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*J Immunol* 2001; 166:1492-1498; doi: 10.4049/jimmunol.166.3.1492

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*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
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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-/- 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. The Journal of Immunology, 2001, 166: 1492–1498.

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

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.

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 G0454/0062, 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 diocadecyl ammonium bromide; SI, stimulation index; hsp, heat shock protein; KCL, King’s College London.
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 (Amersham Pharmacia Biotech, Little Chalfont, U.K.). Broad spectrum 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 collisional detachment (CAD) digestion and facilitate sequence analysis by tandem mass spectrometry (19).

The remaining digested peptides were quantitatively esterified using 1% v/v thionyl chloride in methanol and also analyzed by MALDI to provide acidic residue composition (17). Native and esterified peptide masses were then screened against the MOWSE peptide mass fingerprint database (18). The remaining digested peptides (90% of total digest) were then reacted with SPA to enhance peptide ion abundance and facilitate sequence analysis by tandem mass spectrometry (19). The derivatized peptides were then sequenced by low energy CAD using a Finnigan MAT TSQ7000 fitted with a nanoelectrospray source (20, 21). CAD was performed using 2.5 mV argon with collisional offset voltages and 18 V and 28 V. The product-ion spectra were collected with Q3 scanned at 500 amu's.

Cloning, sequencing, and expression of BiP
Human chondrocytes were isolated and cultured as described (22). Poly(A)+ mRNA (1–2 μg) mass was extracted from a total of 1–2 × 10⁶ 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 ml 20 mM Na₂HPO₄, 500 mM NaCl, 0.1% Triton X-100 and 1 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 mV 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 anti-inflammatory drugs or disease modifying drugs with or without prednisolone (≤7.5 mg daily). Control patients with other inflammatory joint diseases (OIID) 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 proliferation 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-Advanced). All cell preparations were determined to contain 80% viable cells. The cell suspensions were plated in triplicate at 1 × 10⁶ cells per well in 96-well round-bottom microtiter plates (Costar). The wells were pulsed with 0.2 Ci [3H]thymidine/well 24 h before harvesting. Proliferation was expressed as a stimulation index (SI); proliferation in the presence of stimulant/proliferation in the presence of medium alone.

Tissue typing of patients and controls. Tissue typing for HLA-DR was conducted in the Tissue Typing Department, Guy’s Hospital, using PCR-sequence-specific primers as described (24).

Immunological 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 were injected i.v. with 200 μg of BiP emulsified in 0.5 ml complete Freund’s adjuvant, and Freund’s incomplete adjuvant was administered at 2 and 4 weeks. Each mouse was given a total of 300 μg of BiP per animal. On day 90, the mice were challenged i.p. with 200 μg of BiP. Mice were killed on day 10, and popliteal lymph nodes were removed and cellular suspensions were prepared.

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>Mean Age (range)</th>
<th>Rheumatoid Factor Status (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA 23</td>
<td>4:19</td>
<td>65 (25–87)</td>
<td>83</td>
</tr>
<tr>
<td>Controls 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 monoarthritis.
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 OD450 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 = 5) 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 90 days and rats for 42 days for signs of arthritis. Male B10.RIII mice (Harlan Olac, 8–10 wk old, n = 8) were injected intradermally over the scapulae with 100 µg BiP in CFA s.c. at the base of the tail and a second dose of 100 µg emulsified in CFA s.c. at the onset of tail. Arthritis was scored 8 wk later. When 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 dodecyl ammonium bromide (DDA; Eastman Kodak, Rochester, NY), used as adjuvant was prepared as a 20-ng/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 adjuvants 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;
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+/−-, or HLA-DR4+/−-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+/−-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. As shown in Fig. 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.
5. BiP not only delayed the onset of arthritis and but also suppressed the severity of the disease. At the maximum 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-DRB1*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). The same 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 malfolding of proteins (38, 39), heat stress (38), cytokines (40), oxidative stress, and depletion of intracellular Ca$^{2+}$ 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 arthritis, these findings in RA are consistent with the involvement of BiP in the pathogenesis and immunotherapy of RA.

![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 no 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.](http://www.jimmunol.org/)

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**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 wkb (%)</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>

a 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).

b HLA-DR1*$^{-/-}$-transgenic mice were injected i.v. with either PBS (negative control) or recombinant BiP. Either 1 mg protein dissolved in 0.1 ml PBS or 0.1 ml PBS was administered i.v., and mice were immunized with type II collagen (CII) in CFA 7 days after the i.v. dose.

c The incidence of arthritis is reported at 8 wk after immunization.

d Abs represent mean units per group using sera collected 8 wk after immunization. ELISAs were performed, and results are reported as units of activity derived by comparison of test sera with the standard serum, which was arbitrarily defined as having 50 U of activity. Sera were analyzed individually; results are the mean ± SD for each group of animals.

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**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.
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 avridine 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 cells cross-reactive with self-hsp70 that triggered the production of 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.

Acknowledgments

We thank Dr. C. Erhardt, Bromley Hospital; Dr. H. Jones, Queen Mary’s University Hospital; and Dr. P. Williams, Medway Hospital, for clinical samples.

References

27. Wooley, P. H., H. S. Luthra, J. M. Stuart, and C. S. David. 1981. Type II col-
lagen-induced arthritis in mice. I. Major histocompatibility complex (I region) 
toimmune arthritis in HLA-DR4 (DRB1*0401) transgenic mice by immunization 
HLA-DR1 transgene confers susceptibility to collagen-induced arthritis elicited with 
human type II collagen. J. Exp. Med. 185:1113.
2000. A conserved mycobacterial heat shock protein (HSP) 70 sequence prevents 
adjuvant arthritis upon nasal administration and induces IL-10-producing T cells 
that cross-react with the mammalian self-HSP70 homologue. J. Immunol. 164: 
2711.
Oxygen free radicals, inflammation and synovitis: the current status. Ann. Rheum 
Dis. 48:864.
35. Wooley, P. H. 1995. Immunogenetics of animal models of arthritis. In Mechan-
tisms and Models in Rheumatoid Arthritis. B. Henderson, R. Pettifer, and 
36. Watowich, P. H. 1995. Immunogenetics of animal models of arthritis. In Mechan-
tisms and Models in Rheumatoid Arthritis. B. Henderson, R. Pettifer, and 
and K. Nagata. 1996. Reperfusion causes significant activation of heat shock 
transcription factor 1 in ischemic rat heart. Circulation 94:2185.
J. W. Attenello, and A. S. Lee. 1987. Rat gene encoding the 78-kDa glucose-
regulated protein GRP78: its regulatory sequences and the effect of protein glyco-
The presence of malfolded proteins in the endoplasmic reticulum signals the 
induce stress protein formation in cultured cardiac myocytes. Basic Res. Cardiol. 
87:12.
41. Qian, J., R. Moliterno, M. A. Donovan-Peluso, K. Liu, J. Suzow, L. Valdivia, 
reactivity in heterotopic cardiac allografts undergoing cellular rejection. Trans-
delayed-onset sulfonamide hypersensitivity reactions have antibodies recognizing 
of human homologs of the bacterial DnaJ chaperone in the synovial tissue of 
44. Karlsson-Parra, A., K. Soderstrom, M. Fern, J. Ivanji, R. Kiessling, and 
L. Klareskog. 1980. Presence of human 65 kD heat shock protein (HSP) in in-
flamed joints and subcutaneous nodules of RA patients. Scand. J. Immunol. 31: 
283.
45. Res, P. C., C. G. Schaaf, F. C. Breedveld, W. van Eden, J. D., Cohen, 
against 65 kD heat shock protein of mycobacteria in early chronic arthritis. Lancet 
2:478.
46. Crick, F. D., and P. A. Gatensby. 1992. Limiting-dilution analysis of T cell re-
activity to mycobacterial antigens in peripheral blood and synovium from rheu-
dilution analysis of proliferative T cell responses to mycobacterial 65-kDa heat-
shock protein fails to show significant frequency differences between synovial 
21:2937.
48. Schett, G., K. Redlich, Q. Xu, P. Bizan, M. Grogan, M. Tohidast-Akrad, 
protein 70 (HSP70) and heat shock factor 1 (HSF1) activation in rheumatoid 
arthritis synovial tissue: differential regulation of HSP70 expression and HSF1 
activation in synovial fibroblasts by proinflammatory cytokines, shear stress and 