenge with M. tuberculosis. Thus, clearly the immunological mechanisms underlying resistance to AA of Fischer and WKY rats are different. In this regard, study of AA in WKY rats would offer novel insights into the pathogenesis and treatment of this disease. Moreover, this rat strain might also be valuable in studying other animal models of autoimmune disease like EAE (49a).

AA can be induced in the Lewis rat by injection of M. tuberculosis; however, the precise autoantigen(s) responsible for AA are not known. Several candidate autoantigens have been proposed (13, 50, 51), but there is no direct evidence so far that any one of these antigens is arthritogenic in Lewis rats. We (29) and others (52–54) have suggested that self-hsp65 (R hsp65) may be a target of T cells primed by Bhsp65. Based on our earlier study on mouse lysozyme as a model self-protein (29), we suggested that newly displayed cryptic determinants within R hsp65 could serve as targets of autoimmune reactivity for T cells primed with the corresponding dominant determinants within Bhsp65. In addition, any self-antigenic determinants within the tissue components of the joints, with the appropriate cross-reactive homology with determinants within self-hsp65, could also serve as targets for the pathogenic T cells. We are currently characterizing the T cell responses to self-hsp65 to evaluate their direct role in the pathogenesis of arthritis.

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