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Autoimmunity to Heat Shock Protein 60 and Antigen-Specific Production of Interleukin-10

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The immunopathologic sequelae of chlamydial infection are correlated with immune responses to the *Chlamydia trachomatis* heat shock protein 60 (hsp60). One pathogenic mechanism that may explain this association is the induction of autoimmune responses to self hsp60, since these two proteins share a high degree of amino acid sequence identity. To investigate the conditions under which autoimmune responses can be generated against self hsp60, groups of CBA mice were immunized with recombinant mouse hsp60, recombinant chlamydial hsp60, or both proteins. The data show that autoimmune responses characterized by strong T-cell proliferation and high titers of antibody to self hsp60 are induced only by concurrent immunization with mouse and chlamydial hsp60. Immunization with mouse hsp60 alone induced lymphocytes that secreted high levels of interleukin-10 (IL-10) but did not proliferate in response to in vitro stimulation with mouse hsp60; coimmunization with mouse and chlamydial hsp60s induced lymphocytes that proliferated strongly in response to mouse hsp60, secreted 6-fold less IL-10, and exhibited a 12-fold increase in the ratio of gamma interferon/IL-10 production. Switches in cytokine production patterns may mediate the pathogenesis of hsp60-associated diseases such as *C. trachomatis* immunopathology.

Heat shock proteins 60 (hsp60s) belong to a family of related proteins which are inducible and/or constitutively expressed by prokaryotic and eukaryotic cells in response to stressful stimuli and which appear to protect the cell against damage due to stress-induced protein misfolding (17). Immune responses to microbial hsp60s are speculated to initiate chronic inflammatory diseases in which autoimmune responses to hsp60 may be central to pathogenesis (18). Both T-cell clones with self hsp60 reactivity and high titers of antibody responses to self hsp60 have been identified in patients with several chronic inflammatory diseases (5, 9, 10, 31). Autoimmune responses to self hsp60 elicited during infection are possibly consequent to stressful conditions at inflammatory sites that induce expression of hsp60 in both microbial and host cells. For instance, Boog et al. (1) demonstrated increased levels of self hsp60 in the cells of inflamed synovial membranes of patients with juvenile chronic arthritis, a condition that has been associated with immunopathologic response to mycobacterial hsp60 (31).

However, the mechanisms by which microbial hsp60s may induce autoimmune diseases are unclear. One obvious fact is that hsp60s share a high degree of amino acid sequence identity; sequences of hsp60s from over 50 different prokaryotic and eucaryotic species exhibited over 40% sequence identity (33). A high degree of amino acid sequence identity between an antigenic prokaryotic protein and a mammalian protein suggests a structural basis for the initiation of an autoimmune response in which molecular mimicry plays a dominant role. Sequence sharing may be particularly important in autoimmune pathogenesis, since Lin et al. (19) demonstrated that autoreactive B and T cells that respond to self cytochrome *c* can be induced if the host immune system is simultaneously challenged with both a foreign (homologous) and a self cytochrome *c* that are closely related in amino acid sequence. On their own, neither foreign nor self cytochrome *c* could induce autoimmune responses.

The chronic inflammatory histopathology that accompanies *Chlamydia trachomatis* infection is thought to be due to immunopathologic mechanisms, and chlamydial hsp60 has been proposed to induce immunopathology through autoimmune reactions (2, 23). Supportive evidence for autoimmune immunopathology in chlamydial disease states includes the ectopic expression of class II major histocompatibility molecules in uninfected epithelial cells during chlamydia infection (8, 21) and the observation that women with *C. trachomatis*-associated ectopic pregnancies have high titers of serum antibodies to human hsp60 peptide epitopes (36). In the current study, we defined the condition(s) under which autoimmunity to self hsp60 can be induced by chlamydial hsp60 and examined the relationship of autoimmunity to antigen-specific cytokine production in order to further elucidate the basis for hsp60-associated immunopathology.

MATERIALS AND METHODS

Mice. CBA/J ($H\text{-}2^k$) mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). Four- to 8-week-old female mice were used in the experiments.

Antigens. The recombinant *C. trachomatis* hsp60 fusion protein expression plasmid (pGEX-2T) was the generous gift of R. Stephens (University of California, San Francisco) (3). The methods used for production, purification, and site-specific proteolysis with thrombin (Sigma Chemical Co., Mississauga, Ontario, Canada) have been described elsewhere (29). Ovalbumin was purchased from Sigma.

Recombinant mouse hsp60 was cloned and expressed in plasmid pGEX-2T by using the following procedures. Total mouse L-cell mRNA purification, cDNA preparation, and PCR amplification were performed according to the manufacturer's instructions (Micro Fast-track kit, cDNA cycle kit; Invitrogene, San Diego, Calif.). Mouse hsp60-specific oligonucleotides that were used as primers (M1 and MR1.6) were located at the 5′ and 3′ prime ends of the mouse hsp60 gene (32) and were designed with 5′ and 3′ *Bam*HI restriction endonuclease sites. Primers were synthesized on an oligonucleotide synthesizer (Oligo 1000 DNA synthesizer; Beckman Instruments Inc., Fullerton, Calif.) according to the following nucleotide sequence: M1, 5′-GTT CCG CGT GGA TCC GCC AAA GAT GTA AAA TTT GGT GCG-3′; MR1.6, 5′-TTT TCC CGG GGA TCC TTA GAA CAT GCC GCC TCC CAT ACC-3′. The PCR-amplified product (approximately 1,600 bp) was initially cloned into the pUC18 plasmid and subsequently subcloned into the expression vector pGEX-2T. The complete mouse hsp60 gene was expressed as a glutathione-*S*-transferase fusion protein when

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induced by isopropyl-β-D-thiogalactopyranoside (IPTG). The positive clone was identified by Western blot probed with monoclonal antibody (MAb) LK1 (Sigma), which has specificity for mammalian hsp60. The nucleotide sequence of the recombinant mouse hsp60 gene was confirmed as correct by DNA cycle sequencing (BRL Life Technologies, Inc., Gaithersburg, Md.) and comparison with the known nucleotide sequence (32). Purified recombinant chlamydial hsp60 and mouse hsp60 were used after cleavage from glutathione-*S*-transferase with thrombin (29). The purity of recombinant hsp60s was verified by sodium dodecyl sulfate-gel electrophoresis with Coomassie brilliant blue and silver staining and further confirmed by Western blot with antibodies to recombinant proteins. No detectable contamination by *Escherichia coli* proteins was found.

Immunization. Mice were immunized subcutaneously with 50 or 100 μg of antigen(s) emulsified 1:1 in incomplete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). Immunizations were at the base of the tail for antibody production and in the footpad for T-cell assays.

hsp60 ELISA. Solid-phase enzyme-linked immunosorbent assays (ELISA) were used to measure titers of hsp60 antibodies. Chlamydial and mouse hsp60s were separately adsorbed overnight at 4°C to microtiter plates, and the microtiter wells were subsequently blocked with 2% bovine serum albumin. Mouse sera were used at a twofold serial dilution. Goat anti-mouse immunoglobulin G tagged with horseradish peroxidase (Pierce, Rockford, Ill.) followed by ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6 sulfonate; Sigma) was used to detect antibody binding. The serum antibody titers were determined by the highest dilution of the sera which yielded ≥0.3 optical density units at 405 nm.

Lymphocyte proliferation assays. Ten days after immunization with hsp60 in the footpad, regional lymph nodes were aseptically collected, and single-cell suspensions were prepared. The cells (5×10^5/well) were cultured in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) containing 15% fetal calf serum in the presence or absence of specific antigens in flat-bottomed 96-well microculture plates (Costar 3596; Costar, Cambridge, Mass.). The plates were incubated at 37°C in 5% CO_2–95% humidified air for 5 days. [^{3}H]thymidine (1 μCi) was added to each well in 20 μl of RPMI 1640 18 h before harvesting. The cultures were harvested using a PHD cell harvester (Cambridge Technology, Inc., Watertown, Mass.), and the incorporation of tritiated thymidine into DNA was measured by liquid scintillation counting (Ls 5000 CE; Beckman, Palo Alto, Calif.). Results are presented as mean experimental disintegrations per minute.

Cytokine analysis. Mice were sacrificed 10 days postimmunization. Spleen cell suspensions were cultured at 10^7 cells/ml (2 ml/well) in 24-well plates at 37°C in RPMI 1640 medium containing 10% fetal calf serum in the presence or absence of antigen (100 μg/ml). Culture supernatants were harvested at 40 h for gamma interferon (IFN-γ) and at 72 h for interleukin-10 (IL-10) analysis (4). Murine IFN-γ and IL-10 were measured by a two-MAb sandwich ELISA (purchased from Pharmingen, San Diego, Calif.) as described elsewhere (35). The IFN-γ ELISA was carried out using MAb XMG 1.2 as detection antibody and MAb R4-6A2 as capture antibody. The IL-10 ELISA used MAbs JESS-2A5 and SXC-1 as capture and detection antibodies, respectively. Briefly, 96-well plates were coated with capture MAb at 2 μg/ml in buffer (0.1 M $NaHCO_3$, pH 8.6). After overnight incubation at 4°C, the plates were blocked with 4% bovine serum albumin in phosphate-buffered saline for 2 h at room temperature and washed extensively. Culture supernatant and IFN-γ, IL-2, and IL-10 cytokine standards (Pharmingen) were serially diluted and added to the plates. The plates were incubated at 4°C overnight and then washed four times. Biotinylated anti-cytokine-detecting MAb (2 μg/ml) was added for 40 min at room temperature. The plates were washed four times and incubated with streptavidin-peroxidase at room temperature for 30 min. The plates were extensively washed, and ABTS substrate with H_2O_2 was added and read after 30 min.

RESULTS

Autoreactive lymphocyte proliferation in response to self hsp60 is induced by concurrent but not by individual immunization with chlamydial hsp60 and mouse hsp60. Groups of CBA mice were immunized with chlamydial hsp60 alone, mouse hsp60 alone, mouse hsp60 plus chlamydial hsp60, or mouse hsp60 plus ovalbumin. Ten days after immunization, lymph node cells (LNCs) were cultured in the presence or absence of mouse hsp60 or chlamydial hsp60. LNCs from mice primed with chlamydial hsp60 strongly proliferated in response to chlamydial hsp60 (Fig. 1B) and had much lower levels of T-cell proliferation in response to mouse hsp60 (Fig. 1A). LNCs from mice immunized with mouse hsp60 alone failed to proliferate in vitro in response to either mouse hsp60 or chlamydial hsp60 (Fig. 1A and B).

Qualitatively similar but quantitatively different results were found at the B-cell level when antibody responses were measured (Fig. 1C and D). Serum antibody titers to chlamydial and mouse hsp60s were separately measured 30 days following immunization. Mice immunized with chlamydial hsp60 alone showed a 4.2 ± 0.15 $\log_{10}$ antibody titer to chlamydial hsp60 and a 2.2 ± 0.18 $\log_{10}$ titer to mouse hsp60. Mice immunized with mouse hsp60 alone showed a 2.4 ± 0.3 $\log_{10}$ antibody titer to mouse hsp60 (Fig. 1C) and a <2.0 $\log_{10}$ antibody titer to chlamydial hsp60 (Fig. 1D). The data demonstrate that chlamydial hsp60 is highly immunogenic in CBA mice and that it mainly induces T-cell and antibody responses to chlamydia-specific epitopes. As well, CBA mice specifically respond to self hsp60 immunization at the B-cell level although with low antibody responses.

In line with the observations of Lin et al. (19) that concurrent administration of homologous foreign and self proteins may break tolerance to a self antigen, we found that coadministration of chlamydial and mouse hsp60s could induce marked T-cell proliferative autoimmune responses. As shown in Fig. 1, in contrast to immunizations with chlamydial or mouse hsp60 alone, coimmunization with these two homologous hsp60s induced strong lymphoproliferative and antibody responses to mouse hsp60 (Fig. 1A and C).

To determine whether the sequence homology between chlamydial hsp60 and mouse hsp60 was crucial for the induction of autoimmunity to self hsp60, we immunized mice with mouse hsp60 in combination with an unrelated protein, ovalbumin. Unlike coimmunization with mouse hsp60 and chlamydial hsp60, T cells remained proliferatively unresponsive to mouse hsp60 when the mice were coimmunized with mouse hsp60 and ovalbumin. Similarly, antibody responses to mouse hsp60 remained low following coimmunization with ovalbumin (Fig. 1A and C). These data show that autoimmune responses to self hsp60, especially lymphocyte proliferative responses, can be induced when self (mouse) hsp60 and a sequence-related foreign (chlamydial) hsp60 are concurrently exposed to the immune system. As well, the amino acid sequences shared between the two proteins appear to be necessary for the induction of autoimmunity.

T-cell proliferative unresponsiveness to mouse hsp60 is controlled through peripheral anergy. To analyze whether the T-cell proliferative unresponsiveness to mouse hsp60 is due to peripheral anergy or clonal deletion, we repeated the lymphocyte proliferation assays using LNCs collected from mouse-hsp60-immunized mice but added exogenous recombinant IL-2 to the in vitro culture. We reasoned that if peripheral anergy accounted for the failure of T cells to proliferate in response to mouse hsp60 stimulation, then exogenous IL-2 might overcome the anergic state (26, 28). As shown in Fig. 2, LNCs collected from mice immunized with mouse hsp60 were stimulated in vitro with mouse hsp60 alone, mouse hsp60 plus IL-2, or ovalbumin plus IL-2. Cultures that were stimulated with mouse hsp60 supplemented with IL-2 strongly proliferated, whereas unsupplemented cultures and cultures stimulated with a nonrelevant antigen (ovalbumin) plus IL-2 failed to do so. These results indicate that self-hsp60-reactive T cells do exist in the peripheral T-cell repertoire following immunization but appear to be anergic.

Anergy to self hsp60 is associated with antigen-specific IL-10 production that is reduced following coimmunization. To investigate whether a unique cytokine pattern is associated with anergy to self hsp60, we next determined IL-10 and IFN-γ secretions by spleen cells collected from hsp60-immunized mice following antigen-specific in vitro stimulation (Table 1). The level of IL-10 production following mouse hsp60 in vitro restimulation of splenocytes was found to be inversely correlated with the LNC proliferative responses to self hsp60. When mice were immunized with mouse hsp60, the splenocyte cultures following mouse hsp60 restimulation produced high lev-

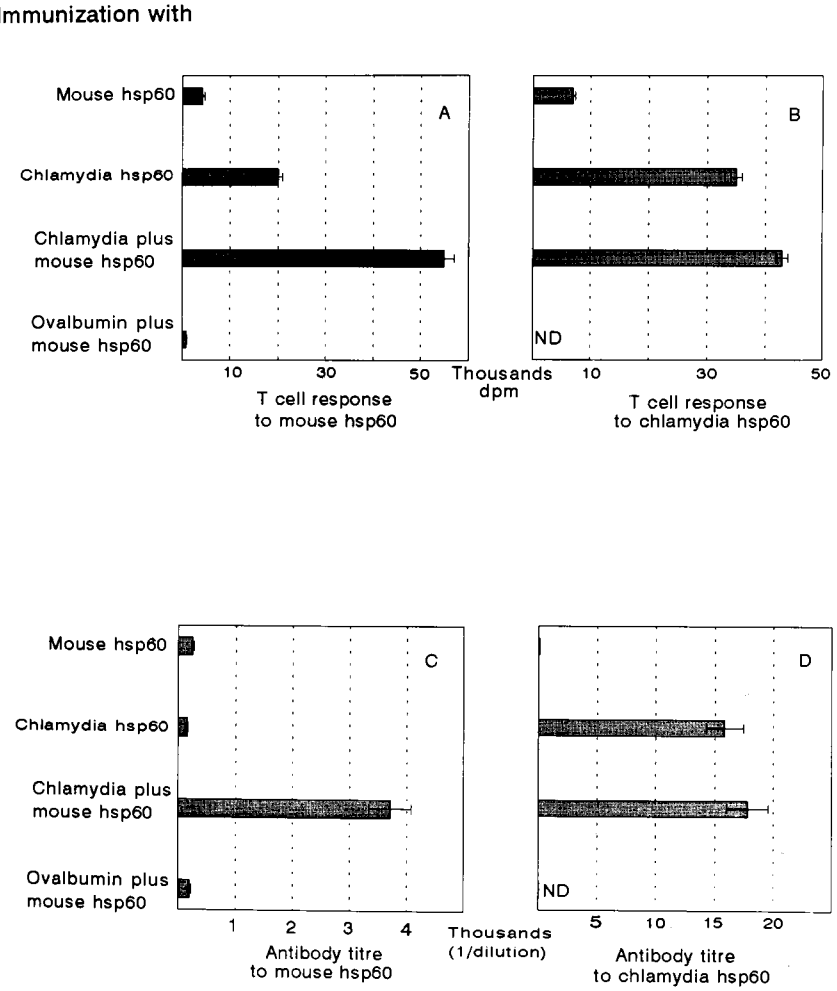

FIG. 1. Four groups of CBA mice (four mice per group) were immunized with purified mouse hsp60, chlamydial hsp60, both mouse hsp60 and chlamydia hsp60, or mouse hsp60 plus ovalbumin in incomplete Freund's adjuvant. LNCs from primed mice were stimulated with 100 μg of mouse hsp60 per ml (A) or 100 μg of chlamydia hsp60 per ml (B) in vitro for 5 days. T-cell proliferation was measured by [^{3}H]thymidine incorporation, which is represented as mean disintegrations per minute (dpm). Sera were collected 30 days postimmunization, and antibody titers are shown as 10^{-3} dilution for binding to mouse hsp60 (C) or chlamydia hsp60 (D) as measured by ELISA. Error bars represent standard deviations of the means.

els of IL-10, and LNCs exhibited poor proliferative responses. Similarly, spleen cells collected from chlamydial-hsp60-immunized mice also produced high levels of IL-10 upon in vitro mouse hsp60 restimulation, and LNCs displayed low levels of proliferation. Therefore, the T-cell tolerance of CBA mice to self hsp60 appears to be a form of immune deviation rather than absolute unresponsiveness.

Among mice coimmunized with both chlamydial hsp60 and mouse hsp60, IL-10 secretion elicited by mouse hsp60 in vitro was markedly reduced and lymphocyte proliferative responses to mouse hsp60 were dramatically increased. The ratio of IFN-γ/IL-10 secretion upon mouse hsp60 in vitro restimulation was increased in mice coimmunized with chlamydial and mouse hsp60s compared to that among mice immunized with mouse or chlamydial hsp60 alone. The data suggest that IL-10 production is associated with low proliferative responses to self hsp60 and that reduction of the IL-10 secretion may functionally break the anergic state to self antigen.

Exogenous IL-10 suppresses IL-2 reversion of T-cell anergy to self hsp60. The mechanism by which IL-10 plays a role in maintaining T-cell anergy is unknown. The "two-signal model" of T-cell activation suggests that engagement of the T-cell receptor by major histocompatibility complex-bound peptide in the absence of costimulatory molecules results in T-cell anergy (26, 28). By down-regulating antigen-presenting cell (APC) expression of costimulatory molecules, IL-10 may cause APCs to preferentially deliver anergizing rather than proliferative signals to antigen-specific T lymphocytes (7, 14, 15, 22). Since exogenous IL-2 was able to reverse the T-cell anergy to mouse hsp60 in vitro, IL-2 presumably bypasses the requirement for second-signal costimulation. We next investigated the effect of exogenous IL-10 on IL-2 reversion of T-cell anergy to mouse hsp60 after reasoning that if IL-10 production in vivo maintains T-cell anergy to mouse hsp60, then high levels of exogenous IL-10 may prevent IL-2 reversion of T-cell proliferative unresponsiveness in vitro. As shown in Fig. 3, LNCs from mice primed with mouse hsp60 strongly proliferated in response to mouse hsp60 restimulation following the addition of exogenous IL-2 (between 0 and 50 U/ml). However, when IL-10 was added to the system in which 50 U of IL-2 per ml was present, the IL-2 reversion of T-cell proliferative unresponsiveness to mouse hsp60 was suppressed in a manner dependent on the concentration of IL-10 added. The data demonstrate that IL-10 blocks the effect of IL-2 on T-cell proliferative responses,

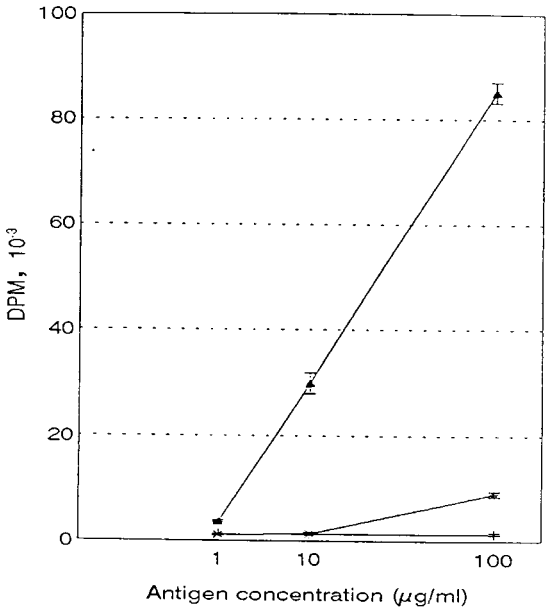

FIG. 2. LNCs proliferated in response to mouse hsp60 when supplemented with IL-2. LNCs were collected from CBA mice 10 days after immunization with mouse hsp60 and cultured in vitro with various concentrations of mouse hsp60 alone (*), mouse hsp60 plus 37.5 U of IL-2 per ml (▲), or ovalbumin plus 37.5 U of IL-2 per ml (+). T-cell proliferation was measured by [^{3}H]thymidine incorporation. Error bars represent standard deviations of the means.

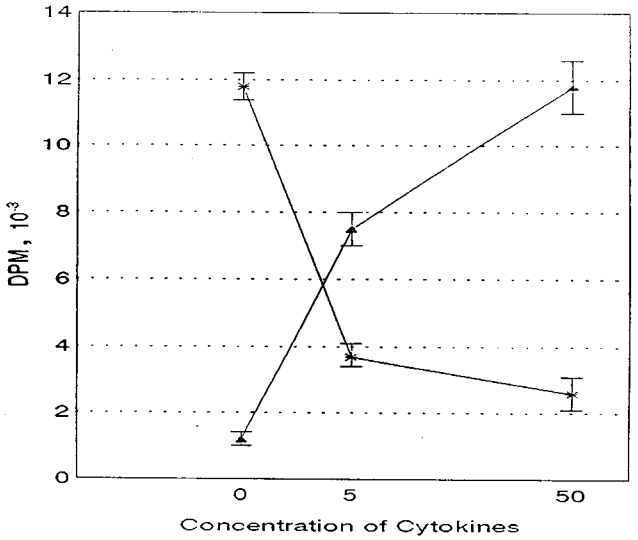

FIG. 3. LNCs were collected from mice immunized with mouse hsp60 and cultured in vitro with mouse hsp60 (20 μg/ml) or mouse hsp60 plus various concentrations of IL-2 (0, 5, and 50 U/ml) (▲). IL-10 (*) was added at various concentrations (5, 50, and 500 ng/ml) to cultures containing mouse hsp60 (20 μg/ml) and a fixed amount of IL-2 (50 U/ml).

suggesting a role for IL-10 in maintaining peripheral T-cell anergy to self antigen in vivo.

DISCUSSION

Tolerance can be defined as "a physiologic state in which the immune system does not react destructively against the organism that harbors it" (27). The establishment and maintenance of immunologic tolerance are based on multiple events, including induction of antigen-specific T-cell anergy. The common characteristic shared by most anergized T cells is loss of the ability to produce and/or respond to antigen by proliferative signals (28). Therefore, anergy may represent a state in which cell populations such as autoreactive T cells are arrested for growth. This may efficiently prevent the amplification of autoreactive cells and consequent autoimmune disease.

In the present study, we found that CBA mice had relative tolerance to self hsp60 in the form of immune deviation (24) characterized by proliferative unresponsiveness and low titers of antibody responses following immunization with self hsp60.

TABLE 1. Correlation of mouse hsp60 antigen-specific immune responses and patterns of cytokine production[a]

hsp60 immunization	T-cell proliferation (10^{-3} dpm)	Production (pg/ml) of:		IFN-γ/IL-10 ratio
		IL-10	IFN-γ	
Mouse	2.0 ± 0.4	2,186 ± 103	194 ± 25	0.09
Chlamydial	8.4 ± 0.1	1,227 ± 80	433 ± 31	0.35
Mouse + chlamydial	20 ± 5.1	366 ± 42	417 ± 31	1.14

[a] Groups of CBA mice (four per group) were immunized with different hsp60 regimens in the footpad, and 10 days later, LNCs were tested for proliferative responses and splenocytes were tested for cytokine production in response to mouse hsp60. T-cell proliferation, IL-10 production, and IFN-γ production were measured. The data are given as means ± standard errors.

The failure of T cells to proliferate in response to mouse hsp60 appeared to be due to T-cell anergy, since it could be overcome by exogenous IL-2. Autoreactive B cells were not made fully tolerant, because low levels of self antibody were detectable following immunization with self hsp60 and because adoptive transfer of chlamydial-hsp60-specific immune T cells primed mice to produce high titers of self antibodies following immunization with mouse hsp60 (data not shown).

As previously reported by Lin et al. (19), autoimmune responses appear to be relatively easily induced when two homologous antigens, one of which is a self antigen, are administrated concurrently. We observed that coimmunization with mouse hsp60 and chlamydial hsp60 induced strong T- and B-cell responses to self hsp60. In particular, T-cell proliferation in response to mouse hsp60 was dramatically increased upon coimmunization. The induction of strong autoimmune responses depended on the homology of amino acid sequences between mouse and chlamydial hsp60s, since a strong response was not observed when a nonhomologous but highly immunogenic protein such as ovalbumin was coadministered with mouse hsp60. The importance of sequence homology implicates cross-reaction between the antigen recognition structures on T and/or B cells in the autoimmune response rather than bystander activation due to cytokine release. These observations suggest that molecular mimicry is the basis for autoimmunity induced by hsp60, at least under the circumstances examined in this study. The mechanism by which molecular mimicry operates to break tolerance was not defined but may relate to the differential effects that altered peptide ligands can have on cytokine secretion patterns and T-cell activation (13, 34).

IL-10 is in part a product of Th2-like cells and has been documented to be crucial in the regulation of immune responses (22). IL-10 inhibits proliferation of Th1-like cells via down-regulation of IFN-γ and IL-2 production (14, 15) and down-regulates Th1 immune responses through inhibition of APC function (6, 7, 22). More recently, IL-10 was implicated in T-cell tolerance (28). For instance, Enk et al. (12) reported that in vivo application of IL-10 before contact allergen treat-

ment caused antigen-specific tolerance in mice, and they suggested that IL-10 might act via inhibition of production of proinflammatory cytokines. It has also been demonstrated that IL-10 suppresses the induction of proliferation of Th1 cell clones by freshly cultured Langerhans cells via inhibition of costimulatory signals on these cells (11). Current evidence favors the idea that IL-10 inhibits production or function of costimulatory molecules such as B7/BB1 (16, 20). Since IL-10 suppresses costimulatory signaling of APCs and since T-cell anergy is readily induced by antigen recognition in the absence of costimulation (25), IL-10 may be a key cytokine in maintaining peripheral anergy to self antigens when clonal deletion has not occurred.

Because of these observations, we evaluated the role that IL-10 responses may play in anergy to self hsp60. Overall, the data show that immunization with mouse hsp60 induced high levels of antigen-specific IL-10 production which correlated with poor T-cell proliferative responses to mouse hsp60. We also observed that autoimmune responses, including strong T-cell proliferation in response to mouse hsp60 following coimmunization, were associated with significantly reduced levels of IL-10 secretion (Table 1). Exogenous IL-2 added to in vitro cultures reversed T-cell anergy, presumably because IL-2 bypassed the requirement for second-signal costimulation. Importantly, exogenous IL-10 prevented IL-2 reversal of T-cell anergy in a dose-dependent manner. These observations suggest that IL-10 in this system may act directly on T lymphocytes in addition to its effect on APCs to maintain tolerance (30).

On the basis of the observations made in this study, we postulate that the following mechanisms may be involved in host immune responses to self hsp60. T cells autoreactive to self hsp60 become activated to produce IL-10 when their T-cell receptors are engaged only by self hsp60. In turn, IL-10 production maintains the proliferative unresponsiveness of T cells autoreactive to self hsp60, perhaps by impeding costimulatory signal expression by APCs or by direct inhibition of T-cell activation. During infection with a pathogen that expresses foreign hsp60, both foreign and autoreactive T cells specific for hsp60 peptide epitopes are induced, with much of the immune response directed to unique determinants of the foreign hsp60. During some infection situations, such as with intracellular pathogens, both foreign and self hsp60s may be present in sufficient amounts to stimulate cross-reactive immune responses. Under these conditions, autoreactive T cells that are induced primarily by foreign hsp60 are expanded by self hsp60. These cross-reactive T cells appear to secrete low levels of IL-10 and to proliferate strongly in response to self hsp60 in comparison to T cells induced only by self hsp60. We propose that the cross-reactive T cells play a major immunopathogenic role in chlamydial-disease pathogenesis. Switches in cytokine patterns and increases in lymphocyte proliferation may provide the basis for inflammatory tissue damage. Clearly, further study of the mechanisms of IL-10 in the induction and maintenance of T-cell anergy and the effects of altered hsp60 peptide ligands on T-cell activation will shed light on our understanding of immune tolerance and immunopathology. It will be of interest to challenge mice that have been primed for an hsp60 autoimmune response with a *C. trachomatis* infection and then examine them for evidence of immunopathology.

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