T Cell Responses to Heat-Shock Protein 60: Differential Responses by CD4+ T Cell Subsets According to Their Expression of CD45 Isotypes

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T Cell Responses to Heat-Shock Protein 60: Differential Responses by CD4⁺ T Cell Subsets According to Their Expression of CD45 Isotypes

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We demonstrate that human T lymphocytes proliferate in vitro to highly purified human heat-shock protein 60 (Hu.hsp60). The response to this self Ag was confined to the CD45RA⁺RO⁻ T cell subset, with minimal responses by adult CD45RA⁺RO⁺ T cells. Experiments using keyhole limpet hemocyanin as a prototypic novel Ag, or tetanus toxoid as a recall Ag, were consistent with the notion that CD45RA⁺RO⁻ and CD45RA⁺RO⁺ T cell subsets can be designated as naive and memory cells, respectively; thus, responses to Hu.hsp60 were confined to the putative naive subset. In contrast, both CD45RA⁺RO⁻ and CD45RA⁺RO⁺ T cell populations proliferated to bacterial hsp60 from Mycobacterium leprae, Escherichia coli, or Chlamydia trachomatis. However, only CD45RA⁺RO⁺ (memory) T cells responded to a mycobacterial hsp60-derived peptide previously defined as a major bacteria-specific epitope. Experiments with cord blood T cells, which are CD45RA⁺RO⁻ and can be considered truly naive, showed that the peptide could elicit responses from naive T cells in vitro; cord blood cells also responded to Hu.hsp60. Since bacterial hsp60 Ags contain both conserved and nonconserved epitopes, we speculate that in vivo challenge with bacterial hsp60 will activate T cells capable of seeing either type of epitope, but only those that see nonconserved epitopes maintain the CD45RA⁺RO⁺ memory phenotype. However, T cells recognizing conserved epitopes, while not apparently being recruited to the memory pool, may nevertheless play a role in immunoregulation, particularly in the context of inflammation, when expression of Hu.hsp60 is increased. The Journal of Immunology, 1999, 162: 704–710.

The peripheral pool of T lymphocytes consists of two broad categories, those that have not yet encountered their cognate Ag, i.e., naive or virgin cells, and those that have (1–4). The latter may be either effector cells, if they have recently encountered Ag and are still in an activated state as a result of antigenic stimulation, or memory cells if they have returned to a quiescent state following Ag encounter, but are primed for subsequent responses to the same Ag. There is evidence that each of these categories of T cell differs in their expression of surface Ags, particularly the isoforms of the CD45 (5, 6). These isoforms are generated by alternative splicing of the CD45 gene to include 0–3 of exons 4, 5, and 6 (termed A, B, and C) (7). High m.w. forms are identified using mAbs specific for different exons, and these vary in different species: Abs to A, B, and C are available for humans, for B in the mouse, and for C in the rat. An Ab identifying the isoform with none of the ABC exons (CD45RO) is also available in humans (8). Responses to recall Ags have been easily demonstrated in human T cells expressing CD45RO and lacking CD45RA (9–12). Their response to stimulation in vitro is faster than cells expressing high m.w. forms, e.g., CD45RA⁺RO⁻ cells, which generally respond to nonrecall Ags. In addition, after in vitro stimulation, CD45RA⁺RO⁻ cells decrease expression of CD45RA and gain expression of CD45RO (5). All of these properties are consistent with the idea that effector/memory cells are CD45RA⁺RO⁺ and naive cells CD45RA⁺RO⁻.

In this study, we have addressed the question of which T cell subset is capable of mounting responses to three categories of Ag: 1) classical recall Ags, i.e., those against which an individual has been specifically immunized; 2) novel Ags, unlikely to have been previously encountered in vivo; and 3) self Ags. In line with previous work, we show that responses to classical recall Ags are indeed mounted by CD45RA⁺RO⁺ T cells, and those to novel Ags by CD45RA⁺RO⁻ T cells. For a self Ag, the human 60-kDa heat-shock protein (Hu.hsp60) responses were confined to the CD45RA⁺RO⁻ subset. We also examined responses to mycobacterial hsp60 (M.hsp60), an Ag that can be considered a recall Ag in populations such as those tested in this study that have been immunized with Mycobacterium bovis bacille Calmette-Guérin (BCG). However, it shares epitopes with Hu.hsp60, since there is considerable sequence homology between prokaryotic and eukaryotic members of the hsp60 family (13, 14). We find that both T cell subsets are capable of responding to M.hsp60, but that the response to a mycobacteria-specific epitope is confined to the CD45RA⁺RO⁻ (memory) subset. Therefore, the peripheral T cell repertoire of healthy individuals contains cells capable of recognizing epitopes in Hu.hsp60 in vitro, although these cells are either not activated in vivo, or following activation, they do not remain within the CD45RA⁺RO⁻ subset in peripheral blood.

There is extensive literature on the role of hsp60-specific T cells in the pathogenesis of autoimmune diseases, particularly arthritis, with evidence for both pathogenic and protective T cells responding to hsp60 (15–17). In adjuvant arthritis, the pathogenic T cells

3 Abbreviations used in this paper: Hu.hsp60, human heat-shock protein 60; BCG, bacille Calmette-Guérin; GroEL, E. coli hsp60; KLH, keyhole limpet hemocyanin; M.hsp60, mycobacterial heat-shock protein 60; PPD, purified protein derivative; TT, tetanus toxoid.
have been found to recognize bacteria-specific epitopes (18), whereas protective cells are directed against epitopes that are conserved (19, 20). This study provides in vitro evidence that T cells specific for epitopes conserved in Hu.hsp60 have the potential to be activated in normal humans following challenge with bacterial hsp60. Unlike bacteria-specific T cells, they are not recruited to the memory pool, a feature that would be consistent with the immunoregulatory role of these cells inferred from experiments in arthritis models.

Materials and Methods

Antigens

The following Ags were used, at previously determined optimal concentrations: tetanus toxoid (TT) (Statens Serum Institut, Copenhagen, Denmark) at 1/200 final dilution; PPD (Statens Serum Institut) at 10 μg/ml; KLH (Calbiochem, Nottingham, U.K.) at 50 μg/ml; GroEL (Sigma, Poole, U.K.) at 10 μg/ml; and Hu.hsp60 and M.hsp60 (prepared as described below) at 20 and 10 μg/ml, respectively. The peptide M1–15 (1–15 amino acids of M.hsp60) was synthesized using FMOC chemistry (a gift from Dr. P. Life, Glaxo/Wellcome, Stevenage, U.K.) and used at 10 μg/ml.

Purification of Hu.hsp60

It was vital that no contaminating Escherichia coli proteins were present in the recombinant preparations. For this reason, two purification steps were performed for Hu.hsp60. To allow purification using nickel affinity columns, the protein was expressed using the histidine-tag system in E. coli (M15)-transformed cells expressing the vector pQE60 and the repressor (pREP4) plasmid (Qiagen, Chatsworth, CA). Lysates of bacteria expressing Hu.hsp60 were resuspended in buffer (pH 7.8, 50 mM sodium phosphate, 300 mM NaCl) and sonicated on ice (three times, 2 min, using 100 W, vibracell sonicator (Jencons, Bedfordshire, U.K.)) before centrifugation at 10,000 × g for 5 min. This procedure was repeated up to six times. Protein was then solubilized in 6 M urea. The protein sample was resolved by electrophoresis using a 3-mm 10% SDS polyacrylamide gel (Bio-Rad Protein II x1 cell; Bio-Rad Hemel Hempstead, Hertfordshire, U.K.). The position of the 60-kDa band was determined and the protein was extracted by electroleution (Bio-Rad model 422 Electro-Eluter; Bio-Rad) from the appropriate strip of gel. Hu.hsp60 was further purified by elution from a nickel-containing HiTrap affinity column (Pharmacia, Herts, U.K.) using 300 mM imidazole (Sigma).

Recombinant M.hsp60 was also prepared from E. coli disrupted by sonication on ice (three times, 1 min) and centrifugation, but in this instance, the pellet was resuspended each time in CIT-E buffer (20 mM Tris-sodium citrate, 10 mM Dl-sodium EDTA). The protein was solubilized in urea (6 M urea, 50 mM sodium phosphate, pH 7.5, 20 mM EDTA). The sample was then subjected to centrifugation at 8000 × g for 10 min, and the supernatant was collected. Addition of ammonium sulfate to a final concentration of 0.9 M (just below the precipitable point of M.hsp60) removed some unwanted proteins. This protocol was adapted from a procedure described by Lamb et al. (21). The remaining proteins were resolved by electrophoresis and electroeluted as described for Hu.hsp60.

Preparation of mononuclear cells

Blood packs were obtained from blood transfusion centers (Birmingham and Cambridge) and from volunteers within the laboratory; cord blood was obtained from the Rosie Maternity Hospital (Cambridge, U.K.). PBMC and cord blood mononuclear cells were obtained following centrifugation on Ficoll-Paque (Pharmacia Biotechnology, Milton Keynes, U.K.) at 2200 × g for 30 min. To remove immature RBC from cord blood mononuclear cells, anti-glycophorin A (PharMingen), and anti-fetal pig IgG (Becton Dickinson, Lincoln Park, N.J.) with the appropriate concentration of Ag. Two-milliliter cultures were sampled at different time points, between days 4 and 10, by transferring three 100-μl aliquots from each well into 96-well microtiter plates and pulsing with 0.5 μg/ml [3H]thymidine/well for 6 h. Samples were harvested onto printed filter mats (Wallac, Milton Keynes, U.K.) by means of a cell harvester (Skatron, Oslo, Norway), and [3H]thymidine incorporation was measured using a beta plate counter (LKB Wallac, Turku, Finland).

Results

PBMC proliferative responses to Hu.hsp60

To determine whether T cells from healthy individuals could respond to Hu.hsp60, PBMC were cultured for up to 10 days with this Ag, and proliferative responses were assessed. Fig. 1 represents one of four such experiments and shows that in vitro responses to this self Ag could be detected. The proliferative response to Hu.hsp60 peaked at day 7, later than the response to the recall Ag TT, but with kinetics similar to the response to the non-recall Ag, KLH.

CD45RA+ T cells are the main responders to self Ags

T cell subsets expressing the isoforms of CD45 associated with memory or naïve subsets were rigorously purified from healthy adults and stimulated with KLH (Fig. 2A), TT (Fig. 2B), or Hu.hsp60 (Fig. 2C). The CD45RA+·RO+ T cells responded to KLH with a response that had not peaked by day 9, whereas the response by CD45RA+·RO+ T cells to the same Ag was very low. For TT, a reciprocal pattern was evident; the CD45RA+·RO+ T cells showed a response that peaked on day 6, whereas there was...
no proliferation by the CD45RA\(^+\)RO\(^-\) T cells (maximum stimulation index, 4.1 \(1456\) cpm), as compared with 315 \(96,361\) cpm for CD45RA\(^-\)RO\(^+\) cells), even though the same cells were capable of responding to KLH. These results are similar to those that we have published previously (10), and are included in this work to show that the purified subsets used in this study to test responses to hsp60 had identical properties. Responses to Hu.hsp60 were similar to those observed with KLH; the largest proliferative responses were demonstrated in the CD45RA\(^-\)RO\(^+\) population, with only minimal responses by the CD45RA\(^+\)RO\(^-\) T cells (Fig. 2C). A similar pattern of response was also observed to another purified human Ag, phospholipid-binding protein (data not shown).

As an additional source of purified CD45RA\(^+\)RO\(^-\) T cells, we obtained cord blood mononuclear cells (98% CD45RA\(^-\), data not shown). Fig. 3 shows that these cells were also able to proliferate in response to Hu.hsp60, with similar kinetics to the Ag PPD. The CD45RA\(^-\) T cells recognize this recall Ag, since cord blood T cells are naive, having not yet encountered PPD or other recall Ags in vivo. Together these experiments are in keeping with the idea that Hu.hsp60-specific T cells are present in the repertoire of the putative naive subset, and absent from the repertoire of the putative memory subset.

Both CD45RA\(^-\)RO\(^+\) and CD45RA\(^+\)RO\(^-\) T cells respond to bacterial hsp60

As heat-shock proteins have highly conserved amino acid sequences, there are regions within bacterial hsp60 that are homologous to Hu.hsp60. When proliferative responses to M.hsp60 were investigated, both the CD45RA\(^-\)RO\(^+\) and CD45RA\(^+\)RO\(^-\) T cells responded (Fig. 4), unlike the findings with either KLH or TT. In
the experiment shown in Fig. 4, the response by CD45RA⁻RO⁺ T cell peaked on day 5, while the response by CD45RA⁻RO⁻ T cells peaked on day 8. In additional experiments, the precise days on which responses peaked varied: days 4 to 6 for CD45RA⁻RO⁺ T cells, and days 6 to 9 for CD45RA⁻RO⁻ T cells, but in all cases, in which cells from the same donor were assessed in parallel, the response by CD45RA⁻RO⁺ T cells always peaked before that of CD45RA⁺RO⁻ T cells. Thus, in contrast to their response to TT, healthy individuals appeared to have both memory and naive T cells capable of responding to M.hsp60 in vitro, as judged by both the responding T cell subsets and the kinetics of their responses. Similar results were found using GroEL, the E. coli hsp60 (data not shown).

Only CD45RA⁻RO⁺ cells proliferate to a previously defined epitope in M.hsp60

We and others have shown previously that a peptide consisting of amino acids 1–15 M.hsp60 (M1–15) contains an epitope that is recognized by M.hsp60-specific T cells in the context of HLA-DR3 (24–27). This peptide is able to bind to HLA-DR3 (shown by using a biotinylated peptide and detecting binding by flow cytometry) and is recognized by M.hsp60-specific T cell clones (data not shown). DR3⁺ individuals commonly mount strong responses to this epitope, and to date, no other DR3-restricted has been described; the sequence of the epitope is not conserved in Hu.hsp60. We examined responses to this peptide mounted by purified CD45RA⁻RO⁺ and CD45RA⁻RO⁻ T cells from a DR3⁺ individual. Fig. 6 shows proliferation by the CD45RA⁺RO⁻ T cells at day 5 both to intact M.hsp60 and to M1–15. In contrast, on day 8, the CD45RA⁻RO⁻ T cells proliferated strongly to M.hsp60, but only minimal responses to M1–15 were observed.

To rule out the possibility that a lack of a response to M1–15 by CD45RA⁻RO⁺ T cells was due to inadequate culture conditions for detecting proliferative responses to peptides by this subset, we again used cord blood as a source of CD45RA⁻RO⁻ T cells. Fig. 7A shows the results using cord blood from a HLA-DR3⁺ neonate. Clear proliferative responses to the peptide M1–15 were observed which peaked on day 7 when the same culture conditions (i.e., cell number and Ag concentration) were used as in those testing adult CD45RA⁻RO⁻ cells. The kinetics of this response was similar to that observed when adult CD45RA⁻RO⁻ T cells were cultured with the novel Ag KLH, and as previously shown, these cells, unlike adult CD45RA⁻RO⁻ cells, also responded to TT (Ref. 10).

Discussion

This work demonstrates that healthy individuals have the potential to mount T cell responses to human hsp60 in vitro, showing that the T cell repertoire post thymic selection includes potentially autoreactive T cells capable of recognizing self hsp60. Studies in transgenic mice have also indicated that a population of T cells able to recognize self hsp60 can be released to the periphery, even when hsp60 is overexpressed in the thymus (28). Nevertheless, the same experiments suggest that hsp60 can participate in negative selection in the thymus, since overexpression abolished recognition of an otherwise dominant epitope by peripheral T cells. Although there are previous reports of human T cell responses to Hu.hsp60, in most cases the studies were in patients with inflammatory conditions such as oligoarticular juvenile chronic arthritis, Behcet’s disease, recurrent oral ulcers, or salpingitis (29–33). Furthermore, in some of these studies, peptides were used, introducing the possibility of observing responses to cryptic epitopes, i.e., those that would not be generated by normal Ag processing in vivo.

The results of the present study also show that the subset of CD4⁺ T cells mainly responsible for the response to Hu.hsp60 is CD45RA⁻RO⁺. Similar results were described for responses to other self Ags, including phospholipid-binding protein in this study, and, in a previous study, the rhesus Ag expressed by human RBC (34). We have also previously shown that highly purified CD45RA⁻RO⁻ T cells respond well in vitro to novel foreign Ags, such as KLH, which the individual is unlikely to have previously encountered, but poorly to recall Ags such as TT (10). Conversely, CD45RA⁻RO⁺ T cells are the main responders to TT and show minimal responses to KLH. These results are consistent with the idea that expression of CD45RO is a marker of memory T cells and CD45RA a marker of naïve T cells. This concept has been challenged, mainly as a result of work in the rat, in which it has been possible to demonstrate that T cells can revert from expressing the low m.w. isoform of CD45 (identified as CD45RC⁺) to expressing high m.w. isoforms (CD45RC⁺) (35, 36). Evidence for
reversion in humans is controversial. Within CD4+ cells, only the CD45RO+ population showed the elevated expression of LFA-1, which is associated with primed cells (37). Contrariwise, studies of patients following radiotherapy indicate that the CD45RO+ population is relatively short-lived (38), a surprising property for the subset that is believed to contain T cell memory that can last many years. In addition, a recent paper claims that the CD45RA+ subset in humans also contains memory T cells, but that they are refractory to normal stimulation with Ag, requiring additional stimulation with similar kinetics.

If T cells responsive to Hu.hsp60 are found within the putative naive subset, does this imply that activation does not normally occur in vivo? Following activation in vitro, CD45RA+ cells switch CD45 isoform expression to CD45RA+ and CD45RA+RO- subsets, there was a reproducible difference in the response and kinetics of the two subsets, which is consistent with the notion that they represent memory and naive cells, respectively. If memory cells specific for TT resided in the CD45RA+ subset, they were either not stimulated by the conditions of our assay or present in very small numbers, since we did not record any responses to TT under conditions in which significant responses to other Ags (KLH, M.hsp60, Hu.hsp60) were readily demonstrated. It should be noted, however, that our studies have been confined to highly purified subsets, and we have not examined responses by the double-positive CD45RA+RO- T cells.

If T cells responsive to Hu.hsp60 are found within the putative naive subset, does this imply that activation does not normally occur in vivo? Following activation in vitro, CD45RA+RO- cells switch CD45 isoform expression to CD45RA+RO-, but it is clear that in vivo T cells recognizing Hu.hsp60 have not been permanently recruited to the CD45RA+RO- T cells subset; otherwise, we would have been able to detect responses in this subset. (It is formally possible that they are recruited to the CD45RA+RO- subset, but are undetectable, having become unresponsive to Ag, i.e., anergic). Therefore, either Hu.hsp60-specific T cells fail to be recruited to the CD45RA+RO- subset following activation, or are never activated in vivo, remaining in an immunologically ignorant state, no different from any other naive T cell population. Activation in vivo without recruitment to the CD45RA+RO- subset could also be a property of a regulatory subset, as has been postulated for Hu.hsp60-specific T cells (see below), and our experiments could not distinguish such cells from those that are truly naive.

To investigate these possibilities further, we studied responses to M.hsp60, a bacterial hsp60, representing a hybrid self/foreign Ag, containing both bacteria-specific epitopes, and epitopes conserved within Hu.hsp60. The subjects were previously immunized with M. bovis BCG 10–20 yr previously. For M.hsp60, responses were clearly demonstrated by both CD45RA+RO- and CD45RA+RO- T cells, in sharp contrast to the result obtained with the recall Ag TT. Similar results were obtained for two other bacterial hsp60 Ags, GroEL from E. coli and chlamydial hsp60. All individuals are exposed to E. coli as a gut commensal, and ~50% of adults have been infected with Chlamydia pneumoniae or Chlamydia trachomatis (mainly the former), organisms with highly conserved hsp60 Ags in which we have recently demonstrated an identical epitope (40).

Having demonstrated responses to bacterial hsp60 by both T cell subsets, we were able to make use of a previously mapped immunodominant epitope in M.hsp60 (24, 25) to show that these subsets were responding to different epitopes. Responses to the bacteria-specific epitope were readily demonstrated in the CD45RA+RO- subset, while they were absent from the CD45RA+RO- subset. Thus, in vitro processing of M.hsp60 allows presentation of epitopes that can be recognized by both subsets; although it is possible to postulate that processing will occur differently in vivo, it is difficult to envision a mechanism whereby bacteria-specific epitopes are presented, but those resembling self are not, simply because of differential processing. The inference must be that in vivo challenge with M.hsp60 should result in the activation of T cells recognizing both bacteria-specific and self-specific epitopes.

To gain further insight into T cell recognition of hsp60, we used cord blood both as a source of CD45RA+RO- cells, and of cells that are unlikely to have previously encountered Ag, i.e., a truly naive population (41). It lacks CD45RA+RO- cells, and a recent study demonstrated that the murine placenta was an efficient barrier against transfer of hepatitis B virus e Ag (42). With respect to Hu.hsp60 and novel Ags such as KLH, the responses of cord blood T cells and the adult CD45RA+RO- cells were similar, consistent with both being naive populations. The principal difference was that adult naive cells failed to recognize the recall Ag TT, or the bacteria-specific epitope in M.hsp60, whereas cord blood cells responded to both (Fig. 7). The apparent lack of responses by the adult naive subset to recall Ags and epitopes requires explanation. It might be supposed that a truly naive subset would be able to respond to any Ag, irrespective of its classification as recall or novel. We have explained previously our results by suggesting that immunization with TT in vivo results in the conversion of all of the CD45RA+RO- T cells capable of recognizing TT into CD45RO- expressing cells, thereby effectively purging the CD45RA+RO- T cell population of this specificity, and that persistent Ag allows purging to continue. The same argument can...
apply to M.hsp60; following immunization with BCG, mycobacterial Ags persist in vivo for long periods of time. M. bovis BCG establishes a low level persistent infection, and strong T cell responses to mycobacterial Ags are maintained indefinitely. This would allow continuous purging of the naive repertoire. These concepts receive support from work in the rat, demonstrating that reexpression of CD45RC by activated T cells only occurs in the absence of Ag; otherwise, the CD45RC⁻ phenotype is retained (43).

In this context, our observation of CD45RA⁻RO⁻ T cells that recognize the recall Ag M.hsp60 in the adult population is surprising. We suggest that the adult CD45RA⁻ T cell response to M.hsp60 is likely to be directed against epitopes other than those selected as targets by the memory T cell population, and that these are most probably epitopes that are also present in Hu.hsp60. Cloning studies are currently underway to address this point directly. If CD45RA⁻RO⁻ T cells are activated by recognition of conserved epitopes in M.hsp60 in vivo, why are they not recruited to the CD45RA⁻RO⁻ subset, as appears to occur in vitro? One possibility is that, following their initial activation by bacterial hsp60 presented by dendritic cells, later exposure to continuous and abundant expression of Hu.hsp60 by nonprofessional APCs could result in the induction of anergy, or even their deletion from the CD45RA⁻RO⁻ T cell subset. It might also be argued that whereas responses to conserved epitopes in Hu.hsp60 would be absent from normal individuals, they might be evident in patients with autoimmune conditions such as rheumatoid arthritis. However, against this hypothesis, we have noted lower responses to M.hsp60 in rheumatoid arthritis patients as compared with controls (J. Ramage and J. S. H. Gaston, manuscript submitted), and in rheumatoid arthritis synovial fluid T cells that are dominated by CD45RA⁻RO⁻ cells, responses to Hu.hsp60 were not evident (M. Lillicrap and H. Gaston, unpublished observations).

Is there any physiologic significance in the presence of a T cell subset that is able to recognize self hsp60? It is known that expression of Hu.hsp60 is up-regulated at sites of inflammation (44), and that these conditions also enhance Ag processing and presentation. In addition, self hsp60-reactive T cells have been demonstrated in both humans and rodents, although in most cases they have been observed in the context of immune responses to bacterial hsp60 (45–47). In the rat, a pathogen T cell model, this population of autoreactive self hsp60-specific T cells has been shown to be important in preventing the onset of arthritis after challenge with adjuvant (20). However, studies in rodents have not determined whether the self hsp60-specific cells are generated from cells expressing the naive phenotype. Likewise, it has not been determined whether, after either activation in vivo or following passive transfer, these effectors are recruited to the memory pool. Therefore, one possible role of human hsp60-specific cells might be the control of the inflammatory response to bacterial Ags through production of cytokines such as TGF-β, IL-4, and IL-10. If, as suggested by results in the rat adjuvant arthritis model, challenge with bacterial hsp60 results in concomitant activation of regulatory self hsp60-specific T cells, this could serve as an important general mechanism in the control of immune responses to bacterial pathogens. Defective function in such a mechanism could have clinical consequences, leading to the chronicity of inflammation that characterizes diseases such as rheumatoid arthritis and juvenile chronic arthritis. This idea receives some support from the recent correlation between favorable outcome and responses to Hu.hsp60 in juvenile chronic arthritis (48) and from our observations of relatively defective T cell responses to M.hsp60 in early rheumatoid arthritis. To assess the significance of these observations, further characterization of the T cell response to Hu.hsp60 in humans is required.

A separate body of work in the rat has shown that cells with the naive phenotype (CD45RC⁺) have the potential to induce autoimmune disease, including diabetes and thyroiditis, when transferred into athymic recipients (49). These effects are prevented by co-transfer of CD45RC⁻ cells (50, 51). Likewise, inflammatory bowel disease is observed in SCID mice when CD45RBhigh cells are transferred and the disease is ameliorated by CD45RBlow cells (52, 53). Hence, it may be that the self-reactive properties of cells within the naive subset, which may be regulated by appropriate responses to foreign Ags, may be capable of inducing autoimmune disease in the absence of reciprocal regulation by cells of the memory subset. Results of our continuing studies will address whether the Hu.hsp60-reactive T cells found within the naive subset in normal adults may function to induce autoimmunity or, as outlined above, regulate inflammation.

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