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Induction of IL-10 and Inhibition of Experimental Arthritis Are Specific Features of Microbial Heat Shock Proteins That Are Absent for Other Evolutionarily Conserved Immunodominant Proteins

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Bacterial heat shock proteins (hsp) are evolutionary conserved immunodominant proteins that manifest amino acid homologies with hsp present in mammalian cells. Preimmunization with mycobacterial hsp65 has been found to protect against various forms of experimental arthritis. As these protective effects have previously been attributed to induction of self homologue cross-reactive T cell responses, the question was raised as to whether this protective effect could be extended to other highly conserved and immunodominant microbial Ags with mammalian homologues. Therefore, we immunized Lewis rats with conserved bacterial Ags (superoxide dismutase, aldolase, GAPDH, and hsp70). Although all Ags appeared highly immunogenic, we only found a protective effect in experimental arthritis after immunization with bacterial hsp70. The protective effect of hsp70 was accompanied with a switch in the subclasses of hsp70-specific Abs, suggesting the induction of Th2-like response. The most striking difference between immunization with hsp70 and all other immunodominant Ags was the expression of IL-10 found after immunization with hsp70. Even more, while immunization with hsp70 led to Ag-induced production of IL-10 and IL-4, immunization with aldolase led to increased production of IFN-γ and TNF-α. Thus, the protective effect of conserved immunodominant proteins in experimental arthritis seems to be a specific feature of hsp. Therefore, hsp may offer unique possibilities for immunological intervention in inflammatory diseases. The Journal of Immunology, 2001, 167: 4147–4153.

H eat shock proteins (hsp)³ are highly conserved cellular proteins that are present in cells of all living organisms (1–3). Despite the high degree of homology among hsp of microbes and their potential mammalian hosts, hsp have been found to be strong immunogens (4, 5). Bacterial hsp are readily recognized by the immune system, which in turn can lead to cross-recognition of self hsp and thus, theoretically, to autoimmunity through antigenic mimicry (4, 6). However, most data collected to date do not support such antigenic mimicry to be a major trigger for autoimmune disease. In contrast, immunization with (mycobacterial) hsp60 has been found to protect animals from the development of arthritis in virtually all forms of experimental arthritis (7–9). Most evidence on the mechanism of the protective role of mycobacterial hsp60 has been obtained in adjuvant arthritis (AA) (10, 11).

The epitopes of mycobacterial hsp60 recognized after induction of AA and after (protective) immunization with mycobacterial hsp60 have been mapped and analyzed (12). Subsequently, various peptides that included these epitopes were tested for their ability to protect against arthritis. Only one peptide, which contained a conserved epitope with a high degree of identity with the homologous rat peptide and which was capable of inducing T cell responses to self rat hsp60, was found to protect in AA. The same epitope also conferred protection in other experimental, nonmicrobial-induced, arthritis models (13). Altogether, these data showed that the protective effect of hsp in experimental arthritis was directly related to the ability of hsp to induce cross-reactive T cell responses with self hsp. There are indications that mycobacterial hsp10 and hsp70 may have similar immunomodulatory effects in experimental arthritis (14–17).

On a theoretical basis, Cohen (18) has postulated an intrinsic inclination of the immune system to focus on Ags of a conserved nature. He used the term “homunculus” to describe a (limited) set of immunologically dominant self Ags, each encoded in a cellular network and comprising the immune system’s picture of self. Based on the experience with conserved microbial hsp in experimental arthritis models, we questioned whether other highly conserved microbial proteins with self homologues present in host tissue might exhibit similar protective effects in experimental arthritis. In addition, we set out to further explore the possible protective effect of mycobacterial hsp70 in experimental arthritis and the mechanisms involved.

We were able to demonstrate in this study a T cell-mediated protective effect of mycobacterial hsp70 in AA, whereas other conserved microbial Ags did not influence the course of experimental arthritis. Hsp70 was found to induce a much greater expression of

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3 Abbreviations used in this paper: hsp, heat shock protein; AA, adjuvant arthritis; DDA, dimethyl dioctadecyl ammonium bromide; DnaK, E. coli hsp70; DTH, delayed-type hypersensitivity; Mt, Mycobacterium tuberculosis; PLNC, primed lymph node cell; SI, stimulation index.
IL-10 than any of the other conserved microbial proteins did, and it also induced a Th2-skewed IgG subclass response of mycobacterial-specific Abs. Even more, hsp70 immunization led to increased intracellular production of IL-4 and IL-10 after in vitro activation with mycobacterial hsp70.

Materials and Methods

Animals

Male inbred Lewis rats (RT1-B') were obtained from the University of Limburg (Maastricht, The Netherlands). Rats were 6–9 wk old at the start of each experiment.

Ads and adjuvants

The following Ags were used for immunization of the animals or in proliferation assays: heat-killed Mycobacterium tuberculosis (Mt; strain H37Rv, Difco, Detroit, MI), Escherichia coli hsp70 (DnaK; Stressgen Biotechnologies, Victoria, Canada), myosine from bovine muscle (catalog no. M 6643; Sigma-Aldrich, St. Louis, MO); superoxide dismutase from E. coli (catalog no. G 5639; Sigma-Aldrich), GADPH from Bacillus stearothermophilus (catalog no. G 589; Sigma-Aldrich), rat GST (catalog no. G 8386; Sigma-Aldrich), and aldolase from Staphylococcus aureus (catalog no. A 2548; Sigma-Aldrich). Purified recombinant hsp60 of Mycobacterium tuberculosis (Mt hsp60) was kindly provided by J. D. A. van Embden (National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands). A purified recombinant preparation of mycobacterial hsp70 was obtained from M. Singh (Gesellschaft fu¨r Biotechnologische Forschung, Braunschweig, Germany) in support of the United Nations Development Program/World Bank World Health Organization Special Program for Research and Training in Tropical Diseases. IFA (Difco) and dimethyl dioctadecyl ammonium bromide (DDA; Eastman Kodak, Rochester, NY) were used as adjuvants. DDA was prepared as a 20 mg/ml suspension in PBS and sonicated/heatd to produce a gel that was mixed 1:1 with Ag solution before immunization.

Immunizations

Rats were immunized with 50 l of protein in 50 l of PBS/DMA in each hind footpad (i.e., 100 l/rat). In some experiments, draining popliteal lymph nodes were removed 14 days after immunization, digested, washed three times, and used as a source of primed lymph node cells (PLNC). In other experiments, PLNC, derived from pooled inguinal and popliteal lymph nodes and splenocytes from AA rats 42–50 days after Mt immunization, were used.

Tissue culture reagents

IMDM supplemented with 2 nM l-glutamine, 100 U/ml penicillin, 100 l/ml streptomycin (all from Life Technologies, Gaithersburg, MD), and 5 x 10^-5 M 2-ME was used as culture medium. Cell populations were washed in IMDM without supplements.

T cell proliferation assays

PLNC and splenocytes were cultured in triplicate in 200-fl-bottom microtiter wells (Costar, Cambridge, MA) at 2 x 10^5 cells/well with or without Ag. PLNC and splenocytes were tested for responsiveness to individual proteins at 5 and 10 l/ml. Con A (2 l/ml) was used as a positive control for T cell proliferation. Cultures were incubated for 96 h at 37 °C in a humidified atmosphere of 5% CO_2. Cultures were pulsed for the final 6 h with ['H]thymidine (0.4 lCi/well, sp. act. 1 Ci/mmol; Amersham International, Little Chalfont, U.K.), and TdR uptake was measured using a liquid scintillation counter. Results are expressed as a mean cpm of triplicate cultures. The magnitude of the proliferative response is expressed as stimulation index (SI): the mean cpm of cells cultured with Ag divided by the mean cpm of cells cultured with medium alone.

Delayed-type hypersensitivity (DTH) analysis

DTH was measured by injecting 10 l of protein in 10 l of PBS s.c. in the left ear. In the right ear, 10 l of PBS was injected. Thickness was measured using micrometer calipers. The difference in thickness between the left and right ear was measured for each rat at 24 and 48 h after the challenge. The results are expressed as the mean for each experimental group ± SE. DTH responses were determined on day 10 after immunization with protein in DDA.

Induction and clinical assessment of experimental arthritis

AA was induced by a single intracutaneous injection in the base of the tail with 0.6 mg of Mt suspended in 100 l of IFA. Rats were examined daily for clinical signs of arthritis. Severity of arthritis was assessed by scoring each paw from 0 to 4 based on degree of swelling, erythema, and deformations of the joints. Thus, the maximum score was 16 (19). In most experiments, the weight of individual rats was scored every other day.

T cell lines

Generation of T cell lines.

The generation of rat T cell lines has been described previously (13). In short, we performed a bulk stimulation of popliteal lymph node cells 10 days after immunization with DnaK or mycobacterial hsp70 (see above). PLNC were cultured at 5 x 10^5/ml in culture medium with 2% normal rat serum and 10 l/ml DnaK or mycobacterial hsp70. After 3 days, viable cells were harvested using a Ficoll gradient. Viable cells were then cultured for 4–7 days in culture medium with 10% FCS (Seratec, Griesbach, IR, Germany) and 10% EL4 supernatant. Cells were restimulated with irradiated (3000 rad) thymocytes as APCs (± 1 x 10^5/well) and 10 l/ml Ag in culture medium with 2% normal rat serum. This cycle was repeated four to eight times. After each cycle, the specificity of the cell line was checked by a lymphoproliferation assay.

Modulation of arthritis with T cell lines.

The above described T cell lines specific for mycobacterial hsp70 and DnaK were restimulated in vitro with irradiated APC and Ag. Three days later, T cell blasts were harvested on a Ficoll gradient, washed, and spun over a second Ficoll gradient to remove all contaminating APC. T cells were washed twice in medium and twice in PBS and finally suspended at 2.5 x 10^6/ml in PBS. Immediately before injection of Mt, 200 l/ml (i.e., 5 x 10^4/ml) of T cells were injected i.v. in the tail vein. In each group, six animals were treated using this T cell transfer protocol.

Analysis of hsp70 Ab responses.

Serum samples were collected at day 50 after the induction of AA and stored at −20°C. In these samples, hsp70-specific Abs and their isotypes were determined as previously described (20). In short, first, hsp70-specific Abs were measured using an ELISA. To determine the isotype distribution of the anti-hsp70 Abs, peroxidase-conjugated polyclonal goat anti-rat IgG2b (Nordic, Tilburg, The Netherlands) and monoclonal mouse anti-rat IgM (catalog no. 63-517; ICN Pharmaceuticals, Costa Mesa, CA), IgG1 (Sanbio, Uden, The Netherlands), and IgG2a (catalog no. 63-512; ICN Pharmaceuticals) were used. After determining the total anti-hsp70 Ab titers, dilutions corresponding to OD values in the linear part of the curve were selected in which equal amounts of anti-hsp70 Abs were present. Polystyrene 96-well microtiter plates were coated with 100 l/ml of hsp70 (2 l/ml) at 4 °C overnight. After washing five times with H_2O, the plates were incubated with 200 l/ml of 10% PBS containing 1% BSA for 30 min at 37°C. After washing, serum samples diluted in 100 l/ml of PBS containing 1% BSA and 0.05% Tween 20 (PBSA-Tw) were incubated for 2 h at 37°C. The wells were washed five times with H_2O, and 100 l/ml of peroxidase-conjugated anti-rat isotype Abs (1/500–1/1000 dilution in PBSA-Tw) were added and incubated for 2 h at 37°C. After washing, 100 l/ml of ABTS with 0.1 M citrate phosphate buffer and 1 l/ml 30% (v/v) H_2O_2 was added to each well. After incubating 30 min at room temperature, absorbance at 405 nm was measured on a Bio-Rad (Hercules, CA) Microplate Reader. The IgM, IgG1, IgG2a, and IgG2b anti-hsp70 titers are expressed relative to the total anti-hsp70 Ab titer, as described before (20).

Analysis of cytokine expression after immunization

PLNC of protein-immunized rats (4 x 10^5) were stimulated with 10 l/ml the corresponding protein for 18 h. RNA from these cells was used for the generation of cDNA by RT-PCR. PCR with rat IL-10-specific primers (5'-TGCCCTTCAGTCAAGTGAAGACT, 3'-AACTGCTCAGTGGCCT) was performed. The signal obtained was measured after gel electrophoresis with ethidium bromide using an imaging densitometer (model GS-700; Bio-Rad) and compared with a control PCR product of a housekeeping gene (rat GADPH primers: 5'-ACCACAGTGCATCCATAC, 3'-TCCACACCTGTTGCTGTA) to compensate for minor variations in cDNA content between samples. Rat IL-2 and IL-4 primers were used as described (16).

Intracellular cytokine staining

PLNC or splenocytes were cultured for 48 h with medium or Ag. During the last 4 h of culture, monensin (GolgiStop; BD PharMingen, San Diego, CA) was added to the cultures. Viable cells were harvested, incubated for
20 min on ice in blocking buffer (PBS with 10% normal rat serum and 0.2 mM sodium azide), and subsequently stained for 20 min on ice with PE- or FITC-conjugated anti-rat CD4 (clone OX-35, mouse IgG2a,κ; BD PharMingen). The cells were washed twice in staining buffer (PBS containing 2% FCS and 0.2 mM sodium azide) and incubated in 100 μl of fixation buffer (CytoFix/Cytoperm; BD PharMingen) for 20 min on ice. The fixed cells were washed twice in permeabilization buffer (Perm/Wash; BD PharMingen), resuspended in 100 μl of permeabilization buffer, and stained with the following conjugated mAbs: PE-conjugated anti-rat IL-4 (clone OX-81, mouse IgG1,κ; BD PharMingen), FITC-conjugated anti-rat IFN-γ (clone 104.14, mouse IgG2b,κ; BD PharMingen), PE-conjugated anti-rat IL-10 (clone A5-4, mouse IgG2b,κ; BD PharMingen), (both Abs from BD PharMingen), and mouse IgG1,κ, FITC-conjugated anti-cytokines (clone 45418.111, mouse IgG1; R&D Systems (Minneapolis, MN), conjugated with FITC conjugation kit; Sigma-Aldrich).

Finally, the cells were washed twice and resuspended in staining buffer. PE- or FITC-conjugated mouse IgG1 (clone MOPC-21; BD PharMingen) and PE-conjugated mouse IgG2b (Caltag Laboratories, Burlingame, CA) were used as isotype controls. As a second specificity control, fixed and permeabilized cells were incubated for 30 min with nonconjugated anti-cytokine Ab (the same clone as the conjugated anti-cytokine Ab) before the actual staining with the conjugated anti-cytokine Ab. Such preincubation procedure blocked the staining to background level. Stained cells were analyzed on a FACSscan cytometer (BD Biosciences, Mountain View, CA). At least 10,000 events were acquired from each sample and subsequently analyzed with CellQuest software.

**Statistical analysis**

Basic descriptive statistics were used to describe the proliferative responses and the characteristics of the different treatment groups. A Mann-Whitney U test was used to compare arthritis scores, weight loss, and proliferative responses to Abs between the different groups. Kolmogorov-Smirnov statistics were used to analyze FACS cytokine staining.

**Results**

**DTH reactions and proliferative responses after immunization with conserved microbial proteins**

Rats were immunized in the hind footpad with 100 μg of the following conserved proteins in DDA: bovine myosine, E. coli superoxide dismutase, GAPDH from *B. stearothermophilus*, and aldolase from *S. aureus*. Rat GST and PBS were included as controls. Ten days after immunization, a skin test was performed as described in Materials and Methods. Five rats were in each group. The results are shown in Fig. 1.

![FIGURE 1. DTH reactions after immunization with conserved immunodominant proteins.](http://www.jimmunol.org/)

After immunization, all conserved proteins showed positive DTH reactions, whereas animals immunized with rat GST and PBS in DDA were negative in this respect. Skin tests performed on animals immunized with mycobacterial hsp70 included in the same protocol were also positive (Fig. 1). Fourteen days after immunization, which is 4 days after performing the skin tests, AA was induced (see below). Fifty days after induction of AA, a lymphocyte proliferation assay was performed on PLNC derived from pooled popliteal and inguinal lymph nodes and splenocytes. Positive proliferative responses to the relevant proteins could be detected in all rats immunized with conserved proteins. In contrast, PLNC and splenocytes from rats immunized with rat GST did not show proliferative responses to GST.

**Effects of immunization with conserved microbial proteins in AA**

Rats were immunized in the hind footpad with 100 μg of the conserved proteins in DDA (five rats in each experimental group). Fourteen days after immunization, rats were immunized with Mt to induce AA. The results of this experiment are shown in Fig. 2A. All rats developed moderate to severe arthritis. No indications of a protective effect of immunization with conserved microbial proteins were found in any of the treatment groups.

**Effects of immunization with mycobacterial hsp70 in experimental arthritis**

**Adjuvant arthritis.** Rats were immunized in the hind footpad with 100 μg of mycobacterial hsp70 in DDA. Control rats were immunized with PBS in DDA. Fourteen days later, AA was induced. The results are shown in Fig. 2B. In rats immunized with mycobacterial hsp70, the onset of arthritis was delayed and the severity of arthritis was reduced compared with control animals (mean maximum arthritis score 3.2; compared with 9.8 in the control rats; p < 0.05).

Weight curves (a sensitive objective measure of physical well-being) of rats immunized with mycobacterial hsp70 were also distinct from the weight curves in PBS-pretreated rats. Mean weight gain at day 23 (day of maximum arthritis score) compared with day 10 (which is before the onset of arthritis) was +23 g for rats immunized with hsp70, compared with −26 g for rats immunized with PBS in DDA (p < 0.05).

**Proliferative responses after immunization with mycobacterial hsp70 in AA rats.** A lymphocyte proliferation assay on PLNC derived from pooled popliteal and inguinal lymph nodes was performed 50 days after the induction of AA. After AA, rats immunized with mycobacterial hsp70 showed clear proliferative responses to mycobacterial hsp70 (mean SI 11.3), but had low proliferative responses to mycobacterial hsp65 (mean SI 1.9). Control animals showed lower responsiveness to mycobacterial hsp70 (SI 4.9). The responses to mycobacterial hsp65 176–190 (major epitope in AA), mycobacterial hsp65, and Con A were not different between the groups (Fig. 2C).

**Transfer of disease protection through adoptive transfer with hsp70-specific T cell lines**

Rat T cell lines specific for mycobacterial hsp70 and DnaK were generated as described in Materials and Methods. These T cell lines were activated during 3 days by antigenic stimulation (mycobacterial hsp70 or DnaK) and injected i.v. in 200 μl of PBS (2.5 × 10^8 cells/ml, i.e., 5 × 10^6 cells/rat) in the tail vein, immediately before arthritis induction. The results of a representative experiment are given in Fig. 3. Although both T cell lines induced some suppression of arthritis, administration of T cells specific for mycobacterial hsp70 (mean maximum arthritis score 1.6, SEM 1.2) reduced the severity of arthritis more than...
DnaK-specific T cells (mean maximum arthritis score 5.8, SEM 1.8) (*p*/H11021 0.05).

Ab responses to mycobacterial hsp70 after AA

Rats were immunized with either mycobacterial hsp70 or PBS in DDA. Fourteen days later, AA was induced with an intradermal injection of CFA. Fifty days after the induction of AA, serum samples from both treatment groups were collected, and the isotype distribution of hsp70-specific Abs was determined. The results are shown in Fig. 4. After AA, hsp70-specific IgM was only found in the PBS treatment group. Hsp70-specific IgG was found in both treatment groups. However, a clear difference in subclass distribution was found. In rats preimmunized with mycobacterial hsp70, the relative amount of hsp70-specific IgG1 and IgG2a Abs was substantially higher than in control rats. In contrast, the relative amount of IgG2b Abs was lower in rats pretreated with mycobacterial hsp70 compared with rats pretreated with PBS ( *p*/H11021 0.05). This was reflected in the IgG1/IgG2b index, which was 1.5 in rats pretreated with mycobacterial hsp70 and 0.14 in rats pretreated with PBS ( *p*/H11021 < 0.05).

Ag-specific cytokine expression after immunization with hsp70 and other conserved proteins

Rats were immunized with 100 μg of the following conserved proteins in DDA: mycobacterial hsp70, bovine myosine, *E. coli* superoxide dismutase (SOD), GAPDH from *B. stearothermophilus*, and aldolase from *S. aureus* (Aldo). Rat GST and PBS were included as controls. Fourteen days after immunization, AA was induced with 0.6 mg of Mt in IFA. Arthritis scores were assessed, in a blinded setup, at least every other day after Mt immunization. Five rats were in each group. Results are shown as mean arthritis scores ± SEM (error bars).

FIGURE 2. A, Effects of immunization with conserved immunodominant proteins in AA. Rats were immunized in the hind footpad with 100 μg of the following conserved proteins in DDA: bovine myosine (Myos), *E. coli* superoxide dismutase (SOD), GAPDH from *B. stearothermophilus*, and aldolase from *S. aureus* (Aldo). Rat GST and PBS were included as controls. Fourteen days after immunization, AA was induced with 0.6 mg of Mt in IFA. Arthritis scores were assessed, in a blinded setup, at least every other day after Mt immunization. Five rats were in each group. Results are shown as mean arthritis scores ± SEM (error bars). B, Modulation of AA development after immunization with 100 μg of mycobacterial hsp70 and PBS in DDA. Fourteen days after immunization, AA was induced with 0.6 mg of Mt in IFA. Arthritis scores were assessed, in a blinded setup, at least every other day after Mt immunization. Five rats were in each treatment group. Results are shown as mean arthritis scores ± SEM (error bars). C, Proliferative responses to mycobacterial hsp65 176–190, mycobacterial hsp70, and Con A after immunization with mycobacterial hsp70 in AA rats on PLNC derived from pooled popliteal and inguinal lymph nodes 50 days after the induction of AA.
superoxide dismutase, GAPDH from *B. stearothermophilus*, and aldolase from *S. aureus*. Fourteen days after immunization, PLNC derived from pooled popliteal and inguinal lymph nodes were isolated and stimulated for 14 h with the protein. RNA was isolated from those cells, and cDNA was generated by RT-PCR. PCR with specific primers was performed, and the strength of the signal obtained was compared with that of a PCR product of a housekeeping gene for which the samples have been normalized. mRNA expression of IL-2 and IL-4 could be found following immunization with any of the conserved proteins given above (data not shown). Immunization with hsp70 led to a high expression of IL-10, whereas IL-10 expression was low or absent after immunization with other conserved proteins (Fig. 5). All of the PLNC used in this experiment proliferated in response to the specific Ag, as had been shown previously (in Results).

**Ag-specific intracellular cytokine production after immunization with hsp70 and other conserved proteins**

Rats were immunized with 100 µg of mycobacterial hsp70, aldolase, or PBS in DDA. Fourteen days after immunization, PLNC derived from inguinal lymph nodes and splenocytes were isolated and stimulated with the immunizing protein for 48 h. Viable cells were harvested, stained for surface markers, permeabilized, and then stained for IL-4, IL-10, IFN-γ, or TNF-α. Cytokine expression was analyzed in CD4+ cells. The results are shown in Fig. 6. CD4+ inguinal lymph node cells from rats immunized with aldolase produce mainly TNF-α and IFN-γ, not IL-4 or IL-10, upon in vitro activation with aldolase (Fig. 6A). In contrast, both CD4+ inguinal lymph node cells and splenocytes from rats immunized with hsp70 produce both IL-4 and IL-10, but not IFN-γ or TNF-α, upon in vitro activation with mycobacterial hsp70 (Fig. 6, B and C). An asterisk marks statistical significance. No Ag-specific cytokine production could be detected in rats immunized with PBS in DDA.

**Discussion**

Bacterial hsp are examples of bacterial Ags that are immunologically dominant, despite a high degree of homology with their host self counterparts. It has been suggested that the immune system may have a vested interest in setting a focus on the recognition of some of these conserved bacterial Ags (18). Evidence is mounting that hsp are critical Ags in the regulation of certain chronic inflammatory diseases, and are important both in disease induction and in protection from disease (21, 22). However, it is unknown whether or not such effects of immune regulation would be restricted to hsp or that, alternatively, all conserved and immunogenic bacterial Ags would share these properties. Therefore, we set out to investigate the potential disease-suppressive effects of hsp.
and other conserved microbial Ags in experimental arthritis. We found that all selected Ags were immunogenic, as measured by their capacity to trigger Ag-specific T cell proliferation, cytokine production, and DTH reactions. However, the non-hsp microbial Ags did not exhibit any immunomodulatory effects in experimental arthritis.

In contrast, immunization with mycobacterial hsp70 clearly reduced the severity of AA. Our present findings confirm a previous report on a protective effect of mycobacterial hsp70 in experimental arthritis (23), and are well in line with the known protective effects of mycobacterial hsp65 and mycobacterial hsp10 (14, 15).

In this study, we showed indications of the mechanisms involved in the protective effects of microbial hsp in arthritis. Pre-immunization with mycobacterial hsp70 gave rise to a shift of Ab isotypes from preferentially IgG2b to IgG1. This is an indication of the induction of Th2 cells through immunization with mycobacterial hsp70, and is in line with a recent report on the putative protective mechanism of hsp65 in pristane arthritis (24). In addition, we showed that immunization with mycobacterial hsp70 strongly triggered the production of IL-10. The last finding was the most striking difference with the other immunodominant Ags. That hsp70, despite the IL-10-inducing potential, generated DTH reactivity is compatible with the fact that IL-10 has only a very moderate capacity to inhibit DTH (25).

The protection of hsp in experimental arthritis is apparently a unique feature of hsp. Several circumstances may provide an explanation for this unique capacity.

First, hsp can be distinguished from other conserved microbial Ags by the fact that hsp are overexpressed on stressed cells. Hsp indeed have been detected at raised levels in synovial tissue and synovial lining cells of arthritic rats and patients with rheumatoid arthritis or juvenile idiopathic arthritis (26–28). This may be decisive for the capacity of hsp to interfere in arthritis. Over the last years, we have obtained evidence indicating that T cells specific for bacterial hsp60 and capable of cross-reacting with self hsp may effectively play a regulatory role in the control of joint inflammation, both in experimental arthritis and in children with juvenile idiopathic arthritis (13, 21, 29).

Second, the capacity to induce IL-4 and IL-10 production may be crucial to the protective capacity of hsp. Although ultimately only in vivo depletion may unravel the role of the two cytokines in hsp70-mediated protection, one may speculate on the role of IL-10. First of all, IL-10 has been shown to be a potent T cell-derived immunoregulatory cytokine (30–32). Furthermore, IL-10 has the capacity to down-regulate monocyte-derived proinflammatory cytokines such as IL-1, IL-6, and TNF-α (31). Because of its capacity to inhibit production of inflammatory mediators by synovial cells, IL-10 is considered an important immunoregulatory component of the cytokine network in rheumatoid arthritis and reactive arthritis (34, 35). Interestingly, passive transfer of (mycobacterial) hsp70-specific T cells, capable of producing significant amounts of IL-10, rendered rats susceptible to listeriosis (36), a disease normally controlled by a Th1-like response. Recently, the same group reported in this journal that activation of T cells recognizing the same epitope of hsp70 could effectively protect against AA (17).

Finally, various anti-inflammatory drugs, which are effective in reducing arthritis, including acetylsalicylic acid, auranofin, and dexamethasone, facilitate expression of hsp70 on macrophages and simultaneously inhibit synthesis of cytokines such as IL-1 and TNF-α (37). Indeed, we have shown that immunization with the second line antirheumatic drug OM-89 induced T cell responses to mycobacterial hsp70 (38). OM-89 is an extract of several E. coli strains, and has proven to be an efficacious, nontoxic second line antirheumatic drug in rheumatoid arthritis.

Hsp have special qualities in the regulation of both chronic inflammatory diseases and peripheral tolerance through the induction of cytokines such as IL-10. Although the milieu in which the immune system recognizes hsp will obviously be an important factor in determining the type of immunomodulation taking place, our present findings indicate that mycobacterial hsp70 is a critical Ag with potential for immunotherapy in human arthritis.

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References


