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The Human Endoplasmic Reticulum Molecular Chaperone BiP Is an Autoantigen for Rheumatoid Arthritis and Prevents the Induction of Experimental Arthritis

Rheumatoid arthritis (RA) is the most common, crippling human autoimmune disease. Using Western blotting and tandem mass spectrometry, we have identified the endoplasmic reticulum chaperone BiP, a 78-kDa glucose-regulated protein, as a possible autoantigen. It preferentially stimulated increased proliferation of synovial T cells from patients with RA but not from patients with other arthritides. Mice with established collagen- or pristane-induced arthritis developed IgG Abs to BiP. Although BiP injected in CFA failed to induce arthritis in several strains of rats and mice, including HLA-DR4-/- mice and HLA-DR1-/-transgenic animals, it completely inhibited the development of arthritis when given i.v. 1 wk before the injection of type II collagen arthritis. Preimmunization with BiP suppressed the development of adjuvant arthritis in Lewis rats in a similar manner. This is the first report of a mammalian chaperone that is an autoantigen in human RA and in experimental arthritis and that can also prevent the induction of experimental arthritis. These findings may stimulate the development of new immunotherapies for the treatment of RA.

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Received for publication June 1, 2000. Accepted for publication November 8, 2000.

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1 This work was supported by Arthritis Research Campaign Program Grant P0075, Project Grant P0059, Integrated Clinical Arthritis Center Grant P0026, The Special Trustees for St. Thomas’ Hospital Grant G054002, and the Dutch Arthritis Foundation.
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4 Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; PIA, pristane-induced arthritis; AA, adjuvant arthritis; OIJD, other inflammatory joint diseases; rhuBiP, recombinant human BiP; SPA, N-succinimidyl-2(3-pyridyl)acetate; CAD, collision-activated dissociation; MALDI, matrix-assisted laser desorption ionization; LB, Luria-Bertani; PB, peripheral blood; SF, synovial fluid; DDA, dimethyl diiodotacetyl ammonium bromide; SI, stimulation index; hsP, heat shock protein; KCL, King’s College London.

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Materials and Methods

Characterization of autoantigen

Whole cell lysates from chondrosarcoma cells (clone SW1353; HTB 94; American Type Culture Collection (ATCC), Manassas, VA) were separated by denaturing SDS-PAGE (10% or 7.5%) (12), and the proteins were transferred to nitrocellulose (13). The membranes were probed with RA, normal or disease control sera (1/100 dilution), and HRP-conjugated anti-human IgG (1/2000) (Sigma, Poole, U.K.) followed by enhanced chemiluminescence (Amer sham Pharmacia Biotech, Little Chalfont, U.K.). Briefly, specific molecular mass markers were used to size the bands. The band of interest was isolated, and matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry was used to identify proteins by peptide mass fingerprinting. To confirm identities, mixtures of tryptic peptides were derivatized with N-succinimidyl-2(3-pyridyl) acetate (SPA), and individual peptides were sequenced de novo using low energy collision-activated dissociation (CAD).

The electroblotted proteins were stained with Ponceau S (0.05% w/v aqueous methanol, 0.1% acetic acid) using a rapid staining protocol (14). The dried, stained proteins were then digested in situ with trypsin (Boehringer Mannheim, Indianapolis, IN; modified), and the peptides extracted with 1:1 v/v formic acid-ethanol (15). One 0.2-μl aliquot (~5% of the total digest) was sampled and directly analyzed by MALDI time-of-flight mass spectrometry using a PerSeptive Biosystems Voyager DE-STR mass spectrometer (Thermo Bioanalysis, Hemel Hempstead, U.K.) (16). A second 0.2-μl aliquot was quantitatively esterified using 1% v/v thionyl chloride in methanol and also analyzed by MALDI to provide accurate residue composition (17). Native and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (90% of total digest) were then reacted with SPA to enhance sensitivity and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (17). Native and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (90% of total digest) were then reacted with SPA to enhance sensitivity and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (17). Native and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (90% of total digest) were then reacted with SPA to enhance sensitivity and esterified peptide masses were then screened against the MOWSE database to provide acidic residue composition (17).

The derivatized peptides were then sequenced by low energy CAD using a Finnigan MAT TSQ7000 fitted with a nanoelectrospray source (20, 21). The derivatized peptides were then sequenced by low energy CAD using a Finnigan MAT TSQ7000 fitted with a nanoelectrospray source (20, 21). The derivatized peptides were then sequenced by low energy CAD using a Finnigan MAT TSQ7000 fitted with a nanoelectrospray source (20, 21).

Cloning, sequencing, and expression of BiP

Human chondrocytes were isolated and cultured as described (22). Poly(A) mRNA (1-2 μg) was extracted from a total of 1-2 × 10^6 cells (Invitrogen, San Diego, CA). One microgram of mRNA was reverse transcribed into cDNA in a 20-μl reaction using 1 μl Moloney murine leukemia virus reverse transcriptase (200 U/μl), 5× first strand buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2, 0.1 M DTT), and an additional high stringency wash using 100 μl 20 mM Na2HPO4, 500 mM NaCl, 0.1% Triton X-100, 50 mM imidazole, pH 7.4. The histidine-tagged recombinant proteins were eluted from the column by stripping with 50 mM EDTA. Eluted proteins were dialyzed against PBS to remove EDTA and nickel contaminants.

The purified protein was concentrated and washed in sterile PBS using a 50,000 m.w. cutoff concentrator column (Millipore, Bedford, MA). The total amount of protein was determined by spectrophotometry using BSA as a standard with the bicinchoninic acid assay (Sigma; according to manufacturer’s instructions). The concentrated BiP recombinant protein was dissolved in PBS and stored at ~70°C.

Confirmation of BiP as Ag. Western blots of chondrosarcoma lysate and recombinant human BiP (rhuBiP) were prepared as previously described (12, 13) and probed with RA sera (1/100 dilution) either before or after absorbing the sera with rhuBiP at 1 μg at 20°C.

Immunological studies in patients with RA

Demographic details of RA patients and controls. The details are given in Table I. Eighty-three percent of patients with RA, diagnosed by the American College of Rheumatology criteria (23) were rheumatoid factor positive. The range of disease duration was 2-25 years. No patient had disease of <2 years duration. Disease controls consisted of patients with ankylosing spondylitis or psoriatic arthritis. RA patients were receiving a mixture of nonsteroidal antiinflammatory drugs or disease modifying drugs with or without prednisolone (~7.5 mg daily). Control patients with other inflammatory joint diseases (OIDD) were on a similar regimen. The normal healthy controls, whose sera were used for the Western blotting, consisted of 8 women and 3 men whose age was 49.3 ± 9.8 and 52.6 ± 12.7 years (mean ± SD), respectively.

T cell responses to BiP. T cell reactivity to BiP, at a range of concentrations from 1-50 μg/ml, was investigated by measuring lymphocyte prolif-eration and IFN-γ production by peripheral blood (PB) and synovial fluid (SF) mononuclear cells. Mononuclear cells were separated from heparinized PB or SF by density centrifugation using Lymphoprep (Nycomed, Oslo, Norway) at optimal dose (10^6 cells/ml). The primary wash was performed using synthetic oligonucleotide primers spanning the entire sequence corresponding to the human gene for the Ig heavy chain binding domain was ligated into the Nde1 site, and the reverse primers contained an XhoI restriction site: BiP forward primer 5′-TATACATATGAGGAGGACAAGAAG GAGGAGC-3′ and BiP reverse primer 5′-CCACCTGGATCTGACG CATTCTCCTCACA-3′. After initial denaturation at 96°C for 2 min, the PCR was performed for 28 cycles using a cycling profile of 94°C for 30s, 60°C for 30s, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR generated a single specific BiP fragment of 1890 bp. The 1890-bp PCR fragment was digested with both NdeI and XhoI. The purified fragment was ligated into the NdeI/XhoI digested bacterial expression vector pET30a (Novagen, Madison, WI). The ligated plasmids were transformed into competent Escherichia coli XL1-Blue (Strategene, La Jolla, CA) and screened by colony-PCR using BiP-specific primers. Positive transformants carrying the required recombinant plasmids were purified and transformed into competent E. coli expression strain BL21-(DE3) (Invitrogen).

Expression and purification of bacterial recombinant proteins

E. coli expression strain BL21-(DE3) containing the recombinant pET30a-BiP plasmid was grown at 37°C in Luria-Bertani (LB) medium containing kanamycin (50 μg/ml). When the cells had reached an OD600 of 0.6 U, isopropyl-β-thiogalactopyranoside (1 mM) was added to the medium to induce expression of the recombinant protein. For maximal expression of the recombinant protein, the culture was incubated for a further 4 h at 37°C. Cells were pelleted by centrifugation and stored at ~70°C. For purification of the recombinant bacterial proteins, the bacterial pellets were lysed in binding buffer (20 mM Na2HPO4, 500 mM NaCl, 5 mM imidazole, 1 mM PMSF, 1 mg/ml lysozyme, 5 μg/ml DNase, 0.1% Triton X-100, pH 7.4). The lysate was cleared by centrifugation and passed over a binding buffer-equilibrated chelating Hi-trap affinity column (Pharmacia). The nonspecifically bound protein was washed from the column under stringent conditions using a series of three wash buffers. The primary wash was performed using 100 ml binding buffer. This was followed by a high stringency low pH wash (20 mM Na2HPO4, 500 mM NaCl, 0.1% Triton X-100, pH 5.5) and an additional high stringency wash using 100 ml 20 mM Na2HPO4, 500 mM NaCl, 0.1% Triton X-100, 50 mM imidazole, pH 7.4. The histidine-tagged recombinant proteins were eluted from the column by stripping with 50 mM EDTA. Eluted proteins were dialyzed against PBS to remove EDTA and nickel contaminants. The purified protein was concentrated and washed in sterile PBS using a 50,000 m.w. cutoff concentrator column (Millipore, Bedford, MA). The total amount of protein was determined by spectrophotometry using BSA as a standard with the bicinchoninic acid assay (Sigma; according to manufacturer’s instructions). The concentrated BiP recombinant protein was dissolved in PBS and stored at ~70°C.

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Immunoological studies in experimental arthritis

Ab response to BiP in experimental arthritis. CIA and PIA were induced in DBA/1 mice according to previously described protocols (25, 26). Mice

Table I. Demographic details of RA patients and disease controls used in the proliferation studies with BiP

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>M:F</th>
<th>Mean Age (range)</th>
<th>Rheumatoid Factor Status (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>23</td>
<td>4:19</td>
<td>65 (25-87)</td>
<td>83</td>
</tr>
<tr>
<td>Controls</td>
<td>12</td>
<td>2:10</td>
<td>44 (21-75)</td>
<td>0</td>
</tr>
</tbody>
</table>

* M:F; Number of male:female subjects.

Controls consisted of patients with other inflammatory diseases such as psoriatic arthritis, reactive arthritis, and undifferentiated seronegative.

Downloaded from www.jimmunol.org on February 21, 2011.
were bled before induction of arthritis (15 animals), at the onset of CIA (16 animals), and at the onset of PIA (14 animals). The Ab in mouse sera against BiP was determined using ELISA with recombinant BiP and expressed as mean ± SEM OD 450, U and analyzed by two-tailed t test for unpaired samples. A similar procedure was used to determine Abs to type II collagen (27).

**Induction of arthritis by injection of BiP.** Male WA/KIR/kcl rats (n = 7) bred at King’s College London (KCL), 10–13 wk old, were injected intradermally over the scapulae with 500 μg BiP in IFA (Sigma), and the same injection was repeated i.v. 26 days later. Male B10.RIII mice (Harlan Olac, 8–10 wk old, n = 8) and male DBA/1 mice (Harlan Olac, 8–9 wk old, n = 8) were injected intradermally over the scapulae with 100 μg BiP in CFA (Difco, Detroit, MI) and repeated by same route on day 26. Mice were observed for arthritis development (31).

Administration of other highly conserved nonself proteins did not protect against tuberculosis in 100 μg BiP emulsified in IFA s.c. at the base of the tail and a second dose of 100 μg BiP emulsified in CFA s.c. at the base of the tail and a second dose of 100 μg BiP emulsified in IFA 3 wk later. Arthritis was scored 8 wk later. Where indicated, arthritis was scored as the number of animals and the number of joints per animal with clinical arthritis and the histological appearance of the joints as previously described (25, 26, 28, 29).

**Modulation of adjuvant arthritis with BiP.** Heat-killed *Mycobacterium tuberculosis* (strain H37Ra) was obtained from Difco. Dimethyl dioctade- cyl ammonium bromide (DDB; Eastman Kodak, Rochester, NY), used as adjuvant was prepared as a 20-μg/ml suspension in PBS and sonicated/ heated to produce a gel, which was mixed 1:1 with Ag solution before immunization. Male Lewis rats (n = 5), obtained from the University of Limburg (Maastricht, the Netherlands) 6–8 wk old, were immunized with 50 μg BiP in 50 μl PBS/DDA in each hind footpad (i.e., 100 μg/rat). Control animals (n = 5) received only the PBS/DDA mixture. Thirteen days later, AA was induced by a single intradermal injection of 0.5 mg *M. tuberculosis* in 100 μl IFA in the base of the tail. Rats were examined daily for clinical signs of arthritis in a blinded setup. Severity of arthritis was assessed by scoring each paw from 0 to 4 based on the degree of swelling, erythema, and deformity of the joints (30). Thus, the maximum score per rat was 16. The weight of individual rats was scored every other day. Differences between experimental groups were evaluated for the maximum arthritis score observed for each rat by means of the two-tailed Mann-Whitney U test. Differences were considered significant at p ≤ 0.05. Vehicle was used as the control for BiP in these experiments as the administration of other highly conserved nonself proteins did not protect against arthritis development (31).

**Results**

**Identification of autoantigen**

Western blotting was used to detect differences between RA and control sera for the identification of chondrocyte Ags. When RA and control sera were blotted against chondrosarcoma extracts, 30% RA sera (n = 54) reacted with a 70–80 kDa protein compared with 10% of control sera (n = 11) (Fig. 1A). No correction has been made for the IgG concentration of individual sera. Peptide mass fingerprint analysis and de novo sequencing of tryptic peptides by low energy CAD identified one of the proteins in the 70– to 80-kDa region as the 78-kDa glucose-regulated protein, a human chaperone also known as Ig heavy chain binding protein (BiP). DNA sequence analysis of BiP from articular chondrocyte cDNA showed a number of deviations from the original published sequence (accession number X87949). These differences were confirmed by sequencing of BiP cDNAs isolated from PBMC of six individuals. A total of six single nucleotide substitutions and a codon insertion result in three amino acid substitutions and an arginine insertion at position 834–836 of BiP (accession number AF188611). To confirm that BiP was the Ag detected at 70 kDa, Fig. 1B shows Western blots of both chondrosarcoma lysate and rhuBiP. In both cases, the RA sera showed a band, indicating Ab to the protein on the nitrocellulose; however, incubation of RA sera with rhuBiP completely neutralized the Ab response.

**Immunological studies in RA**

T cell-proliferative responses were determined for mononuclear cell preparations from paired PB and SF samples obtained from 23 patients with RA and from 12 disease controls. Twelve of 23 (52%) patients with RA and only 2 of 12 (17%) of disease controls showed increased synovial proliferation to BiP (Fig. 2A). The proliferative response to BiP of RA synovial T cells was significantly higher than that of the paired PB (SI, mean ± SEM: SF 3.5 ± 0.7;

![FIGURE 1. Abs to chondrosarcoma lysate. A Western blot showing six rheumatoid sera (lanes 1–6), four disease control sera (lanes 7–10), and five normal control sera (lanes 11–15) reacting with chondrosarcoma lysates. Ordinates, Molecular mass markers. The negative control, secondary Ab only, is shown as the ‘‘–ve’’ lane. B, Western blots using chondrosarcoma lysate or recombinant human BiP were probed with RA sera (1/100 dilution) before (S) or after addition of recombinant human BiP (S + BiP). A representative example is shown.](Downloaded from www.jimmunol.org on February 21, 2011)
PB 1.6 ± 0.2; p < 0.01, Wilcoxon paired test). A significant difference was also seen between SF responses to BiP between RA patients and disease controls (SI: RA 3.5 ± 0.7; OIJD 1.4 ± 0.2; p = 0.03, Mann-Whitney U test). There was no significant difference between the proliferation of PB and SF cultures for the inflammatory disease controls (p = not significant, Wilcoxon paired test). The increased proliferation by RA SF T cells could have been due to contaminating E. coli proteins. β-Galactosidase was prepared in the same expression system as the BiP but did not induce RA SF T cell proliferation (Fig. 2B). Another possible contaminant could be endotoxin, but six separate RA SF experiments did not show any T cell proliferation at 20 ng/ml endotoxin (SI 1.4 ± 0.2, mean ± SEM), whereas BiP showed the expected proliferation (SI 3.5 ± 0.7).

There was no association with HLA-DR given that 50% of responders and nonresponders were HLA-DR4 positive (data not shown). Rheumatoid SF T cell proliferation to BiP was inhibited by 66–84% by anti-HLA-DR mAb L243 (ATCC) (data not shown).

**Immunological studies in experimental arthritis**

**Induction of experimental arthritis with BiP.** BiP did not induce arthritis in DBA/1-, BALB/c-, B10.RIII-, HLA-DR1/1-, or HLA-DR4/2-transgenic mice or WA/KIR/kcl rats (data not shown).

**Immune response to BiP in experimental arthritis.** We next investigated whether DBA/1 mice made Abs against BiP during the course of CIA or PIA (Fig. 3). DBA/1 mice developed serum anti-BiP Abs at the onset of collagen arthritis (0.189 ± 0.042) and PIA (0.504 ± 0.074) when compared with prebleed sera (0.070 ± 0.019; p < 0.02 vs CIA and p < 0.00001 vs PIA, respectively). The concentration of these Abs was significantly higher in PIA mice than in CIA mice (p < 0.007).

**Prevention of CIA by i.v. administration of BiP.** The presence of Abs to BiP in the sera of mice with CIA or PIA suggested that manipulating the immune response to BiP might prevent the subsequent development of CIA. HLA-DR1/1-transgenic mice were injected i.v. with 1 mg BiP before immunization with type II collagen in CFA 1 wk later (Table II). Whereas 5 of 6 animals had 11 of 24 limbs that were involved with arthritis at 8 wk when pretreated with saline, only 1 of 10 animals had 1 of 40 limbs involved with arthritis in the group previously given i.v. BiP. These differences are highly significant (p ≤ 0.008 and p ≤ 0.0001, respectively). Table II also shows that there was a significant reduction in anti-collagen Abs in the BiP-pretreated animals to one-third the level in the controls. The control mice, pretreated with PBS, had twice as much IgG2 as IgG1 anti-collagen Abs, whereas mice pretreated with BiP had almost equivalent amounts of IgG1 and IgG2 anti-collagen Abs. (Table III). The histology of the joints of these animals (Fig. 4) confirmed the clinical findings in that there was no synovitis in the joints of BiP-pretreated mice.

**Suppression of AA by preimmunization with BiP.** Earlier we found that preimmunization with mycobacterial heat shock protein (hsp)70 suppressed the development of AA, which was related to the induction of regulatory T cells cross-reactive with self hsp70. Under the same conditions, other highly conserved nonself proteins were completely devoid of any disease-reducing effect (31). Therefore, BiP (97% identical with rat BiP) was used to preimmunize rats 13 days before the induction of AA.

![FIGURE 2.](image1.png) **FIGURE 2.** Cell proliferation to BiP. Lymphocyte proliferation in mononuclear cells cultured for 6 days expressed as SI, namely, proliferation in the presence of Ag/proliferation in the presence of culture medium alone. SI ≥ 2.5 was considered significant. A. Proliferation to 20 μg/ml BiP. B. Proliferation to 20 μg/ml β-galactosidase (∇) and to 20 μg/ml BiP (●) by four RA SF mononuclear cell preparations. + indicates mean SI ± SD.

![FIGURE 3.](image2.png) **FIGURE 3.** Abs to BiP in experimental arthritis. IgG Abs to recombinant human BiP in the sera of mice measured by ELISA and expressed as OD₄₅₀. Shown are the values for the animals bled before the induction of experimental arthritis (prebleed), and at the onset of CIA and of PIA.
Table II. Prevention of CIA by i.v. injection of recombinant BiP

<table>
<thead>
<tr>
<th>Tolerogen</th>
<th>Incidence of Arthritic Mice at 8 wk (%)</th>
<th>Incidence of Arthritic Limbs at 8 wk (%)</th>
<th>Abs (IgG) to CII</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiP (1 mg)</td>
<td>1/10 (10)*</td>
<td>1/40 (3)**</td>
<td>22 ± 9***</td>
</tr>
<tr>
<td>PBS</td>
<td>5/6 (83)</td>
<td>11/24 (46)</td>
<td>68 ± 10</td>
</tr>
</tbody>
</table>

* Sera were diluted to 1/100,000 and analyzed individually; results represent the mean ± SD for each group of animals with statistical analysis done using Student’s t test. **, p ≤ 0.008 (Fisher’s exact test); ***, p ≤ 0.0001 (Fisher’s exact test); ***, p < 0.05 (Student’s t test).

5. BiP not only delayed the onset of arthritis and but also suppressed the severity of the disease. At the hypothesis of the disease in the control animals (days 15–21), a significant suppression was observed in the BiP-treated animals (p < 0.05). Weight curves (a sensitive objective measure of physical well-being) of rats immunized with BiP were also significantly distinct from the weight curves in PBS-pretreated rats (data not shown).

A lymphocyte proliferation assay on spleen cells was performed 57 days after the induction of AA. After AA, rats immunized with BiP showed weak, but clear, proliferative responses to BiP (1.5–2.5 higher than the background response). Control animals showed no responses to BiP. The responses to Con A and M. tuberculosis were not different between both groups (data not shown).

Discussion

We have shown that (1) the human ER chaperone BiP is an autoantigen in patients with RA, (2) DBA/1 mice with CIA or PIA concomitantly produce anti-BiP Abs, and (3) BiP preimmunization will prevent the induction of CIA in HLA-DR1*0101-transgenic mice and will delay the onset and reduce the severity of adjuvant-induced arthritis in the Lewis rat. The hypothesis motivating this work was that autoantigens driving T cells would be of chondrocyte origin. In that event, we have shown that BiP, which is expressed in all cells of the body, is a major autoantigen in RA. However, an inflammatory, destructive arthritis has been induced in mice to the ubiquitous enzyme glucose-6-phosphate isomerase (32). The localization of arthritis in this model is not fully understood but may be due to the unusual situation within the joint where there is hypoxia with reperfusion injury as well as the release of inflammatory reactive oxygen species (33, 34).

Table III. IgG1 and IgG2 Ab isotypes to type II collagen in mice treated i.v. with either recombinant BiP or PBS

<table>
<thead>
<tr>
<th>Tolerogen</th>
<th>IgG1 Abs to Type II Collagen</th>
<th>IgG2 Abs to Type II Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiP (1 mg)</td>
<td>9 ± 2*</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>PBS</td>
<td>24 ± 7</td>
<td>44 ± 4</td>
</tr>
</tbody>
</table>

* Ab expressed as units of activity using the methods described in the legend to Table II. **, p ≤ 0.05 (Student’s t test) comparing BiP- vs PBS-treated groups.

FIGURE 4. Histology of joint of animals pretreated with BiP. Histology of joint from mice in which CIA was induced by the injection of type II collagen in CFA. Top, Joint from animal injected i.v. 1 wk before CIA induction showing erosive pannus and damaged articular cartilage. Bottom, joint from animal injected i.v. with BiP 1 wk before CIA induction showing no erosive pannus and intact articular cartilage.

reasoning might apply to BiP and the induction of RA (see below). Despite intensive efforts, we were not able to induce inflammatory arthritis in several strains of mice and rats including HLA-DR1*+/− and HLA-DR4+/− transgenic mice. However, it is well known that arthritis induction is very strain specific and is subject to multiple gene regulation including class II MHC and Mls genes (35).

Induction of BiP expression, which is primarily due to transcriptional activation (36), may be brought about by a number of cellular stress mechanisms including ischemia and/or reperfusion injury (37), glucose starvation (36), failure of glycosylation or misfolding of proteins (38, 39), stress heat (38), cytokines (40), oxidative stress, and depletion of intracellular Ca2+ stores. There is evidence that up-regulation of BiP may be involved in the immune response to tumors or during allograft rejection. Cells staining positively for BiP are found among the inflammatory cell infiltrate of rejecting rat cardiac allografts (41) and T cells from the allograft proliferate when cultured with BiP in the presence of autologous APCs (41). These observations are of relevance to our findings in RA as they confirm that T cell autoimmunity can arise to BiP. The presence of anti-BiP Abs in the sera of 4 of 21 patients with delayed onset reactions to sulfonamide antibiotics (42) further suggests that an immune response to BiP may be stimulated under appropriate conditions. Because of the prominent role of microbial HSP in the pathogenesis and immunotherapy of experimental
forms of arthritis, much effort has been expended in defining their role in the pathogenesis of RA. Human HSP60 (43) and human homologues of the bacterial chaperone DnaJ (44) are expressed in the rheumatoid synovial membrane. Although some investigators have provided evidence for preferential T cell responses by RA patients to mycobacterial hsp65 (45), the majority have been unable to do so (46, 47).

Earlier studies have shown that immunization with mycobacterial hsp70 reduces the severity of both AA and avian arthritis, a nonmicrobial agent-induced experimental arthritis. Disease suppression was found to be related to the induction of regulatory T cells cross-reactive with self-hsp70 that triggered the production of IL-10 (31). This phenomenon was specific for hsp70, because other highly conserved nonself proteins did not protect and did not induce IL-10 (31). In the present study, we show for the first time that a similar suppression of arthritis can be induced with BiP, a member of the hsp70 family, when BiP is given i.v. before the induction of CIA in DBA/1 mice or AA in Lewis rats. In the CIA model, mice pretreated with i.v. PBS had twice as much IgG2 and IgG1 anti-collagen Abs as did the mice pretreated with BiP, in which they were almost equal. This suggests that BiP may have immunomodulatory properties because it appears to be able to significantly suppress a Th1 Ab. This suggests that regulatory, self-hsp70-reactive T cells can be activated and expanded not only by immunization with M. tuberculosis hsp70 but also with homologous self-hsp70. As it has been demonstrated that the synthesis of hsp, such as hsp70, is up-regulated in arthritic joints (48), it is likely that MHC presentation of self-hsp peptides is also enhanced in arthritic joints. Therefore, migrating BiP-specific T cells may encounter their Ag in the joints (or the respective draining lymph node) on “stressed” APC or MHC II-positive activated T cells to exert their predicted regulatory activity. The immunomodulatory properties of BiP-activated T cells in these experimental systems are presently under investigation.

The observations described in this work are the first, to our knowledge, that implicate an endogenous chaperone in the pathogenesis of RA and the immunotherapy of experimental arthritis. BiP is, therefore, a strong candidate for the immunotherapy of RA.

References


