Clin Exp Immunol 1996; **103**:77–82

A 71-kD heat shock protein (hsp) from *Mycobacterium tuberculosis* has modulatory effects on experimental rat arthritis

A. E. KINGSTON, C. A. HICKS, M. J. COLSTON* & M. E. J. BILLINGHAM† *Lilly Research Centre Ltd., Eli Lilly and Company, Windlesham, *Laboratory for Mycobacterial Research, National Institute for Medical Research, Mill Hill, and †Rheumatology Unit, Department of Medicine, University of Bristol, Bristol, UK*

(Accepted for publication 2 October 1995)

SUMMARY

The effects of a mycobacterial 71-kD hsp antigen have been investigated for its ability to modulate arthritis in rats. Subcutaneous injection (base of tail) of increasing amounts of hsp71 from *Mycobacterium tuberculosis* (MTB) produced dose-dependent differential inhibitory effects on induction of arthritis by MTB and CP20961 in rats. As little as 1 μg of the hsp71 produced a reduction in MTB arthritis, whereas complete protection was observed when 50 μg were administered. When 71-kD-treated rats were challenged with CP20961, all developed reduced symptoms of arthritis compared with control rats, but in this model no complete protection was observed over the dose range studied. The effects of 71-kD pretreatment on collagen II arthritis were not significant, but in general symptoms of arthritis were milder than in the control group. The same pattern of results was observed previously when hsp65 was used in the different models. These results show that the modulatory effects of hsp on adjuvant arthritis are not restricted to the hsp65 series, but are also mediated by a member of the hsp70 family.

Keywords experimental arthritis hsp70-induced suppression

INTRODUCTION

The etiopathogenesis of rheumatoid arthritis (RA) in humans has been proposed to involve immunity to mycobacterial antigens and related antigens of other infectious organisms [1]. It has been suggested that the autoimmunity observed in RA is a result of cross-reactive immunological reactions between infectious organisms and host cartilage components [2]. Investigation of humoral and cell-mediated immune responses during infectious diseases such as leprosy, tuberculosis, malaria and trypanosomiasis has shown that bacterial stress proteins belonging to the 65-kD and 70-kD hsp families are major immune targets, and can be considered immunodominant antigens [3–6].

As a result of the proposed link between autoimmunity and infection, the role of mycobacterial hsp as antigens in RA has been a subject of expanding interest. Whether heat shock proteins are actual target antigens in arthritis still remains to be determined, however. The expression of hsp has been reported in both synovial membrane cells of RA and osteoarthritic joints, and also in chondrocytes [7,8]. Moreover, T lymphocytes specific for recombinant *Mycobacterium bovis* bacille Calmette–Guérin (BCG) hsp65 have been isolated from joints of RA patients, and hsp65-specific T cell clones were found to modulate the development of arthritis in rats [9–11].

Rat adjuvant arthritis is a widely used experimental model for RA, and is observed after animals are injected subcutaneously with heat-killed *Myco. tuberculosis* (MTB) suspension in paraffin oil [12]. The disease is T lymphocyte-driven and can be transferred to naive animals with T cells from spleens and lymph nodes of adjuvant-treated rats [13]. When animals are pretreated with the 65-kD antigen, it has been shown that arthritis induction can be prevented against streptococcal cell wall [14], MTB [15], pristane [16] and collagen II [17] challenge. Pretreatment with hsp65 also reduces the severity of lipoidal amine-induced arthritis [15]. Epitope mapping studies have further demonstrated that a non-apeptide sequence contained within the 65-kD protein (amino acids 180–188) was recognized by arthritogenic T cell clones and was able to prevent arthritis induction [18–20].

Taken together, these studies suggest that a T cell immunity to the 65-kD antigen is important in the induction and protection against adjuvant arthritis. In this study, we have investigated the effects of another stress protein, hsp71 of *Myco. tuberculosis*, for its ability to modulate arthritis in different experimental models of rat arthritis. The 71-kD protein is distinct from the 65-kD hsp antigen, and belongs to the 70-kD hsp gene family as defined by biochemical and antigenic analysis [5,21]. Garsia *et al*. [22] have

Correspondence: Dr A. E. Kingston, Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK.

shown no homology at the gene level between the 65 and 70 hsp family members, and moreover, there is no cross-reactivity between antibodies directed at the two antigens [23]. Like hsp65, hsp71 antigen can induce immune responses in human individuals exposed to mycobacteria [3,4]. Distribution studies have shown that immunogenic sequences contained within hsp70 gene products may be restricted to the more pathogenic types of mycobacteria, including *Myco. leprae*, *Myco. bovis* and *Myco. tuberculosis* [3]. This contrasts with hsp65, which is found in both pathogenic and non-pathogenic mycobacteria. In view of the concordant immune reactivity to both the 65-kD and 70-kD antigens during infection, it is of interest to investigate the activity of hsp71 in experimental models of RA. The results of this study show that the effects of the 71-kD antigen are consistent with those seen previously for the 65-kD antigen.

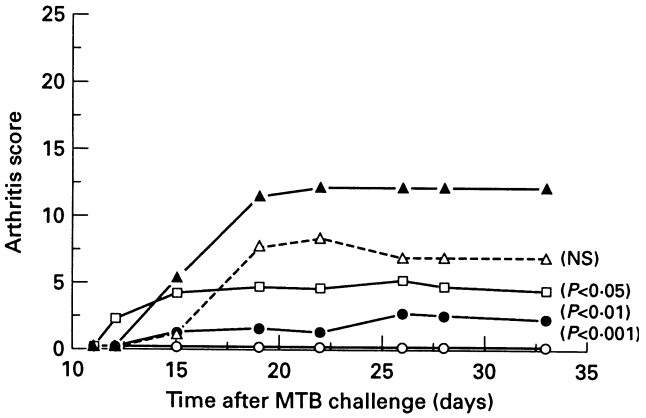

Fig. 1. The effects of hsp71 pretreatment on *Mycobacterium tuberculosis* (MTB)-induced arthritis. Rats were pretreated with recombinant hsp71 in oil at 1–50 μg per rat subcutaneously at the base of tail for 21 days before challenge with MTB (250 μg/rat) at base of tail. Results represent mean arthritis scores of five rats per group. *P* values were calculated at day 33. NS, Not significant. ○, 50 μg hsp71; ●, 5 μg hsp71; □, 1 μg hsp71; △, oil alone; ▲, disease control.

MATERIALS AND METHODS

MTB adjuvant arthritis

Age-matched female Lewis rats (5–6 weeks old; Bantam and Kingman Ltd., Hull, UK) were injected with 250 μg of *Myco. tuberculosis* (heat-killed human strains C, DT and PN; Central Veterinary Laboratory, Weybridge, UK), subcutaneously in the base of the tail. The MTB was finely ground in a pestle and mortar and suspended in paraffin oil at a concentration of 2·5 mg/ml. Arthritis was assessed by scoring symptoms at 10 sites: hind feet, fore feet, ears, eyes, tails and nose on a scale of 0–4, where 0 represents no signs and 4 = severe arthritis. A total score of 32 was possible for each rat, but arthritis development was usually terminated when the rats reached a score of 25. Five rats were used in each experimental group.

CP20961 arthritis

A synthetic lipoidal amine CP20961 (LA; N,N-dioctadecyl-N′,N-bis (2-hydroxyethyl) propanediamine; Avridine; Pfizer, Groton, CT) induces an arthritis in Lewis rats [24]. Female Lewis rats were injected subcutaneously in the base of the tail with 5 mg of CP20961 in paraffin oil (50 mg/ml). Arthritis development was evaluated as described above.

Type II collagen-induced arthritis

Native bovine type II collagen was prepared according to previously described methods in a 1 : 1 emulsion of 0·1 M acetic acid and Freund's incomplete adjuvant (FIA; Difco Labs Inc., Detroit, MI). Female Lewis rats were injected subcutaneously with 2 mg of collagen II, and arthritis development was scored as described for MTB-induced arthritis. The arthritis produced by collagen II was usually localized in the hind feet and therefore did not achieve scores in excess of 8.

Pretreatment of Lewis rats with hsp71

A purified recombinant preparation of hsp71 from *Myco. tuberculosis* was obtained from Dr Ruurd van der Zee (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) in support of the UNDP/World Bank/WHO Special Programme. Hsp71 was dissolved in 0·1 M PBS and mixed with an equal volume of paraffin oil. Emulsions containing 5–500 μg/ml were prepared and 0·1 ml volumes injected subcutaneously in the base of the tail of female Lewis rats. Rats were immunized for 21 days before challenge with arthritogens: MTB, CP20961 or collagen II. Hsp65 was prepared and purified as described previously [25]. The differences in arthritis scores between groups were analysed statistically by the Wilcoxon rank test and *P* values were calculated for a two-tailed test.

Lymphoproliferation assay

Femal Lewis rats injected with MTB adjuvant or paraffin oil alone were killed 12 days after challenge when cell-mediated immune responses to antigens were found to be optimal (unpublished results). Rats injected with 50 μg of either hsp65 or hsp71 were killed 21 days after immunization. Spleens were removed and pooled from five rats, and single-cell suspensions were prepared. Cells were resuspended in RPMI 1640 culture medium containing 5% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5×10^{-5} M) and HEPES (10 mM). Lymphocytes were cultured in 96-well round-bottomed microtitre plates (2×10^5 cells/well; Nunclon, Paisley, UK) for 6 days in an atmosphere of 5% CO_2 in air at 37°C. ^{3}H-thymidine (Amersham, Aylesbury, UK); specific activity 5 Ci/mmol) was added to the cell cultures (0·25 μCi/well) during the last 6 h of culture. Cells were harvested using an automatic cell harvester (Skatron, Newmarket, UK) and radioactivity was determined by means of a Wallac Betaplate liquid scintillation counter.

RESULTS

Effect of hsp71 on MTB-induced arthritis

Rats pretreated with different amounts of hsp71 in oil showed dose-dependent reduction in arthritis scores as a result of MTB challenge (Fig. 1). Pretreatment with oil *per se* brought about a modest reduction in the severity of arthritis, but it was evident that pretreatment with 71-kD superimposed greater suppression of arthritis scores in a dose-dependent manner. It was evident that injection of only 1 μg of hsp71 produced a significant effect against MTB challenge, with complete protection observed when rats were pretreated with 50 μg of the heat shock protein. Control rats that were injected with ovalbumin (50 μg) all developed arthritis scores similar to the MTB disease control group (Fig. 2).

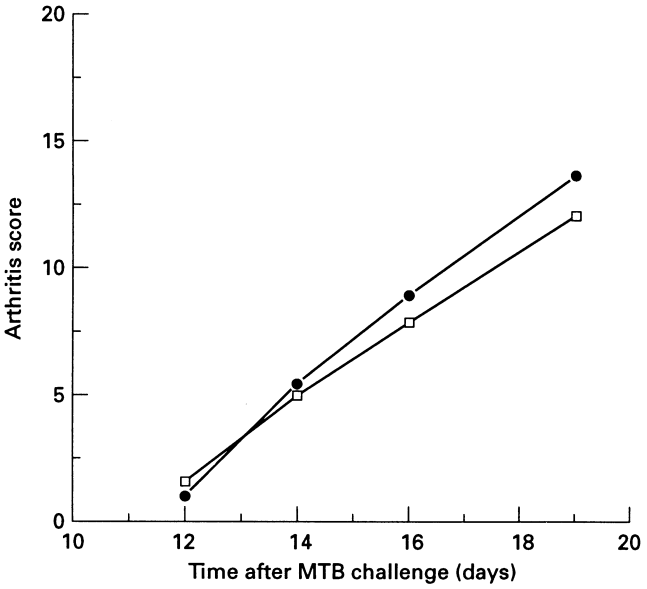

Fig. 2. The effect of ovalbumin pretreatment on *Mycobacterium tuberculosis* (MTB)-induced arthritis. Rats were pretreated with 100 μg ovalbumin (OVA) in oil subcutaneously at the base of tail for 21 days before challenge with MTB (250 μg/rat) subcutaneously at the base of tail. Results represent the mean arthritis scores of five rats per group. □, 50 μg OVA; ●, disease control.

Effect of hsp71 on CP20961 arthritis

Lewis rats pretreated with the hsp71 antigen and subsequently challenged with 5 mg of CP20961 all developed varying degrees of arthritis symptoms (Fig. 3). It was evident, however, that there was a significant reduction in the severity of score which was proportional to the dose of hsp71 administered. A maximum of around 50% protection was observed at the highest level of 50 μg of hsp71.

Effect of hsp71 pretreatment on collagen II-induced arthritis

The arthritis observed in Lewis rats pretreated with hsp71 and subsequently challenged with 2 mg of collagen II did not appear to show marked differences to the arthritis control groups (Table 1). Although there appeared to be a trend in reduction of arthritis scores in rats treated with 71-kD, because of the degree of variation in mean arthritis severity for the groups in this model, there were no significant dose-related protective effects against collagen II arthritis.

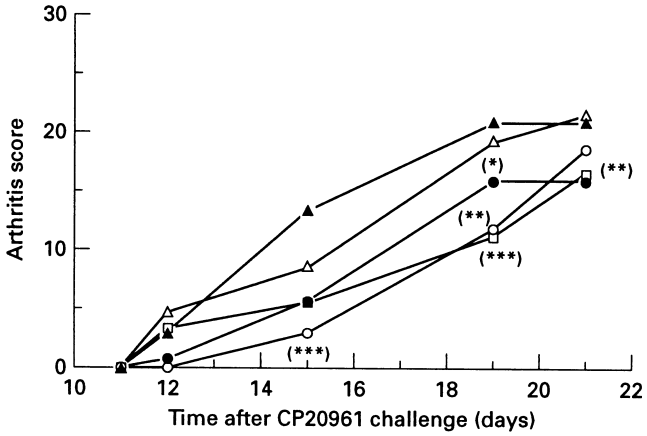

Fig. 3. The effects of hsp71 on CP20961-induced arthritis. Rats were pretreated with recombinant hsp71 at 1–50 μg per rat in oil subcutaneously at the base of tail for 21 days before challenge with CP20961 (5 mg/rat) subcutaneously at the base of tail. Results represent the mean arthritis socres of five rats per group. *P* values were calculated at days 15, 19 and 21. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}002$. ○, 50 μg hsp71; ●, 5 μg hsp71; □, 1 μg hsp71; △, oil alone; ▲, disease control.

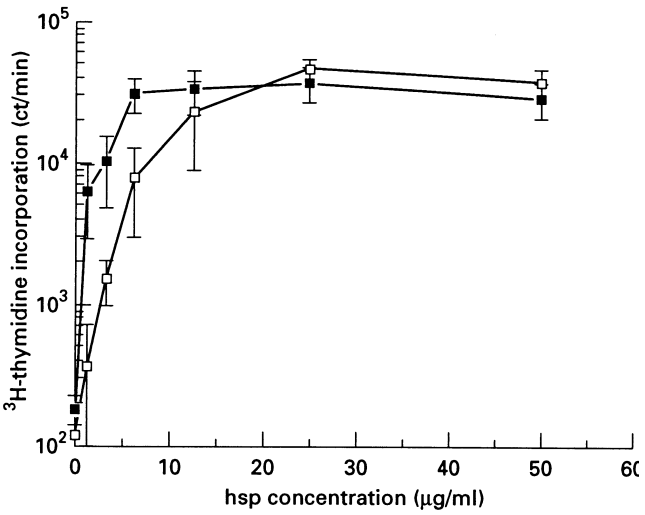

Fig. 4. Spleen cell proliferative responses to hsp antigens. Spleens were removed and pooled from 12 day *Mycobacterium tuberculosis* (MTB) adjuvant-treated rats. Single-cell suspensions were prepared and cells were cultured in microtitre plates for 6 days in the presence of either recombinant hsp65 (□) or hsp71 (■). Cell proliferation was measured by ^{3}H-thymidine incorporation, and results represent the mean ± s.d. of quadruplicate cultures.

In vitro *response of MTB-treated rats to hsp71 antigen*

Figure 4 results show that for MTB-treated rats, hsp71 stimulated spleen cell proliferation in a dose-dependent manner that was comparable to the responses observed for the 65-kD antigen. Lymph node lymphocytes also responded to hsp71 (results not shown). Results in Table 2 show that in normal untreated control rats there was a modest stimulation by hsp65 and hsp71 above

Table 1. The effect of hsp71 on collagen II arthritis

	Arthritis score			
71-kD treatment* (μg/rat)	Day 21	Day 33	Day 37	Day 40
10	4·4 ± 1·1	2·4 ± 0·9	2·5 ± 0·59	2·6 ± 1·1
5	3·2 ± 1·1	3·0 ± 1·0	3·7 ± 1·5	3·5 ± 1·5
1	2·7 ± 1·8	4·4 ± 2·2	4·3 ± 1·9	3·3 ± 1·1
0·5	3·4 ± 1·5	7·6 ± 2·04	7·6 ± 2·6	4·8 ± 1·23
Oil	4·8 ± 1·7	5·8 ± 1·25	6.6 ± 1·25	6.4 ± 1·5
Collagen II control	5.6 ± 1·5	4.4 ± 1·03	3.3 ± 0·8	2·7 ± 1·3

* Rats were pretreated with recombinant hsp71 at 0·5–10 μg rat in oil subcutaneously at the base of tail for 21 days before challenge with collagen II (2 mg per rat) subcutaneously at base of tail. The results represent the mean arthritis scores of five rats per group.

Table 2. *In vitro* lymphorpoliferative responses to hsp antigens

Antigen (10 μg/ml)	^{3}H-thymidine incorporation (ct/min) in rat group*			
	Normal	Paraffin oil	hsp65	hsp71
hsp65	1274 ± 659	28 814 ± 3982	42 050 ± 3649	21 350 ± 1892
hsp71	712 ± 220	25 213 ± 3361	4033 ± 503	59 000 ± 5419
Unstimulated	373 ± 56	6003 ± 371	3594 ± 881	6123 ± 528

* Rats were injected with 50 μg of either hsp65 or hsp71 in Fruend's incomplete adjuvant (FIA) subcutaneously at the base of tail and killed 21 days later. Paraffin oil-treated rats were killed 12 days after s.c. injection. Spleen cells were prepared and pooled from each group and the proliferative responses to predetermined optimal concentrations of hsp65 or hsp71 were assayed. The results show the mean ± s.d. of quadruplicate cultures and are representative of four experiments.

basal ^{3}H-TdR incorporation, i.e. 3·4- and 1·9-fold, respectively. Rats injected with paraffin oil alone showed spleen cell proliferative responses to the 65-kD and 71-kD antigens (4·8- and 4·2-fold, respectively) that were greater than those observed for control rats. In contrast, for the hsp65-immunized group, there was a clear differential stimulation of spleen cell proliferation to hsp65 producing an 11·7-fold response compared with only modest stimulation by hsp71 (Table 2). Similarly, for 71-kD immune rats, lymphoproliferation was specific for the hsp71 antigen (9·6-fold stimulation) with weaker stimulatory effects produced by hsp65 (3·5-fold).

DISCUSSION

Several reports to date have shown that the hsp65 antigen can protect against experimental arthritis in rodents [14–17]. The mechanism whereby the 65-kD protein is able to confer resistance to arthritis is unknown, although studies have shown that 65-kD-reactive T lymphocytes may mediate its effects *in vivo* in rats [14]. Most evidence would suggest that hsp65 is an immune target in both human and experimental RA, and that the mechanism of its experimental effects may be due to the highly conserved nature of the antigen and its ability to induce a tolerization to the arthritogenic effects of autologous hsp65 itself or to a cross-reactive cartilage-associated molecule present within the joint [26]. Others have shown that hsp65 can induce expansion of T cell clones that suppress arthritis development [11] and that immunization with hsp may cause an immune deviation away from pathogenic pathways [16]. Consistent with these reports are further studies that have shown the presence of epitopes on the hsp65 molecule capable of regulating responses to arthritogenic epitopes also contained within the molecule [27].

In this study we have shown that a mycobacterial 71-kD hsp belonging to the hsp70 gene family is also able to induce a suppression of adjuvant arthritis, and like hsp65 is not itself arthritogenic. Stress proteins are classified according to their molecular weight. At least three major families of hsp have been described: the 90-kD family, the DnaK or 70-kD family, and the GroEL or 60-kD family. Members of the hsp60 and hsp70 families are thought to act as molecular chaperones preventing aggregation of newly synthesized polypeptides and participate in the unfolding and renaturation of damaged proteins [28]. Since both hsp65 and hsp70 are able to bind polypeptides, they may play a role in the presentation of antigenic peptides to immune cells [29]. Hsp70 has been shown to bind to unfolded proteins by means of a region that has been proposed to be similar to the peptide binding region of MHC class I proteins [30,31]. Both antigens have been used experimentally as adjuvant carrier molecules for vaccination purposes [32,33]. It is conceivable, therefore, that such molecules may promote the formation of immunogenic complexes when up-regulated or administered *in vivo*.

In experimentally induced adjuvant arthritis, immune cell reactivity to mycobacterial antigens has been implicated in the pathogenesis of joint inflammation. In a previous report, Billingham *et al*. [15] showed that mycobacterial 65-kD produced a potent resistance to MTB-induced arthritis, with lesser effects on arthritis induced by either a synthetic non-immunogenic adjuvant CP20961 or type II collagen. In this study pretreatment of rats with 71-kD showed an analogous order of effect to that of hsp65 in preventing arthritis induction by the different arthritogenic stimuli. Moreover, evidence that these effects were hsp-specific was shown by the inability of ovalbumin pretreatment to suppress arthritis induction. Investigation of the cell-mediated responses to hsp71 in MTB disease rats showed that, like hsp65, there is an immune recognition of the antigen during induction of arthritis, as judged by *in vitro* lymphoproliferative responses. The observed parallelism between the *in vivo* and *in vitro* effects of hsp65 and hsp71 would support that the two antigens share a similar mechanism of action. The observation that oil alone produced some inhibitory effects on adjuvant disease is of interest, and may also be attributed to an hsp-mediated mechanism, since paraffin oil-treated rats have enhanced T cell reactivity to hsp *in vitro*. In this study, it is evident that the *in vivo* effects of hsp71 are clearly superimposed over vehicle effects *per se*, and it is worth noting that in experiments in which rats were pretreated with hsp65 in saline, the same inhibitory effects have been observed as described for hsp65 in oil (manuscript in preparation). Given that the relative purities of the recombinant mycobacterial hsp preparations used in these studies are not absolute, the possibility that contaminating *Escherichia coli* protein homologues may also contribute an effect can not be excluded. However, Thompson *et al*. [16] have shown that mice injected with *E. coli* equivalent GroEL have no effect on pristane-induced arthritis. Further experiments are in progress to determine whether the *E. coli* hsp, GroEL and DnaK, can also mediate the same effects

as the mycobacterial homologues on rat adjuvant arthritis. Moreover, the ability of both hsp65 and hsp70 to ameliorate the induction of arthritis induced by a non-mycobacterial arthritogen CP20961 as well as moderate the symptoms of collagen II arthritis suggests that their mechanism of action(s) may not be as a result of a direct tolerization to species-specific mycobacterial antigens. Instead, these results would support a role for cross-tolerization to common endogenous antigen(s) that may be expressed differentially in all three arthritis models. The nature of such antigens is speculative, since they may still be endogenous hsp; close structural mimics of the hsp or self-peptides bound to the hsp. It is clear that hsp synthesis in all cell types is up-regulated and induced by exposure to physical and chemical challenge. Factors such as free radicals, cytokines, prostaglandins and lipid mediators generated during immune and inflammatory responses may induce a series of changes in the metabolic state of immune and connective tissue type cells within the joint site, and contribute to the expression of a number of different hsp types [34–36]. The pattern of hsp induction following tissue trauma has been shown to have characteristics specific to the type of cell involved. This has been described for the mammalian brain, where it is apparent that hsp70 plays an important role in the response of the tissue to injury, hyperthermia and ischaemia, and would support a role of the hsp70 in the protection and survival of cellular systems [37].

It is conceivable that increased expression of chaperonins in tissue may cause an increased exposure and presentation of nascent and/or denatured self proteins and nucleic acids that are normally sequestered intracellularly, leading to an autoimmune response. Udono & Srivastava [38] showed that vaccination of mice with hsp70 preparations derived from a Meth A sarcoma protects the mice against subsequent challenge with the same sarcoma. The authors reported that several low molecular weight peptides were found associated with hsp70 and were responsible for its vaccination effects. In another study, Lukacs *et al*. [39] showed that murine macrophage tumour cells transfected with mycobacterial hsp65 lose tumorigenicity, and that immunization with the cells induces a protection against tumour formation. The authors suggest that increased hsp65 chaperone activity in transfected cells may enhance cytotoxic T cell recognition of tumour-associated antigens, or may confer the correct conformational and functional characteristics on a tumour suppressor protein such as p53. Further similar studies need to be undertaken to investigate the role of endogneous hsp antigen in the presentation and/or alteration of the conformational characteristics of self antigens in autoimmune diseases.

Patients with chronic inflammatory diseases such as Crohn's disease and ulcerative colitis have been shown to produce antibodies that recognize human and mycobacterial hsp60 and 70 stress proteins [40]. Furthermore, immune responses against hsp have been associated with autoimmune diseases such as systemic lupus erythematosus and polymyositis [41,42], as well as the non-obese diabetic (NOD) mouse model [43]. It is conceivable that in such diseases, where the identity of the autoantigen is not defined, molecular analysis of self peptides bound to immunoreactive hsp may provide a means of identifying the autoantigenic determinants.

In sum, the results of this study, together with evidence from both clinical and experimental studies, suggest that a number of different hsp may contribute to a significant immune reactivity in pathological conditions, and would support a continued investigation of hsp function in autoimmune diseases such as RA.

REFERENCES

1 Inman RD. The role of infection in chronic arthritis. J Rheumatol 1992; **19**:98–104.

2 Vaughan JH. Infection and rheumatic disease: a review. Bull Rheum Dis 1990; **39**:1–8.

3 Britton WJ, Garsia RJ, Hellquist L, Watson JD, Basten AR. The characterisation and immunoreactivity of a 70kD protein common to *M. leprae* and *M. bovis* (BCG). Lep Rev 1986; **57** (Suppl. 2):67–75.

4 Britton WJ, Hellquist L, Basten AR. Immunoreactivity of a 70kD protein purified from *M. bovis* BCG by monoclonal antibody chromatography. J Exp Med 1986; **164**:695–708.

5 Young D, Lathgria R, Hendrix R, Sweetser D, Young RA. Stress proteins are immune targets in leprosy and tuberculosis. Proc Natl Acad Sci USA 1988; **85**:4267–70.

6 Young D, Mehlert A, Smith DF. Stress proteins and infectious diseases. In: Morimoto R, Tissieres A, Georgopoulus C, eds. Stress proteins in biology and medicine. New York: Cold Spring Harbor, 1989.

7 Mapp PI, Cherrie AH, Blake DR, Archer JR, Winrow VR, McLean L. 70kD stress protein in the arthritic synovial membrane (abstract). Arthritis Rheum 1989; **32**(Suppl. 4):S152.

8 Kubo T, Towle CA, Mankin HJ, Treadwell BV. Stress induced proteins in chondrocytes from patients with osteoarthritis. Arthritis Rheum 1985; **28**:1140–5.

9 Res PC, Schaar CG, Breedveld FC, Van Eden W, Van Embden JDS, Cohen IR, De Vries RRP. Synovial fluid T cell reactivity against 65kD heat shock protein of mycobacteria in early chronic arthritis. Lancet 1988; **II**:478–80.

10 Gaston JSH, Life PF, Jenner PJ, Colston MJ, Bacon PA. Recognition of a mycobacteria-specific epitope in the 65kD heat shock protein by synovial fluid derived T cell clones. J Exp Med 1990; **171**:831–41.

11 Van Eden W, Thole JER, Van der Zee R, Noordzij A, Van Embden JDA, Hensen EJ, Cohen IR. Cloning of the mycobacterial epitope reocgnised by T lymphocytes in adjuvant arthritis. Nature 1988; **331**:171–3.

12 Pearson CM. Studies of polyarthritis and other lesions induced in rats by injections of mycobacterial adjuvant. General clinical and pathological characteristics and some modifying factors. Arthritis Rheum 1959; **2**:440–59.

13 Pearson CM, Wood FD. Passive transfer of adjuvant arthritis by lymph node or spleen cells. J Exp Med 1964; **120**:547–60.

14 Van den Broek MF, Hogervorst EJM, Van Bruggen MCJ, Van Eden W, Vand der Zee R, Van den Berg W. Protection against streptococcal cell wall induced arthritis by pretreatment with the 65kD heat shock protein. J Exp Med 1989; **170**:449–66.

15 Billingham MEJ, Carney SL, Butler R, Colston MJ. A mycobacterial 65kD heat shock protein induces antigen specific suppression of adjuvant arthritis, but is not itself arthritogenic. J Exp Med 1990; **171**:339–44.

16 Thompson SJ, Rook GAW, Brealey RJ, Van der Zee R, Elson CJ. Autoimmune reactions to heat shock proteins in pristane induced arthritis. Eur J Immunol 1990; **20**:2479–84.

17 Ito J. Preadministration of a 65kD heat shock protein GroEl, inhibits collagen induced arthritis in mice. J Cell Biochem 1991; **15A**:284.

18 Van der Zee R, Van Eden W, Meloen RH, Noordzij A, Van Embden JDA. Efficient mapping and characterisation of a T cell epitope by the simultaneous synthesis of multiple peptides. Eur J Immunol 1989; **19**:43–7.

19 Yang X-D, Gasser J, Feige U. Prevention of adjuvant arthritis in rats by a nonapeptide from the 65kD mycobacterial heat shock protein 60. Clin Exp Immunol 1990; **81**:189–94.

20 Golden HW, Maniglia CA, Ranges GE. A synthetic mycobacterial heat shock peptide prevents adjuvant arthritis but not proteoglycan-induced synovitis in the rat. Agents and Actions 1991; **34**:148–50.

21 Mehlert A, Young DB. Biochemical and antigenic characterisation of the *Mycobacterium tuberculosis* 71kD antigen, a member of the 70kD heat shock protein family. Molec Microbiol 1989; **3**:125–30.

22 Garsia RJ, Hellqvist L, Booth RJ *et al*. Homology of the 70-kilodalton antigens from *Mycobacterium leprae* and *Mycobacterium bovis* with the *Mycobacterium tuberculosis* 71-kilodalton antigen and with the conserved heat shock protein 70 of eukaryotes. Infect Immun 1989; **57**:204–12.
23 Engers HD. Results of a WHO-sponsored workshop to characteristic antigens recognised by mycobacteria specific monoclonal antibodies. Infect Immun 1986; **51**:718–20.
24 Chang YH, Pearson CM, Abe C. Adjuvant arthritis. IV. Induction by a synthetic adjuvant: immunologic, histopathologic and other studies. Arthritis Rheum 1980; **23**:62–71.
25 Lamb FI, Kingston AE, Estrada-GI, Colston MJ. Heterologous expression of the 65-kilodalton antigen *Mycobacterium leprae* and murine T cell responses to the gene product. Infect Immun 1988; **56**:1237–41.
26 Van Eden W, Hogervorst EJM, Van der Zee R, Van Embden JDA, Hensen EJ, Cohen IR. The mycobacterial 65kD heat shock protein and autoimmune arthritis. Rheumatol Int 1989; **9**:187–91.
27 Hogervorst EJM, Boog CJP, Wagenaar JPA, Wauben MHM, Van der Zee R, Van Eden W. T cell reactivity to an epitope of the mycobacterial 65kD heat shock protein (hsp65) corresponds with arthritis susceptibility in rats and is regulated by hsp65 specific cellular responses. Eur J Immunol 1991; **21**:1289–96.
28 Hurst NP. Stress (heat shock) proteins and rheumatic disease. New advance or just another band wagon? Rheumatol Int 1990; **9**:271–6.
29 Wand-Wurttenberger A, Schoel B, Ivanyi J, Kaufmann SHE. Surface expression by mononuclear phagocytes of an epitope shared with mycobacterial heat shock protein 60. Eur J Immunol 1991; **21**:1089–92.
30 Sadis S, Ragavendra K, Hightower LE. Secondary structure of the mammalian 70kD heat shock protein cognate protein analysed by circular dichroism spectroscopy and secondary structure predictions. Biochem 1990; **29**:8199–206.
31 Rippmann F, Taylor WR, Rothbard JB, Green NM. A hypothetical model for the peptide binding domain of HLA. EMBO J 1991; **10**:1053–9.
32 Barrios C, Lussow AR, Van Embden J *et al*. Mycobacterial heat shock proteins as carrier molecules II. The use of the 70kD mycobacterial heat shock protein as a carrier for conjugated vaccines can circumvent the need for adjuvants and BCG priming. Eur J Immunol 1992; **22**:1365–72.
33 Lussow AR, Barrios C, Van Embden J *et al*. Mycobacterial heat shock proteins as carrier molecules. Eur J Immunol 1991; **21**:2297–302.
34 Takenaka IM, Hightower LE. Regulation of chick hsp70 and hsp90 family gene expression by transforming growth factor beta-1. J Cell Physiol 1993; **155**:54–62.
35 Koller M, Hensler T, Konig B, Prevost G, Alouf J, Konig W. Induction of heat shock proteins by bacterial toxins, lipid mediators and cytokines in human leukocytes. Int J Med Microbiol Virol Parasitol Infect Dis 1993; **278**:365–76.
36 Holbrook NK, Carlson SG, Choi AM, Fargnoli J. Induction of hsp70 gene expression by antiproliferative prostaglandin A2–a growth dependent response mediated by activation of heat shock transcription factor. Mol Cell Biol 1992; **12**:1528–34.
37 Brown IR. Induction of heat shock (stress) genes in the mammalian brain by hyperthermia and other traumatic events: a current perspective. J Neuroscience Res 1990; **27**:247–55.
38 Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 1993; **178**:1391–6.
39 Lukacs KV, Lowrie DB, Stokes R, Colston MJ. Tumour cells transfected with a bacterial heat shock gene lose tumorigenicity and induce protection against tumours. J Exp Med 1993; **178**:343–8.
40 Elsaghier A, Prantera C, Bothamley G, Wilkins E, Jindal S, Ivanyi J. Disease association of antibodies to human and mycobacterial hsp70 and hsp60 stress proteins. Clin Exp Immunol 1992; **89**:305–9.
41 Minota S, Winfield J. Autoantibodies to the constitutive 73kD member of the hsp70 family of heat shock proteins in systemic lupus erythematosus. J Exp Med 1988; **168**:1475–80.
42 Minota S, Kolasu S, Yamara I, Winfield J. Autoantibodies to the heat shock protein hsp90 in systemic lupus erythematosus. J Clin Invest 1988; **81**:106–9.
43 Elias D, Markovits D, Reshof T, Van der Zee R, Cohen IR. Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65kD heat shock protein. Proc Natl Acad Sci USA 1990; **87**:1576–80.