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Chlamydial Heat Shock Proteins and Trachoma

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Two chlamydial proteins (HSP-60 and HSP-70) have marked homology with bacterial and mammalian heat shock proteins. Previous studies have indicated that when inoculated into the eyes of immune animals, a Triton X-100 extract of chlamydia containing HSP-60 induces an ocular delayed-type hypersensitivity reaction. The potential for HSP-70 to induce a similar reaction was tested in six cynomolgus monkeys that had been sensitized to both antigens by previous ocular chlamydial infection. Whereas the chlamydial extract containing HSP-60 induced a marked clinical response within 24 h of inoculation, no response followed inoculation of HSP-70 in the contralateral eye. The lack of a response to HSP-70 suggests that further assessment of its potential as a trachoma vaccine is warranted.

The chronic inflammatory response in blinding trachoma is characterized by the heavy infiltration of the conjunctiva by lymphocytes, both B cells and T cells, and the eventual development of scarring (17). This response has the features of a delayed-type hypersensitivity (DTH) reaction, and this has led to the notion that much of the pathogenesis of trachoma is immunologically mediated (11). The changes observed during chlamydial infection of the fallopian tubes are very similar to those seen in the eye, and it seems likely that the pathogenesis of chlamydial genital tract infection is similar (10).

The chlamydial protein that induces the ocular DTH reaction in immune animals is extractable with Triton X-100 detergent (12, 14). It has a molecular mass of 57 kilodaltons (kDa) and has considerable homology with the general group of heat shock proteins with molecular masses of approximately 60 kDa (HSP-60) (9).

Recently, another chlamydial heat shock protein has been characterized (5). This protein has a molecular mass of 75 kDa and belongs to the general group of heat shock proteins of approximately 70 kDa (HSP-70). Antibodies against HSP-60 have no apparent neutralizing ability; however, antibodies against HSP-70 do neutralize chlamydia (8). This suggests that HSP-70 is promising as a candidate for vaccine development. The present studies were conducted to determine whether HSP-70 was capable of eliciting an ocular DTH response in ocular-immune monkeys in a way similar to HSP-60. This was particularly relevant because both mycobacterial HSP-60 and HSP-70 can induce DTH responses in appropriately sensitized animals (1).

MATERIALS AND METHODS

Six adult cynomolgus monkeys (Charles River Primates Corp., Port Washington, N.Y.) were studied. Previously, they had been infected in the eye with *Chlamydia trachomatis* serovar C (TW-3) and had recovered completely. Animals received a single topical inoculation of HSP-60 in one eye and HSP-70 in the other. The allocation of inoculum was determined randomly.

The eyes were examined with a slit lamp before and after inoculation. The examiner did not know which inoculation had been given to each eye or the results of the previous clinical examination. The presence and severity of the clinical response were graded by scoring 10 features (the presence and extent of follicles and injection in different parts of the conjunctiva, ocular discharge) on a scale of 0 to 3 as described in detail previously (13). The grading was summed to give the clinical disease score for each eye.

HSP-60. A Triton X-100 extract from *C. trachomatis* serovar B (provided by C. Morrison and H. Caldwell, Rocky Mountain Laboratories, Hamilton, Mont.) was prepared by incubating 10^9 EB in 1 ml of phosphate-buffered saline with 0.5% Triton X-100 for 1 h at 37°C. The supernatant was recovered after centrifugation for 1 h at 100,000 × *g* at 6°C. The Triton X-100 was removed by passage over an Extracti Gel D column (Pierce Chemical Co., Rockford, Ill.) (14, 16). The final ocular inoculum contained approximately 1 mg of chlamydial proteins.

HSP-70. Recombinant HSP-70 was purified from *Escherichia coli* $DH5_x$ containing plasmid pERU52 after lysis with 2% Sarkosyl (5). The cell supernatant was extracted from an affinity column containing the capture antibody UM-13 by eluting with 0.1 M sodium acetate–0.15 M sodium chloride. The purified HSP-70 was lyophilized and reconstituted to 0.1 mg/ml in distilled water.

Western immunoblot analysis of anti-HSP serologic responses. Both preparations were analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Chlamydial proteins were transferred to nitrocellulose paper, and 2- to 3-mm nitrocellulose paper strips were incubated overnight (4°C) with either monkey serum (diluted 1:100) or tears (diluted 1:50) obtained 1 week before heat shock protein challenge. Cross-reacting alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) (diluted 1:500) or IgA (diluted 1:250) was used to develop the antibody-binding bands. Optimal concentrations of anti-HSP-70 and anti-HSP-60 monoclonal or polyclonal antibodies with appropriate secondary antibodies were also included in these assays (Fig. 1).

RESULTS

Serum IgG antibodies to HSP-60 and HSP-70 were demonstrable in five of six monkeys before ocular challenge.

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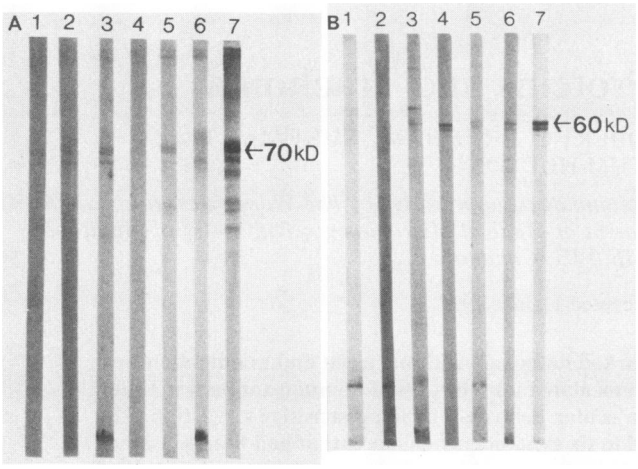

FIG. 1. (A) Immunoblot analysis of serum IgG anti-HSP-70 reactivity of different monkeys. Lanes represent HSP-70 reacted with serum obtained 1 week before challenge with HSP-60 and HSP-70 from monkeys D5, D9, 1955, D6, D7, and D8 (lanes 1 through 6, respectively) and with monoclonal anti-HSP-70 (lane 7). (B) Immunoblot analysis of serum IgG anti-HSP-60 reactivity of different monkeys. Lanes represent HSP-60 reacted with sera from monkeys D5, D9, 1955, D6, D7, and D9 (lanes 1 through 6, respectively) and with monoclonal anti-HSP-60 (lane 7).

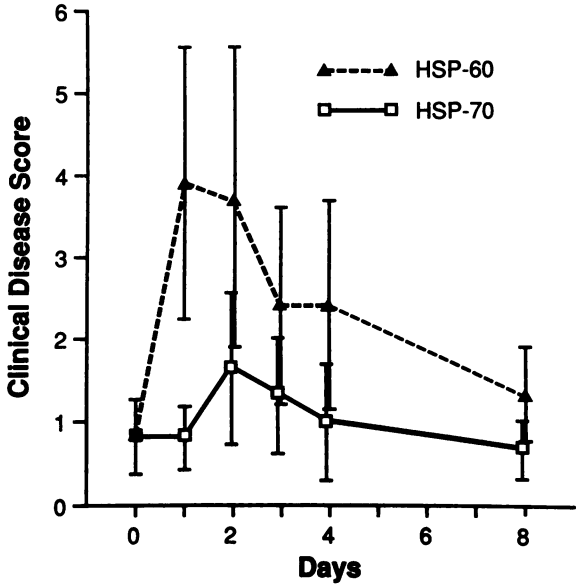

FIG. 2. Clinical response of the eyes of six monkeys after inoculation with either HSP-60 or HSP-70 on day 0, shown as the mean clinical disease score (error bars show the standard errors of the means).

Western blot analysis of anti-HSP-60 and anti-HSP-70 reactivity against recombinant HSP-70 (Fig. 1A) and purified Triton X-100-extracted chlamydial antigen, which contains HSP-60 (9) (Fig. 1B), demonstrated serum IgG directed against both of these heat shock proteins. Anti-HSP IgA antibodies were not detected in tears from any of the six monkeys. All sera were positive by Western blot analysis for anti-chlamydial IgG antibodies directed against four or more chlamydial proteins continued in the solubilized elementary body preparation (data not shown).

The baseline clinical disease score of the eyes randomized to receive HSP-70 was identical to that of eyes that received HSP-60 (0.83 ± 0.98 [mean ± standard deviation] clinical disease units). At 24 h after inoculation with HSP-60, there was a marked increase in clinical disease score (3.92 ± 3.73) that was significantly higher than that at baseline (Wilcoxon sign rank test, $P = 0.03$) and also higher than that induced in the other eye that received HSP-70 (0.83 ± 0.81; rank sign test, $P = 0.03$). The response in the HSP-60-inoculated eyes then waned over the next 7 days (Fig. 2). The eyes that received HSP-70 remained uninflamed.

DISCUSSION

In marked contrast to HSP-60, HSP-70 did not elicit a DTH response when inoculated into the eyes of immune monkeys. The preparation of HSP-70 was a purified recombinant protein, whereas the HSP-60 preparation was a detergent extract from whole organisms and contained multiple bands although the HSP-60 band predominated. By using recombinant HSP-60, Morrison and colleagues have shown that the ocular DTH response induced by the crude extract is due to the HSP-60 and that both preparations behave similarly (9).

Proteins of the HSP-60 family seem to be responsible for aggregation and packaging of other proteins (3, 7). It seems likely that HSP-60 is involved in the assembly of the outer membrane of the elementary body during the change from the large replicating reticular body to the compact infective elementary body. Chlamydial HSP-60 does not appear to be a promising protective molecule. Certainly after Triton X-100 extraction, elementary bodies are still infective (N. G. Watkins, personal communication; data presented at the Edna McConnell Clark Foundation Workshop on Trachoma, Woods Hole, Mass., May 1987), and ocular immunization of monkeys with HSP-60 does not lead to any protection against subsequent ocular challenge (16). HSP-60 appears to be the key antigen in eliciting the deleterious, immunopathogenic component of the host immune response to chlamydial infection.

On the other hand, HSP-70 offers significant promise as a vaccine candidate, because monospecific antibodies to HSP-70 neutralize *C. trachomatis* in vitro (8) and antibodies to HSP-70 are prominent in the immune response after infection (2). Proteins of the HSP-70 family are generally responsible for the unfolding of proteins (4, 6) and may also have ATPase activity (1). HSP-70 may be important in the initial steps of chlamydial replication, since infective elementary bodies reorganize and expand into the reproductive reticular bodies. The analogies that are apparent between chlamydia and *Mycobacterium leprae* are noteworthy: in both infections an HSP-60 appears pathogenic, and some have suggested that an immunoreactive HSP-70 may offer protection (15). Studies to assess the protective capacity of chlamydial HSP-70 are now underway.

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