Antibodies to Mycobacterial 65-kDa Heat Shock Protein and Other Immunodominant Antigens in Patients with Psoriasis

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An association of microbial agents and autoimmunity has been suggested for the pathogenesis of psoriasis. Mycobacteria are common environmental microbes and their antigens, especially the highly conserved mycobacterial 65-kDa heat shock protein (hsp65), have been implicated in the pathogenesis of autoimmune arthritis and other idiopathic diseases. In this context, we investigated a possible mycobacterium-induced humoral immune response in psoriasis. Sera from 17 patients with chronic plaque-type psoriasis were studied by immunoblotting using the whole sonicate of Mycobacterium tuberculosis and purified recombinant mycobacterial hsp65. Immunoblot analysis demonstrated that 58% of the psoriasis patients compared to patients with acne and DLE, and normal controls showed strong antibody activity to 65-kDa and 48/45 doublet antigens from M. tuberculosis sonicate, whereas 47% of the patients showed antibody activity to mycobacterial hsp65. Only 10–20% of the patients had an antibody response to 16-kDa and 80-kDa antigens. Similar antibody activity to 65 kDa and 48/45 kDa was also found consistently with eight different sonicated mycobacterial species by immunoblotting, indicating that these seroreactive antigens are crossreactive and are present in common environmental mycobacteria. Antibody activities to both mycobacterial 65-kDa and hsp65 showed a positive correlation (r = 0.76) with the psoriasis disease activity, whereas antibodies to 48/45-kDa doublet antigens showed a weak correlation (r = 0.54). By enzyme-linked immunosorbent assay (ELISA), 47% of the psoriasis patients showed significantly elevated antibody titers to hsp65 (p < 0.003) as compared to control groups, and the antibody response by ELISA also showed a significant positive correlation (r = 0.76) with disease activity. Anti-mycobacterial antibody activity may be related to severity of disease and may be useful in monitoring disease activity in psoriasis. J Invest Dermatol 100:87–92, 1993

Autoimmunity and microbial agents have often been suggested to play a role in the pathogenesis of psoriasis [1–7]. Previous studies have clearly shown that some psoriatic flares are associated with microbial infection and colonization, and the removal of these microbial foci has resulted in clearing of the disease in some cases [4,6].

Mycobacteria have long been suspected of having an etiologic role in autoimmune and other idiopathic diseases [8,9]. Detailed antigenic analysis of several dominant antigens of mycobacteria has resulted in their identification as members of highly conserved heat shock or stress protein families [10,11]. Heat shock proteins (hsp) are a focus of interest as potential antigens in the autoimmune diseases. They are highly immunogenic proteins that show a high degree of sequence homology between many bacterial and human proteins [11–13].

Autoimmunity to hsp may be explained on the basis of so-called antigenic mimicry [9,11,12]. Such a pathologic mechanism may underlie human arthritic disorders in which an association with previous bacterial infection has been suggested [14,15]. The notion that mycobacteria may be involved in autoimmune diseases is further supported by the experimental autoimmune ajuvant arthritis, which is morphologically similar to human rheumatoid arthritis [14,15]. In adjuvant arthritis, mycobacterial hsp65, the well-characterized bacterial hsp, was the antigen considered to be responsible for the pathogenesis of the disease [13–15]. In addition, an increased antibody and T-cell responses to mycobacterial hsp65 has been shown to occur in human rheumatoid arthritis [15].

In psoriasis, some patients exhibit symptoms of arthritis [16]. In view of this and of the increasing evidence that mycobacteria may directly or indirectly be involved in the pathogenesis of autoimmune and other diseases with unknown etiology [5,8,9], we investigated the presence of serum antibody responses of psoriasis patients.

Manuscript received February 11, 1992; accepted for publication October 6, 1992.

Part of this study was presented at the Fifth International Psoriasis Symposium, July 10–14, 1991, San Francisco, CA, USA, and the 21st Annual Meeting of the European Society for Dermatological Research (ESDR), April 4–7, 1992, London, UK.

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Abbreviations:

DLE: discoid lupus erythematosus
ELISA: enzyme-linked immunosorbent assay
HLA: human leukocyte antigen
hsp: heat shock protein
PASI: psoriasis area severity index
r: correlation coefficient
SDS-PAGE: sodium dodecylsulphate–polyacrylamide gel electrophoresis

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to mycobacterial hsp65 and other mycobacterial antigens. Our study demonstrated that psoriatic patients had significant IgG antibody against mycobacterial hsp65 and other dominant mycobacterial antigens of various molecular sizes as compared to normal controls. This antibody response was found to be positively correlated with the disease activity.

**MATERIALS AND METHODS**

**Patients** Sera from 17 chronic plaque type psoriasis patients were studied. There were 13 men and four women, age range 29–71 years. All were suffering from severe plaque-form psoriasis unresponsive to topical therapy. Duration of the disease of these patients was from 4 to 41 years. The status of disease was scored by means of the psoriasis area severity index (PASI), using the sum of mean erythema, thickness, area of the involved skin, and scaling [17]. The disease activity as compared to previous visits has been scored as regressive (−), stable (0), progressive (+), or strongly progressive (+++). Clinical parameters of individual patient are illustrated in Table I. All patients were treated with oral cyclosporin A with or without conventional topical corticosteroid treatment at the time of sample collection. None of the patients had any clinical history of tuberculosis or leprosy. Although 10 patients had a history of arthropathic complaints, only five had mild to moderate complaints at the time of sample collection (Table I). Arthropathic complaints of these patients were defined by one or more of the following clinical symptoms: pain in the joints, redness, swelling of the joints, or impairment of daily living activities. None of the patients had severe arthritis. Control sera used in this study were obtained from five acnes patients, six DLE patients, and 15 normal healthy individuals.

**Antigen Preparations** The mycobacterial antigens were prepared from sonicats of fresh bacilli as described previously [18,19]. The species used were Mycobacterium tuberculosis, Mycobacterium bovis BCG, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium gordonae, Mycobacterium marinum, Mycobacterium ulcerans, Mycobacterium kansasii, and Mycobacterium davisii. Strains of these mycobacterial species are similar to those described in our previous report [19].

The native 65-kDa mycobacterial hsp was derived from Escherichia coli K12 carrying M. bovis BCG recombinant DNA with the 65-kDa protein gene [20], and was obtained in purified form from Dr. J. van Embden, National Institute of Public Health, Bilthoven, The Netherlands.

**Sodium Dodecylsulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting** SDS-PAGE was performed in slab gels (12%) in discontinuous Tris-buffer system as described previously [18,19]. All antigen preparations were incubated with sample buffer at 65°C for 20 min. Pre-evaluated concentration of 15 μg of proteins were applied from different species of mycobacterial sonicates, whereas 2 μg were loaded from purified mycobacterial hsp65. Proteins were transferred to nitrocellulose papers according to Towbin et al [21], and immunoblotting was performed with sera of optimal dilution (1:100) as previously described [18]. The bound antibodies were visualized by using HRP-conjugated goat anti-human IgG diluted at 1:4000 (Tago, Burlingame, CA) as described previously [18].

**Enzyme-Linked Immunosorbent Assay (ELISA)** ELISA was performed essentially according to our earlier report [18], except that in the present study affinity-purified mycobacterial hsp65 (0.5 μg per well) was used as the coating antigen and the optimum serum dilution used was 1:100. Serum IgG, IgM, and IgA antibody classes against mycobacterial hsp65 were determined in all patient groups and normal individuals.

**RESULTS AND DISCUSSION**

**Antibody Response to M. Tuberculosis Antigens** Immunoblot analysis using total sonicate of M. tuberculosis was employed for the initial screening of sera of psoriasis patients. Our results demonstrated that more than 58% of the psoriasis patients showed a consistent strong antibody reactivity to antigens of apparent molecular masses of 65-kDa and 48/45-kDa doublet as compared to control groups. Figure 1 shows the variable serum IgG antibody reactivity of eight psoriasis patients to M. tuberculosis antigens of various molecular weights. Antibody activity to 16-kDa and 80-kDa antigens was found only in 10–20% of the patients, whereas most of the psoriasis patients showed a moderate activity to 40 kDa and to multiple bands of high–molecular-weight antigens. The intensity of the bands reflects the antibody titers in the following order: +++ to ++++. The moderate to the highest intensity of the bands at a dilution of 1:100. Because some of the control sera also showed weak antibody
Figure 1. Representative example showing serum IgG antibody responses of eight patients with plaque type psoriasis shown at left. Sera were diluted 1:100. Molecular weights in kilodalton (kDa) are shown at left.

Activity (+), especially to the 65 kDa and occasionally to 48/45 kDa, we used + intensity as the cutoff value for the evaluation of the positive antibody titer in psoriasis patients. Moreover, antibody activity could be detected up to 1:500 to 1:1000 in psoriasis patients who showed high titer to 65 kDa and 48/45 kDa. At these dilutions, all control sera gave no staining. The latter was also taken as a criteria of evaluation of ++ and +++ antibody activity of psoriasis patients.

It is important to note that serum antibodies to high-molecular-weight mycobacterial antigens has also been identified as a preferential antibody response to mycobacteria in patients with autoimmune diseases and autoimmune type dermatoses [22]. Some of these mycobacterial antigens recognized by psoriasis sera are known to be the major cross-reactive antigens involved in the immune response of active mycobacterial diseases [18,23]. It is interesting that high antibody activity of psoriasis patients to 48/45-kDa doublet antigens has not been shown to occur consistently even in active mycobacterial diseases. However, in our preliminary report we demonstrated that patients with Crohn's disease showed a significant antibody response to 48/45-kDa antigens.* On the other hand, patients with Crohn's disease did not show any significant antibody response to 65-kDa antigen. Serum samples obtained from patients with acne and DLE as well as normal controls showed no significant antibody activity to these antigens, although DLE patients showed a certain antibody response to antigens other than 65 kDa and 48/45 kDa (data not shown).

Occurrence of Antibody Response to Similar Antigens from Other Mycobacterial Species Immunosblots containing sonified extract of eight different mycobacterial species were developed with a pool of positive sera from psoriasis patients to determine whether immunodominant protein bands identified in Mycobacterium tuberculosis are also present in other mycobacterial species, especially those that are known to be associated with dermatologic lesions [24,25]. Indeed, similar 65-kDa and 48/45-kDa bands were observed in all the mycobacterial species tested (Fig 2), indicating that these seroreactive mycobacterial antigens are cross-reactive antigen components [18]. This was further confirmed by absorption of pooled positive sera with different mycobacterial sonicates (data not shown). Non-tuberculous mycobacteria have been found in association with infection of the skin and subcutaneous tissues [24]. In addition, M. marinum and M. ulcers are known to be predominantly skin pathogens with affinity to the low-temperature areas of the body, and some dermatologic lesions and ulcers are known to be caused by M. aviumintracellularur and M. gordonae [25,26]. It may be that persistence of serum antibodies in psoriasis patients is due to previous skin infections with these mycobacterial species because mycobacteria are quite widespread in the environment.

Antibody Response to Mycobacterial hsp65 The finding of serum antibody response to 65-kDa antigen from various species of mycobacteria is of particular interest. Mycobacterial 65-kDa is known to be a common bacterial antigen [10,20] and belongs to the highly conserved heat shock protein family of hsp60 that has been implicated in cross-reactive autoimmunity in arthritis [14]. Therefore, we investigated the serum antibody activity of psoriasis patients to purified mycobacterial hsp65 derived from recombinant DNA strain of E. coli K12 [20]. It is interesting that those patients who had strong antibody response to antigen from mycobacterial sonicates in immunoblotting showed significantly elevated antibody reactivity to mycobacterial hsp65 in immunoblotting (Fig 3A). Individual anti-mycobacterial hsp65 IgG antibody levels in ELISA are shown in Fig 3B. Because no differences were found between normal and control non-psoriatic patient groups, the optical density (OD) corresponding to 0.2 was taken as the cutoff point or normal upper limit. Accordingly, we found that 47% of psoriasis patients showed significantly elevated serum IgG antibody titer to mycobacterial hsp65 (p > 0.003). None of the psoriasis patients showed elevated IgM or IgA antibody response to mycobacterial hsp65 in ELISA (data not shown).

![Figure 2](image-url) Immuno blot analysis of total sonicates of various mycobacterial species with positive pool sera from five psoriasis patients (diluted 1:100). Lanes: 1, M. avium; 2, M. marinum; 3, M. ulcers; 4, M. kansasii; 5, M. gordonae; 6, M. dawali; 7, M. intracelullare; 8, M. bovi BCG. Note the consistent strong antibody activity to 65-kDa and 48/45-kDa antigens in all mycobacterial species.
Figure 3. A) Immunoblot analysis showing the serum antibody activity to mycobacterial hsp65 in psoriasis patients with varying intensities. Sera were grouped as strong positive (lanes 1 and 2), moderate positive (lane 3), and weak positive (lane 4). Most of the normal serum reactivity corresponds to the intensity of lane 4. B) Individual serum IgG antibody titers to mycobacterial hsp65 in patients with psoriasis, acne, DLE, and normal controls in ELISA. Sera were diluted 1:100.

The variability in the antibody response to mycobacterial hsp65 and other immunodominant antigens, both in intensity and specificity, in psoriasis patients, is intriguing. When the data were examined for the association with disease activity of psoriasis, we found a significant positive correlation between the anti-mycobacterial serum IgG response and the clinical parameters of the patients. Antibodies to mycobacterial 65-kDa and hsp65 in immunoblotting showed an excellent relationship with the disease activity ($r = 0.76$), whereas antibodies to 48/45 kDa showed a weak correlation ($r = 0.54$). $r$ value was calculated as the relative antibody activity in relation to the serum dilution (relative intensity of bands as a parameter of + to +++ positivity). Moreover, anti-hsp65 ELISA titers showed a similar correlation coefficient ($r = 0.76$) with disease activity. Table I summarizes the overall comparison of antibody response (both immunoblotting and anti-hsp65 ELISA titers) and the clinical parameters of the psoriasis patients. It is worth noting that the patients who developed a very severe disease had the most prominent antibody response to various mycobacterial antigens as well as the highest ELISA titers to mycobacterial hsp65. Among the patients who showed strong anti-mycobacterial antibody response (+ to ++++ in immunoblotting and ELISA), 77% had the active form of psoriasis. On the other hand, none of the patients who showed weak (±) or no (−) antibody response (corresponding to antibody activity of control groups) had disease activity and thus showed a negative correlation. We found no correlation of anti-mycobacterial antibody activity with arthropathic complaints of the patients.

Although the present data do not necessarily suggest a pathogenic role of anti-mycobacterial antibodies in psoriasis patients, serum antibody responses to specific group of mycobacterial antigens could be regarded as an indicator for an increased risk of developing psoriasis in genetically predisposed individuals. This possibility needs to be investigated further. However, no association was found between the antibody activity and the duration of the treatment of the disease. We have followed up some of the patients who are undergoing cyclosporin A treatment for up to 3–6 months and found no difference in antibody activity to mycobacterial antigens during and after the treatment. However, this does not exclude the possibility that major deviations in antibody titer may be present before the cyclosporin A treatment. In order to evaluate the direct effect of cyclosporin A treatment on anti-mycobacterial antibody titer of psoriasis patients, further studies are needed.

The stress proteins are important targets of the immune response to mycobacterial infection and have been implicated for the pathogenesis of autoimmune diseases [9,11,12]. The major stress protein recognized by antibodies in bacterial infection is hsp60. In addition, there are many microbial candidates that account for the development of anti-hsp 60/65 immune response in humans [9]. Significantly elevated antibody binding to mycobacterial hsp65 has been reported in patients with rheumatoid arthritis [27], Behçet's syndrome [28], and superficial candidiasis [29]. This suggested that hsp60 is likely to be an immune target in many bacterial infections [9,11]. Moreover, previous studies have shown that a polyclonal rabbit antisemur against mycobacterial hsp65 recognized a similar-molecular-size protein from streptococcus strains, suggesting that some of the streptococcal antigens are cross-reactive with mycobacterial hsp65 [28]. A recent report on group A streptococcal cell wall–induced arthritis in rats also presented evidence of T-cell-
based cross-reactivity with M. tuberculosis antigens [30] and has shown that pretreatment with mycobacterial hsp65 prevented the arthritis [31]. In this regard, it is interesting to note that streptococcal infection is considered to be a trigger of guttate psoriasis, which may precede chronic plaque type psoriasis [7,32]. Such clinical observations have been partly explained by immunologic cross-reactivity between streptococcal proteins and components of the human skin [33]. Published data further suggest that a conserved part of the streptococcal M protein may trigger the psoriatic process [33]. In this context, it is pertinent to note that we have recently shown that two novel MoAbs (Ne5/Nd4) raised against mycobacterial hsp65 cross-reacted with human epidermal cytokeratin 1/2 [34,35]. In psoriasis, this particular epitope was found to be absent in lesional sites.

These relevant findings, together with our present observation of elevated antibody response of psoriasis patients to mycobacterial hsp65, suggested that shared or common antigens such as mycobacterial or streptococcal hsp might play a role in the pathogenesis of psoriasis. Moreover, psoriasis has been associated with an increased frequency of certain human leukocyte antigen (HLA) haplotypes including HLA-Cw6, B13, B17, and B27 [36,37]. It is possible that an association between HLA haplotype and the observed variable serum antibody response to mycobacterial hsp65 and other mycobacterial antigens exists in psoriasis patients. Finally, our study further suggests that because mycobacteria are ubiquitous environmental pathogens, and because the mycobacterial antigens are potent immunomodulators, certain mycobacterial antigens may trigger psoriasis in genetically predisposed individuals. On the other hand, most of the patients in the present study are BCG vaccinated. Consequently, an alternative explanation can be that the mycobacterial antibodies are somehow stimulated in active psoriasis, but nonspecifically. Therefore, further studies in a non-BCG-immunized population would be of interest in this respect.

We thank Dr. Stefanie Meridith for critical reading of the manuscript, and Dr. Marc Nahows for the careful analysis of patients' clinical data.

This work was carried out under project GNK/DA/004 (AHG-49) of the Faculty of Medicine, University of Amsterdam, and Dutch Medical Research Council project 511-02-2, The Netherlands, and was partly supported by Q. M. Gasmann-Wichers-Stichting, The Netherlands.

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