Annu. Rev. Immunol. 1991. 9:567–89

AUTOIMMUNITY TO CHAPERONINS IN THE PATHOGENESIS OF ARTHRITIS AND DIABETES

Irun R. Cohen

Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

KEY WORDS: autoimmunity, heat shock proteins, immune networks, adjuvant arthritis, diabetes

Abstract

The immunology of the 65 kd heat shock protein (hsp65) is paradoxical. Microbial and mammalian hsp65 molecules are 50% identical in amino acid sequence and immunologically cross-reactive, so microbial hsp65 looks like self; yet hsp65 is a dominant antigen in infection. Immunity to hsp65 can cause autoimmune diabetes in mice and may be related to autoimmune arthritis in rats and humans, so immunity to hsp65 should be forbidden; yet healthy persons manifest T-cell responses to self-hsp65. The aim of this chapter is to explore the immunological dominance of hsp65 and its role in autoimmunity—benign and pernicious.

INTRODUCTION: THE IMMUNOLOGICAL CHALLENGE

Chaperonins are molecules belonging to the family of stress or heat shock proteins (hsp's) that have a molecular mass of about 60 kd (1). The chaperonins have been referred to by various names; here we shall call them *hsp65 molecules* or simply *chaperonins*. They are called *hsp molecules* because historically they were noted to be expressed in increased amounts by cells responding to heat or other stresses (2). They are called *chaperonins* because of their function as molecular chaperones (1). The term chaperone originally denoted a person, usually an older maiden aunt, who was

0732–0582/91/0410–0567$02.00

attached by the family to young girls of marriageable age to escort, supervise, and protect them from undesirable liaisons. Similarly, molecular chaperones are thought to protect cells by escorting unfolded proteins and preventing their undesirable liaisons. For example, a hydrophobic domain, which in the properly folded protein is buried in the protein's interior, may become exposed during synthesis or denaturation of the protein. Once accessible, the domain might inadvertently glue the protein to unnatural partner molecules, thereby hindering function, disrupting cellular physiology, and poisoning the cell. Since stresses denature proteins, cells respond to stress by producing large amounts of chaperonins.

The evolution of human technology and social behavior has rendered the human chaperone obsolete, at least in some societies. Molecular chaperones, in contrast to human chaperones, have not yet been replaced in biological evolution; it appears that no cell can survive without them (1). The conservation of the molecular chaperones not only includes their functions but extends to their amino acid sequences (3). Indeed, it is this chemical conservation that has made the chaperonins so important to the immune system and so attractive to immunology.

Despite evolutionary divergence of over a billion years, the human and Mycobacterial hsp65 molecules are identical in about 50% of their amino acid residues (3). This chemical conservation includes many stretches of amino acid identity or near identity. Consequently every organism will necessarily share immunological cross-reactivity with the hsp65 molecules of any foreign cell, including the cells the organism eats (its food) and the cells that eat the organism (its parasites). The chaperonins of the host and the parasite, similar at some epitopes, also will differ immunologically at other epitopes. Hence, the hsp65 molecule of any infectious cellular agent will be part self and part nonself to any host with an immune system. The molecular chaperones of microbes embody the problem of self–nonself discrimination at the level of a single physiologically vital molecule.

The problem of discriminating between self and foreign is not theoretical; the confrontation between host and parasite and its attendant inflammation provide all the adjuvants and accessory signals required to stimulate a strong immune response to available antigens. Therefore, microbial chaperonins, at the interface between self and non-self, are nature's testing ground for self-tolerance. Learning how the immune response relates to the chaperonins, those both of host and of parasite, should furnish some useful insights into the clockwork and the logic of the immune system.

This chapter aims to describe the role of immunity to hsp65 in two autoimmune diseases: adjuvant arthritis (AA) of rats and insulin dependent diabetes mellitus (IDDM) of NOD mice. Attention will also be

directed to the paradox that, despite their potential for inducing autoimmune disease, hsp65 molecules are immunologically dominant antigens of microbes.

ONE HSP65 AND TWO AUTOIMMUNE DISEASES

Adjuvant Arthritis (AA)

AA is a type of arthritis inducible in certain strains of rats by immunization with dead Mycobacteria in oil, a concoction called complete Freund's adjuvant (4, 5). The disease appears as inflammation of the joints of the extremities, beginning 11–14 days after intracutaneous inoculation of about 1 mg of Mycobacteria, usually at the base of the tail. The acute inflammation peaks about 10–15 days after its onset and slowly regresses, often leaving the rat with deformities of the paws.

The disease is influenced by the mode of immunization, by factors intrinsic to the rat, and by elements in the environment (D. Markovits, N. Karin, F. Mor, I. R. Cohen, to be published). For example, intramuscular administration of the adjuvant, rather than producing arthritis, may induce a state of resistance to later attempts to induce AA by intracutaneous inoculation. If the Mycobacteria are emulsified in the oil of the adjuvant, the AA obtained is weaker than the AA obtained when the bacteria and oil are merely mixed without emulsification. Pulverized bacteria are more arthritogenic than are whole bacteria. Genes inside and outside the MHC are required to render a rat susceptible to AA (6). Gender is important; male rats suffer more severe and prolonged AA than do female rats. Rats maintained in clean animal quarters are much more sensitive to AA than are rats exposed to a heavily contaminated environment; indeed the disease may spontaneously remit in dirty rats but not in clean rats.

All this tells us that the expression of disease is the integral of many factors acting before, during, and after the contact between rat and Mycobacterial antigen. The presence of the antigen and the capacity to respond are required for the disease but are not sufficient to produce the disease. How can we analyze such complexity?

Over a century ago the founders of microbiology demonstrated that the complexities of pathogenesis of infectious diseases could be simplified by isolating in culture the etiologic agents (7). The specific microbe could then be used as a key to unlock the intricacies of the relationship between the host and parasite. Similarly, the past decade has seen the adaption of the pure-culture strategy to autoimmune diseases; the difference being that, rather than an invading microbe, the isolated etiologic agent of an autoimmune disease is an autoimmune lymphocyte, usually a T cell. Ben-Nun and his colleagues demonstrated that purified cultures of T cells reactive

to myelin basic protein were able to cause experimental autoimmune encephalomyelitis (EAE) after intravenous inoculation into naive recipient rats (8, 9). This observation laid the foundation for investigation of a wide range of questions about the pathophysiology of EAE, including the molecular relationship between T cell receptors and peptide epitopes (10, 11), the migration of T cells into the central nervous system (12) and the influence of specific T cells on nerve conduction (13). The stratagem of isolating T cells responsive to myelin basic protein is now being applied to clinical diseases like multiple sclerosis (14).

Beyond pathogenesis, the pure culture of virulent autoimmune T cells has made it possible to demonstrate the asymptomatic carrier state of autoimmunity: the persistence in the healthy body of potentially virulent T cells in a state of quiescence enforced by regulatory mechanisms (15). These mechanisms for controlling autoimmune T cells can be augmented by vaccination with the autoimmune T cells themselves (16); we return to this subject later on.

Holoshitz and his associates adopted the methods established in EAE to analyze AA. Our primary objective was to isolate arthritis-causing T cells and to use them as probes to detect important antigens. There seemed, however, to be a catch in this plan; how could we hope to isolate the arthritogenic T cells without using the specific antigen to select them. This problem was solved by allowing the arthritogenic T cells to select themselves and grow into a line by repeatedly incubating AA lymph node cells with pulverized whole Mycobacteria (17). The disease-producing T cells simply used their receptors to pick out from the undefined mixture the antigen they required. We now know that actively autoimmune T cells taken from a rat with AA enjoy a selective advantage in culture over naive resting T cells, even in the absence of specific antigen (18).

From the arthritogenic line an arthritogenic clone of T cells, clone A2b, was isolated (19). This clone was similar to the clones capable of causing EAE (9) or experimental autoimmune thyroiditis (EAT) (20) in having the helper phenotype: $CD4^+$ $CD8^-$. But unlike the pathogenic T cells in the other diseases, A2b could cause arthritis only in syngeneic rats that had been heavily irradiated (750 R).

Analyzing the responsiveness in vitro of clone A2b to various materials, van Eden and associates uncovered immunologic cross-reactivity between Mycobacteria and a protein moiety of the cartilage proteoglycan (21). Antigenic mimicry between Mycobacteria and an element in joint cartilage could well explain AA (22).

Attention was drawn to a chaperonin as a key antigen in AA when van Eden and associates used clone A2b to screen an expression library of Mycobacteria genes transfected into *E. coli*; A2b responded strongly and

specifically to hsp65 (23). Through genetic engineering and peptide synthesis, the A2b epitope was identified as the nine amino acids in positions 180–188 of Mycobacterial hsp65.

The association of AA with T-cell recognition of hsp65 motivated Res and his colleagues to investigate the T cells of patients with rheumatoid arthritis (RA) (24). They found that helper T cells from the joints of RA patients also recognized the Mycobacterial hsp65 molecule, thereby extending an earlier observation that T cells of RA patients responded to a Mycobacterial antigen (25). Thus, RA as well as AA seemed to involve T cells with specificity for a microbial chaperonin.

What, however, is the exact relationship between Mycobacterial hsp65, its 180–188 epitope, and the joints that could account for arthritis in rats and humans? This question is the subject of much current research and the definitive answer is not yet in. Nevertheless some information is available.

Although human hsp65 is similar in sequence to Mycobacterial hsp65, amino acids 180–188 are in a variable segment of the molecule and there is no epitope homologous to the 180–188 peptide in the known human hsp65 molecule (see 3). Thus, mimicry between the 180–188 epitope and a molecule in the joints could occur in either of two circumstances: An isotype of the hsp65 molecule not yet identified does carry this epitope, or the 180–188 epitope is present in a non-hsp65 molecule. Indeed, four of the nine amino acids of the 180–188 sequence are present in the cartilage link protein of the rat (23); such similarity could explain the earlier finding that A2b recognized a part of the cartilage proteoglycan (26). However, A2b did not appear to respond to a peptide comprising the homologous link protein sequence (D. Markovits, I. R. Cohen, in preparation). This means that we do not yet know what A2b sees in the joints when it produces arthritis in irradiated rats. Perhaps irradiation of the recipient rats (17, 19) is needed to induce enhanced expression of some stress protein (27) that does bear the 180–188 epitope.

The responses of human RA patients to hsp65 are even less clear. It has been reported that the T cells of RA patients respond to a Mycobacterial antigen but that the antigen is not the hsp65 molecule (18). Others confirm the RA response to hsp65 (29, 30). The isolated T cells may respond either to mycobacteria-specific epitopes (30) or to human epitopes (A. Lohse, personal communication). In contrast to adult RA patients, children suffering from Juvenile RA (JRA) have T cells that recognize the 180–188 peptide and the partially homologous peptide of the cartilage link protein, as well as the hsp65 molecule (31). Do the differing T cell responses of JRA and adult RA patients stem from immunologically different etiologies, or are the immunological differences merely the secondary expressions of age or chronicity?

Another puzzle is the observation that reactivity to hsp65 may also be present in inflammatory infiltrates outside of the joints such as pleural effusions (R. R. P. de Vries, personal communication). Finally, it appears that Mycobacterial antigens, including hsp65, may preferentially be recognized by γ/δ T cells (32–34). The particular function of this class of T cells is unknown (35). Therefore the involvement of γ/δ T cells in the hsp65 story is curious but not yet very enlightening.

Particularly perplexing is the effect of the hsp65 molecule itself on AA. Because hsp65 is recognized by T cells capable of causing arthritis, one might suppose that active immunization with hsp65 would induce AA. On the contrary, administration of hsp65 in incomplete Freund's adjuvant (oil) was found to induce not arthritis but resistance to arthritis (23, 26). This finding is not compatible with the notion that AA is caused by an immune response to hsp65, a notion derived from the evidence that arthritogenic T cells recognize hsp65. Nevertheless, if hsp65 can protect from AA, hsp65 must surely be involved in some way in the pathogenesis of AA. The rub is that administration of hsp65 seems to protect against arthritis induced by streptococcal cell walls (37), a model disease not thought to be related to AA.

Additional observations appear to undermine the notion that immunity to hsp65 has an exclusive or causal relationship to AA: AA can be induced by defined adjuvants free of hsp65 (38, 39); AA can be prevented by the induction of tolerance to collagens (40); and the severity of arthritis induced by collagen or by a synthetic adjuvant can be reduced to some degree by administering hsp65 (36).

IDDM

As in the case of AA, there was no reason to suspect before the experiments were done that immunity to hsp65 might be involved in diabetes. In fact, having already discovered the association of hsp65 with arthritis, it seemed improbable to us that the same hsp65 molecule might be involved in a second autoimmune disease, especially a disease as distinct clinically from arthritis as is diabetes. Nevertheless, Dana Elias and her colleagues have found that immunity to hsp65 fulfills most of the criteria required logically to implicate an antigen as a target in an autoimmune disease (41).

Unlike AA which is artificially induced, the IDDM of NOD mice develops spontaneously (42). Female mice have a higher incidence of diabetes than do male mice; about 80% compared to about 40% in our colony. The disease begins at the age of 4–6 weeks as a mononuclear cell infiltrate, an insulitis, evident first around the periphery of the islets and later penetrating and disrupting them. The insulitis causes the gradual and

cumulative destruction of insulin-producing beta cells. Finally at about 4–6 months of age the number of beta cells remaining in the affected mice is not sufficient to supply the amount of insulin required for glucose homeostasis. The hour of beta cell decompensation is the hour at which IDDM becomes clinically overt. Note that the hyperglycemia and glycosuria marking IDDM are not the disease but the result of the disease; the disease is the autoimmune process that destroys the beta cells (43).

The etiologic agents of the IDDM of NOD mice appear to be autoimmune T cells that attack the beta cells; such cells have been shown to cause early diabetes in young, prediabetic recipient mice (44). What target self-antigen attracts the diabetogenic T cells to the islets? The results of the experiment described below indicate that the mouse's own hsp65 molecule can serve the purpose.

ANTI-HSP65 AUTOIMMUNITY PRECEDES IDDM About two months before the outbreak of overt IDDM, there appears in the blood of NOD mice material that specifically binds antibodies to hsp65 (43). We termed this material hsp65 cross-reactive antigen. It seems likely that the hsp65 cross-reactive antigen is produced as a result of the stress of insulitis, possibly by the islet cells being destroyed: NOD mice that did not develop IDDM did not manifest the hsp65 cross-reactive antigen in their blood.

About two weeks after the appearance of the hsp65 antigen the mice spontaneously developed antibodies to Mycobacterial hsp65 (43). We now know that the hsp65 antibodies are preceded by T cells specifically reactive to mammalian hsp65; the reactivity to the Mycobacterial hsp65 molecule is only about 20% of that to the human hsp65 molecule (45). It seems that the T-cell reactivity to hsp65 is coincident with the appearance in the blood of the hsp65 cross-reactive antigen. These observations indicated that the process of islet cell destruction was associated with the expression of hsp65, which was not surprising, and that there was an autoimmune response to the hsp65, which was surprising. Was the autoimmune response merely the result of developing diabetes, or could it be a cause?

ANTI-HSP65 T CELLS ARE DIABETOGENIC From prediabetic NOD mice suffering from advanced insulitis we isolated clones of T cells of the $CD4^+$ $CD8^-$ phenotype spontaneously reactive to the human hsp65 molecule. We tested the virulence of these T cells by inoculating them into one-month-old prediabetic recipient mice; within 1 week of receiving 5–10×10^6 T cells the mice developed severe insulitis and hyperglycemia (43, 45). The implication was clear: anti-hsp65 T cells might really operate in the destructive process.

It was conceivable that the anti-hsp65 T cells merely accelerated the destruction of NOD beta cells which might abnormally express endogen-

ous hsp65 as a consequence of stress; NOD mice are genetically programmed to stress their beta cells (42). To learn whether healthy beta cells could also be destroyed by anti-hsp65 T cells, we used as recipients mice of the NON.H-2^{NOD} strain. Similar to their NON (non-obese normal) parent strain (42), these H-2 congenic mice do not develop IDDM spontaneously. Consequently their beta cells should not be in a state of stress, despite the fact that the I-A allelle of the NOD strain has been bred into them. Thus NON.H-2^{NOD} mice can serve as H-2 compatible, nondiabetic recipients of NOD T cells.

The results of the experiment were clear; a clone of NOD T cells specifically reactive to human hsp65, designated C9, caused diabetes in the NON.H-2^{NOD} mice within 1 week of inoculation. So the ability of anti-hsp65 T cells to cause diabetes is not limited to mice with manifestly stressed islets (45).

ACTIVE IMMUNIZATION TO HSP65 INDUCES DIABETES If diabetes is actually caused by a T-cell response to hsp65, then immunization to hsp65 might activate such T cells in vivo to accelerate the process of destruction of beta cells. This expectation was fulfilled: immunization of 1-month-old NOD mice with hsp65 in oil induced hyperglycemia and marked insulitis well before the diabetes would have spontaneously appeared in the mice (43). Moreover, immunization of NON.H-2^{NOD} mice with hsp65 in oil also induced diabetes (45). Since NON.H-2^{NOD} do not develop diabetes spontaneously, we can conclude that active immunity to hsp65 may cause diabetes in nondiabetic mice as well as hasten the onset of diabetes in genetically prone mice.

INHIBITION OF ANTI-HSP65 IMMUNITY PREVENTS DIABETES The above results suggest that immunity to hsp65 in mice of the NOD and NON.H-2^{NOD} strains is sufficient to produce diabetes. But is immunity to hsp65 necessary for NOD diabetes? This question might be answered by specifically inhibiting the immune response to hsp65 and observing whether the development of diabetes was also inhibited.

One way of specifically inhibiting autoimmune responses is by T-cell vaccination: the use of attenuated autoimmune T cells to induce anti-idiotypic T cells to downregulate the autoimmune response (16, 46, 47). We therefore vaccinated one-month-old NOD mice with $5–10 \times 10^6$ anti-hsp65 T cells attenuated by gamma irradiation (3000 R). The T-cell vaccines were composed of anti-hsp65 T-cell blasts isolated from 3-month-old female NOD mice or of T cells of the C9 clone. Control T-cell vaccines were made of T cells reactive to recombinant hsp70 (a 70-kd Mycobacterial hsp) or to bovine serum albumin.

We found that treatment with the anti-hsp65 T cells led to resistance of

NOD mice to three types of diabetes: that developing spontaneously, that produced by transfer of virulent clone C9 T cells, and that induced by active immunization to hsp65 in oil (45). NOD mice vaccinated with control T cells remained susceptible to all three types of diabetes.

Inhibition of diabetes following specific T-cell vaccination was preceded by a marked reduction in the spontaneous anti-hsp65 reactivity of T cells and of antibodies in the mice. The mice treated with control T cells showed no reduction in their immunity to hsp65, spontaneous or induced. In other words, impairment of hsp65 immunity blocked diabetes. Thus, immunity to hsp65 seems to be necessary as well as sufficient for the development of diabetes in NOD mice.

AN HSP65 TARGET EPITOPE Recently we have used diabetogenic T-cell clones such as C9 to map a key epitope on the hsp65 molecule. Assaying the responses of C9 to various peptides synthesized according to the sequence of human hsp65, we hit upon a peptide of 24 amino acids we call p277 (45). Peptide p277, but not an adjacent peptide, was also found to stimulate the splenic T cells of NOD mice developing diabetes. Peptide p277 must contain a key epitope; T cells recognizing p277 can either produce diabetes or can be used to vaccinate mice successfully against diabetes. Affirming its importance is the finding that p277 by itself may be used to prevent diabetes. A single inoculation of 50 μg of p277 in oil into the peritoneum of NOD mice endowed the mice with resistance to diabetes developing spontaneously or to diabetes produced by clone C9 T cells (45). An adjacent peptide, had no effect. The regulatory mechanisms activated by peptide p277 are being studied, but whatever they turn out to be, the effectiveness of peptide treatment of diabetogenic autoimmunity is promising.

We have cloned the mouse hsp65 molecule from a beta cell tumor and its sequence differs from the human sequence by one amino acid among the 24 (O. S. Birk, A. Rosen, A. Weiss, I. R. Cohen, M. D. Walker, in preparation). Thus, the T-cell response of NOD mice to p277 is probably autoimmune.

Comparison of Anti-hsp65 Immunity in AA and in Diabetes

AA in Lewis rats and diabetes in NOD mice are both associated with T-cell immunity to hsp65, but each disease is related to a different epitope. Each disease also suffers from a different fault in the logic of its connection to hsp65 immunity. AA has not been inducible by immunization to hsp65; rather, administration of hsp65 only prevents arthritis (23, 36). Thus, hsp65 immunity may be necessary for the expression of arthritis, but there is no evidence yet that it is sufficient. Despite this paradox, cross-reactivity between hsp65 and cartilage, as defined by clone A2b, explains how the

joints might be injured by T cells activated by a particular hsp65 epitope (21, 22, 26).

Although the evidence for causality is more complete in mouse diabetes than in rat arthritis, the case for diabetes lacks the connection between the immune response and destruction of the target tissue: why should autoimmunity to 24 amino acids in the sequence of human hsp65 lead T cells to destroy beta-cells? What is the necessary connection between hsp65, expressible in any cell, and beta cells? Could the p277 epitope also be present on a non-hsp65 molecule that is specifically expressed on beta cells, a molecule that serves as the true target? Genes of the hsp family, probably because of their great antiquity, have been reduplicated and donated to construct molecules for purposes unrelated to stress. For example the lens crystalline of the eye is encoded by hsp genes (48). Perhaps the p277 epitope has been donated by hsp65 to a tissue-specific beta cell molecule.

HEALTHY IMMUNITY TO HSP65

Immunity to hsp65 is associated with autoimmune disease, but it is also associated with health.

Munk and colleagues observed that the peripheral blood T cells of 8 of 9 normal persons recognized self-epitopes of hsp65 after stimulation of the T cells with killed Mycobacteria. In other words, at least part of the T cells responding to a microbe were directed against hsp65 epitopes shared by the responding human with the microbe (49). Koga and his associates found that mouse T cells stimulated by Mycobacterial hsp65 were cytotoxic to the mouse's own macrophages, provided that the macrophages had been stimulated by signals usually accompanying an immune response such as gamma-interferon (50). Thus, physiological stress induces expression of self-hsp65 epitopes, and these epitopes are recognizable by the mouse's own killer T cells. Note, these examples of autoimmune recognition are natural; they are not related to autoimmune disease.

Chaperonins Are Dominant Microbial Agents

Immunologically dominant antigens are those antigens to which immune systems predictably respond in the face of competing antigens. Immunological dominance is another way of saying that the immune system focuses its response habitually on a few antigens out of a large number that may be available. To extract signal from noise, an immune system (for that matter, a nervous system too) must be able to ignore many competing potential stimuli and pay attention to only a few (51). Without focus, any system processing information would be hopelessly jammed.

Chaperonins fulfill the definition of dominant antigens because infection

or immunization with many different bacteria induces antibodies and T cells specific for hsp65 molecules. Witness the agents of tuberculosis (52, 53), leprosy (53), syphilis (54), Q fever (55), Legionnaires disease (56), Lyme disease (57), and trachoma (58). Indeed the hsp65 molecule has been called the common bacterial antigen (59, 60). The responses to hsp65 are great: One in five T cells of mice immunized to *M. tuberculosis* organisms responds specifically to hsp65 (61).

The Riddle of hsp-65: Autoimmunity, Microbial Immunity, and Dominance

The hsp65 molecule came to the attention of immunologists only a few years ago, yet most of what we are finding out about its immunology contradicts the expectations of traditional thinking. True, a self-like microbial molecule should be a reasonable inducer of autoimmunity (26); but why two diseases and why are they organ-specific?

IDDM of mice and AA of rats prove that immune responses to microbial hsp65 are costly; the penalty for such immunity can be a progressive autoimmune disease. To the extent that a phenotype detracts from fitness, that phenotype will be lost during evolution. Since autoimmune disease reduces fitness one would expect immunity to microbial hsp65 to be down-regulated, if not forbidden entirely.

Why then do healthy individuals respond to hsp65 naturally (49, 50)? How are we to understand the paradox of natural hsp65 autoimmunity in health and noxious hsp65 autoimmunity in disease. Why is microbial hsp65 immunologically dominant?

A few firm facts tolerate a multitude of hypotheses, but the following pair of ideas may be a reasonable way to begin thinking about these questions: The first idea is that the hsp65 molecule is immunologically dominant because of the special way in which it is expressed and processed. The second idea is that antiidiotypic networks determine whether immunity to hsp65 remains benign or progresses to autoimmune disease. MHC alleles take part by selecting the hsp65 epitope and hence the clinical expression of the disease.

DOMINANCE OF HSP65 AND ANTIGEN PROCESSING T cells are restricted to recognizing antigens in the form of processed peptides clasped by MHC molecules (62). Therefore to be immunogenic, hsp65—or any other molecule recognized by T cells—must be processed by antigen-presenting cells to yield peptides readily accommodated in the MHC cleft. The peptides most amenable to processing and MHC-packaging should therefore have a competitive edge in attracting T cells. Could this mechanism account for the immunological dominance of hsp65?

Not enough is known about the processing of hsp65 to allow a definitive answer. Nevertheless it would seem reasonable to expect that hsp65 might enjoy an advantage in processing, certainly during microbial infection. In the stress of inflammation (63) and phagocytosis, both host hsp65 (50) and microbial hsp65 (64) are expressed in increased amounts by activated macrophages, cells that specialize in presenting peptide antigens.

An important physiological function of hsp65 is to interact with unfolded peptides (1). Therefore, it is conceivable that hsp65 takes part in the processing of antigens in general (65). Perhaps involvement in the processing mechanism also enhances the processibility of hsp65 relative to competing antigens.

DOMINANCE OF HSP65 BOOSTED BY INFECTION Any advantage of hsp65 in processing would be amplified by repeated contact with microbes; immunological memory of hsp65 is boosted with each microbial contact, subclinical as well as clinical. Because of the marked conservation of hsp65 among all cells, contact with different bacteria can reinforce immunity to the same shared epitopes.

One might argue that different bacteria also share cross-reactive epitopes on molecules that are not hsp65 and not self-antigens to the responding host. The immune response directed to common microbial antigens that are safely foreign would be free of autoimmune hazards. However, consider the possibility that a high frequency of memory T cells directed to self-epitopes of hsp65 as well as to microbial hsp65 epitopes might be advantageous.

Successful resistance to an infectious agent should be promoted by an early arrival of activated T cells irrespective of their specificity. Activated T cells can serve to open up access to the tissues, activate macrophages and other effector cells responsive to lymphokines, stimulate antiviral effects, and amplify inflammation in general. To carry out this job, the T cells need not actually recognize the specific invader; they need only recognize the invasion.

The expression of host hsp65 is a reliable sign of trouble in general (63). Therefore, host hsp65 can serve as an alarm signaling the recruitment of anti-hsp65 memory T cells to areas of tissue damage (66). These T cells can quickly become activated at the site and augment the processes needed to fight the infection. Thus, the immune response need not remain idle waiting for the few microbe-specific but naive T cells to proliferate and differentiate into effector cells. Autoimmune hsp65 memory T cells could serve as a system of early warning and early response in the service of microbial immunity. These T cells are demonstrated in healthy humans and mice; what better purpose could they serve than help fight infection?

REGULATION OF HSP65 AUTOIMMUNITY

Granted there are good reasons for the dominance of hsp65, how is autoimmune disease avoided if anti-hsp65 T cells become activated? Note that autoimmune lymphocytes are necessary for an autoimmune disease to develop, but their mere presence does not make a disease inevitable. Disease results from the differentiation of the autoimmune lymphocytes into effector cells and, more critically, from the perpetuation of the effector response or its repeated exacerbation. In other words, the course of the autoimmune response determines the nature and the degree of tissue damage; tissue damage is the disease. An autoimmune response that is regulated so as to be limited in severity and time will not produce clinical disease. Regulation of the autoimmune lymphocytes present in us all is decisive.

How is this regulation carried out?

Effects of MHC

The products of MHC genes could regulate autoimmune disease in two ways: by molding the T-cell-receptor repertoire through positive and negative selection of T cells differentiating within the thymus (67) and by favoring the presentation to T cells of particular peptide epitopes after processing of an antigen (62).

The autoimmune diseases we are examining here result from the activation of anti-hsp65 T cells that exist outside of the thymus. Regulation of these peripheral autoimmune T cells therefore cannot be easily explained by a process of selection within the thymus. Peripheral regulation must depend on peripheral regulatory mechanisms. This does not mean to imply that thymic selection is entirely irrelevant to autoimmune disease; it only restates the truism that thymic selection is irrelevant once a T cell has left the thymus. Since the autoimmune anti-hsp65 T cells detected in healthy individuals have already left the thymus, any mechanism that could control the behavior of these cells must also be post-thymic. I suspect that most common varieties of autoimmune disease are subject to peripheral regulation; the autoimmune diseases that directly result from abnormal thymic selection are probably rare.

Epitope selection at the time of presentation of hsp65 is likely to be the more important contribution of the MHC to regulation of autoimmune disease (68). For example MHC allelic products that allow the presentation of the p277 epitope (45) would render a mouse (perhaps a human too) susceptible to IDDM rather than to arthritis, while MHC alleles that allow the presentation of the 180–188 epitope (23) would favor arthritis instead. MHC alleles that exclude both epitopes would make both diseases unlikely.

However, MHC molecules cannot account for two other important

features of immunity to hsp65: the dominance of the hsp65 molecule as a whole and the fact that the numbers of individuals bearing the alleles encoding susceptibility for a disease but who never get the disease are much greater than the numbers of individuals who actually develop the disease. For example, the MHC alleles that encode for susceptibility to IDDM, a disease that affects 1% or less of the population, can be carried by large numbers of healthy people (42). Even in NOD mice, the males who resist diabetes have the same MHC genes as the females who get diabetes. To state it in another way, an MHC molecule that excludes the key epitope will tell us that the person who bears it is unlikely to develop a particular disease (43). In contrast, having an MHC molecule that can present the key epitope does not make the disease inevitable, it does not even make having the disease very likely. The MHC is a better predictor of who will not get a disease than of who will get a disease.

Regulation by T-Cell Networks

The results of studying the regulation of AA and NOD diabetes lead to the conclusion that T cell antiidiotypic networks are regulators of disease; networks govern the transition from benign hsp65 autoimmunity to pernicious autoimmune disease (16, 69, 70).

This conclusion is based on the following general observations:

1. Antiidiotypic T cells specific for anti-hsp65 T cells exist naturally,
2. The development of disease is inversely correlated with the level of activity of the antiidiotypic T cells, and
3. Deliberate activation of antiidiotypic T cells can prevent or abort the development of disease.

The detailed results have not yet been published; I briefly describe them here.

Antiidiotypic Network in AA

To detect T-cell networks in AA, Nathan Karin and his colleagues in this laboratory investigated the development of antiidiotypic T-cell immunity to T-cell clones A2b and M1. The M1 clone is a newly isolated T cell that recognizes a part of the hsp65 sequence other than the 180–188 peptide recognized by clone A2b. M1 does not recognize human hsp65; the 10-amino-acid peptide it sees is in a variable part of the hsp65 molecule. However, this hsp65 epitope, which we call the M1 epitope, does have strong sequence homology with a peptide present in a mammalian acute phase protein; it mimics self, although not self-hsp65.

Lewis rats were found to be primed for a T-cell response to the M1 clonotype: Within 3 or 4 days after immunization with Mycobacteria, the lymph node cells of the responding rats demonstrated a strong T cell–

proliferative response to clone M1 (N. Karin, D. Markovits, I. R. Cohen, in preparation). There was no detectable response to the anti-hsp65 clone A2b or to syngeneic helper T cell clones that recognize myelin basic protein. Thus the response to M1 could be defined as antiidiotypic. As one would expect of a preformed network, the antiidiotypic response to clone M1 preceded the response of the idiotypic T cells to the hsp65 antigen.

The magnitude of the antiidiotypic response to M1 seems to be inversely related to the severity of the arthritis developing two weeks later; the most severe arthritis was found to be preceded by a low response to clone M1 and mild arthritis was preceded by a high anti-M1 response.

Lewis rats kept in unsanitary quarters demonstrate a much higher level of anti-M1 activity than do Lewis rats maintained in gnotobiotic conditions (N. Karin, D. Markovits, I. R. Cohen, in preparation). The conventional Lewis rats, with high anti-M1 activity, develop AA that is much milder and shorter-lived than is the AA developing in the clean Lewis rats, who have low anti-M1 activity. Thus, the magnitude of naturally expressed anti-M1 T-cell activity appears to influence the clinical expression of AA. The differences between the anti-M1 networks of the clean and dirty rats imply that the antiidiotypic network controlling AA may be acquired through immunologic experience with bacteria in the environment. Factors that establish immunological dominance may also establish immunological regulation.

The importance of T-cell antiidiotypic networks to regulation is affirmed by premediated activation of such networks by T-cell vaccination; the use of autoimmune T cells as vaccines to activate specific T-cell antiidiotypic immunity (16, 69–72). We have previously demonstrated that clone A2b could vaccinate rats against AA (73). Clone M1 also was found to be an effective vaccine; rats treated with M1 T cells developed augmented anti-M1 activity and resisted the induction of AA.

As expected, the vaccinated disease-resistant rats showed decreased T-cell responses to hsp65 at the time the control rats had clinical AA. However, when we studied the kinetics of T-cell immunity to hsp65, we were surprised to discover that the low response to hsp65 associated with resistance to AA was actually preceded by an accelerated response to hsp65 (N. Karin, I. R. Cohen, in preparation; 47). The low response to hsp65 we originally detected at a given moment in time was in effect the trough following an early peak of activity. In other words, T-cell vaccination, which induces heightened antiidiotypic activity and resistance to clinical AA, leads to a change in the kinetics of the response to hsp65 and not to general suppression of the response itself.

How can an antiidiotypic network both transiently enhance reactivity to hsp65 and downregulate disease? We have no specific evidence yet from AA or IDDM to answer this question. However, an earlier investigation

of the T-cell network activated by T-cell vaccination against EAE may provide an explanation. Ofer Lider and his associates isolated clones of antiidiotypic T cells from the lymph nodes of Lewis rats that had been vaccinated against EAE using autoimmune T cells recognizing myelin basic protein (72, 74). The antiidiotypic T cells were found to be of two types: $CD4^+$ $CD8^-$ helpers and $CD4^-$ $CD8^+$ suppressors. We studied the effects of these antiidiotypic clones on the autoimmune T cells reacting to myelin basic protein. The $CD4^-$ $CD8^+$ T cells suppressed the response to the self-antigen in vitro. Sun and coworkers have demonstrated that such antiidiotypic suppressors can prevent EAE in vivo too (75). In contrast to the suppressor T cells, the $CD4^+$ $CD8^-$ helper T cells amplified the autoimmune response. In fact, the $CD4^+$ $CD8^-$ antiidiotypic T cells could stimulate antibasic protein T cells even in the absence of basic protein (72).

If the hsp65 antiidiotypic network was also to be found to contain helper and suppressor T cells, one would suppose that the accelerated response to hsp65 might be the work of the antiidiotypic helpers; the helpers would thus contribute to the immunological dominance of hsp65. The rapid decay of the response to hsp65 in turn might be attributed to the antiidiotypic suppressor cells. Regulation and dominance are a wedded pair (69, 70).

Antiidiotypic Network in IDDM

Investigation of the regulation of diabetes in NOD mice has also demonstrated the existence of T cells reactive to the C9 anti-hsp65 clone (D. Elias, T. Reshef, I. R. Cohen, in preparation). As NOD diabetes develops spontaneously, the antiidiotypic T cells in this model appeared even without experimental immunization to hsp65. Similar to AA, the nature of the antiidiotypic network influences the expression of IDDM. For example, we found that the development of diabetes in female NOD mice is preceded by a fall in the level of antiidiotypic T-cell activity to the C9 clone (D. Elias, T. Reshef, I. R. Cohen, in preparation). Male mice that escape diabetes maintain a persistently high degree of anti-C9 T cell activity. In other words, persisting natural antiidiotypic activity is associated with escape from diabetes. Indeed, T-cell vaccination with anti-hsp65 T cells boosts the anti-C9 antiidiotypic T-cell response and aborts the development of diabetes in female mice (45).

PATHOPHYSIOLOGY OF ANTI-HSP65 AUTOIMMUNITY

The pieces of the hsp65 puzzle may be put together to form the following picture (see Figure 1).

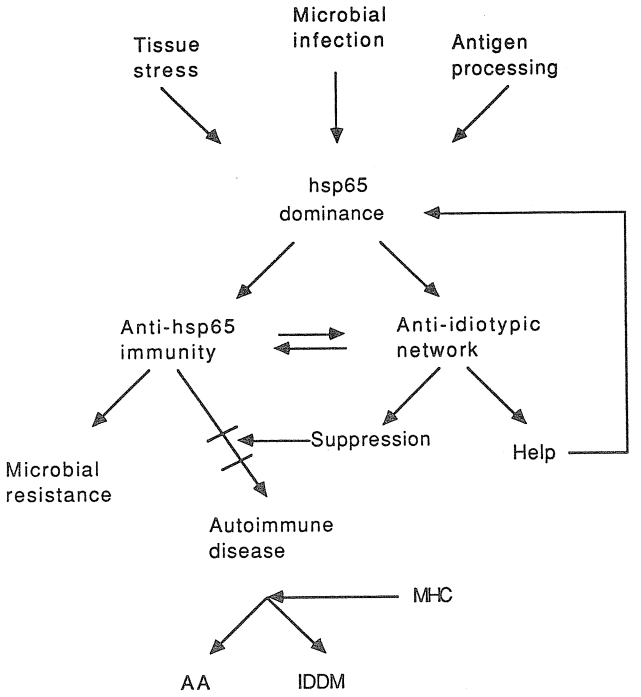

Figure 1 A schematic representation of the physiology of hsp65 immunity.

The hsp65 molecule is immunologically dominant because of its repeated expression in the immunogenic context of inflammation and its likely participation in the machinery of antigen-processing.

These factors expand the numbers of anti-hsp65 memory T cells and lead to the formation of an antiidiotypic network of regulatory T cells. The helpers in the network contribute to the immunological dominance of the hsp65 and stabilize the network (69).

The dominance of hsp65 promotes resistance to microbial infection and repair of damaged tissue by mobilizing an anti-hsp65 memory-T-cell brigade to the site of action. Hsp65 may chaperone polypeptides, but anti-hsp65 can chaperone infections.

The suppressor cells in the hsp65 antiidiotypic network dampen the anti-hsp65 response and shut it off entirely when it is no longer needed. Regulation of the anti-hsp65 response prevents its progression into autoimmune disease.

Autoimmune disease results from weakness of the suppressor arm of

the network (69). The weakness can result from a variety of seemingly nonspecific factors, such as the style of life (compare AA in clean and dirty rats), sex differences (compare male and female NOD mice), the nature of contact with inciting antigens (compare subcutaneous with intramuscular injection of Mycobacteria in AA), and so forth. Autoimmune T cells are the etiologic agents of AA or IDDM; but the actual inducers of disease are the circumstances that unleash the T cells from network restraints. Various stresses—infectious, toxic, or even traumatic—can enhance the expression of hsp65 or other self-antigens along with class-II MHC molecules (76). However, it is doubtful that these stresses by themselves will spark off the progressively destructive autoimmune process we record as clinical disease. The multitude of factors—genetic, environmental, and mechanical—required for successful induction of AA hint at the coincidences involved in precipitating an autoimmune disease in a human. The antigen can trigger the disease, but the antigen can also raise the level of resistance to the disease. Administration of hsp65 in oil protects Lewis rats from AA (23) while it induces diabetes in NOD mice (41); whole mycobacterium in oil induces AA in Lewis rats (4) while it protects NOD mice from diabetes (77).

The MHC can influence the expression of disease by focusing the T-cell response to hsp65 epitopes associated with beta cells (diabetes), with joint cartilage (AA), or with no self-organ (no defined disease). However, studies of NOD mice bearing MHC transgenes suggest that the role of MHC gene products in IDDM is probably much more complicated (78). Some of this complexity might become simplified as we measure the effects of MHC molecules on specific immune responses to the p277 epitope (45) and to other beta cell target antigens (79).

EPILOGUE: GENERAL PRINCIPLES

Do the lessons of hsp65 immunity apply to autoimmunity and microbial immunity in general, or is hsp65 unique; is it an exception or a rule? Elsewhere, Douglas Young and I review evidence suggesting that the case of hsp65 is not unique (80). The papers cited are many, but the main points can be summarized briefly:

First, many immunologically dominant microbial antigens are conserved, self-like molecules. These include the other hsp types, essential enzymes, and even structural proteins such as myosin.

Secondly, many self-antigens associated with autoimmune diseases are immunologically dominant: Individuals suffering from the same autoimmune diseases tend to have very similar immune reactivities to common

self-antigens. Autoimmune disease may have an element of randomness in whom it visits, but its immunological expression is highly structured. Persons with lupus have predictable autoantibodies as do persons with myasthenia gravis or autoimmune hepatitis (see 81). If autoimmunity were truly random, would not each patient have their own private disease? But this is not so: autoimmunity is ordered.

Thirdly, healthy persons also harbor autoantibodies and autoreactive T cells to the dominant self-antigens.

Fourthly, immunity to many dominant self-antigens is controlled by antiidiotypic networks. These networks establish both dominance and regulation. The finding that all Lewis rats have anti-M1 and all NOD mice have anti-C9 antiidiotypes suggests that the regular structure of autoimmunity includes a few dominant T-cell idiotypes interacting with regulatory T-cell antiidiotypes. Dominant idiotypes may be an expression of the restricted usage of T cell receptor genes that has been detected in some autoimmune responses (11).

Borrowing a term used in neurology, I have referred to the collection of autoimmune lymphocyte networks as the immunological homunculus (70). Just as vital neurological information about the self is organized by neural networks, so is vital immunological information about the self organized by lymphocyte networks. The immunological homunculus is limited to the set of dominant self-antigens, some few dozen. How the immunological homunculus works for other self-antigens is illustrated by what it does for immunity to hsp65.

ACKNOWLEDGMENTS

The work reviewed here was supported by grants from the National Institutes of Health (USA), the Juvenile Diabetes Foundation International, the Minerva Foundation, Institut Merieux, and Mr. Rowland Schaeffer. Mrs. Doris Ohayon organized the manuscript with help from Mrs. Malvine Baer. Many of the ideas written here developed through help and suppression provided by a network of friends.

Literature Cited

1. Ellis, R. J. 1990. The molecular chaperone concept. *Semin. Cell Biol.* 1: 1–9
2. Lindquist, S., Craig, E. A. 1988. The heat-shock proteins. *Annu. Rev. Genet.* 22: 631–37
3. Jindal, S., Dudani, A. K., Harley, C. B., Singh, B., Gupta, R. S. 1989. Primary structure of a human mitochondrial protein homologous to bacterial and plant chaperonins and to hsp65 K D mycobacterial antigen. *Mol. Cell. Biol.* 9: 2279–83
4. Pearson, C. M. 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc. Soc. Exp. Biol. Med.* 91: 95–101
5. Pearson, C. M. 1964. Experimental models in rheumatoid disease. *Arthritis Rheum.* 7: 80–86
6. Battisto, J. R., Smith, R. N., Bechman,

K., Sternlight, M., Wells, W. L. 1982. Susceptibility to adjuvant arthritis in DA and F344 rats. A dominant trait controlled by an autosomal gene locus linked to the major histocompatibility complex. *Arthritis Rheum.* 25: 1194–1200
7. Rosenberg, E., Cohen, I. R. 1983. *Microbial Biology*. Philadelphia: Saunders
8. Ben-Nun, A., Wekerle, H., Cohen, I. R. 1981. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol.* 11: 195–99
9. Ben-Nun, A., Cohen, I. R. 1982. Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines. Process of selection of lines and characterization of the cells. *J. Immunol.* 129: 303–8
10. Zamvil, S. S., Steinman, L. 1990. The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* 8: 579–621
11. Acha-Orbea, H., Steinman, L., McDevitt, H. O. 1989. T cell receptors in murine autoimmune diseases. *Annu. Rev. Immunol.* 7: 371–405
12. Naparstek, Y., Ben-Nun, A., Holoshitz, J., Reshef, T., Frenkel, A., Rosenberg, M., Cohen, I. R. 1983. T lymphocyte lines producing or vaccinating against autoimmune encephalomyelitis (EAE): Functional activation induces PNA receptors and accumulation in the brain and thymus of line cells. *Eur. J. Immunol.* 13: 418–23
13. Yarom, Y., Lev-Ram, V., Naparstek, Y., Holoshitz, J., Ben-Nun, A., Cohen, I. R. 1983. Immunospecific inhibition of nerve conduction in isolated rat optic nerve exerted by a line of T lymphocytes reactive to basic protein of myelin. *Nature* 303: 246–47
14. Ota, K., Matsui, M., Milford, D., Mackin, G., Weiner, H. L., Hafler, D. A. 1990. T cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature* In press
15. Naparstek, Y., Holoshitz, J., Eisenstein, S., Reshef, T., Rappaport, S., Chemke, J., Ben-Nun, A., Cohen, I. R. 1982. Effector T lymphocyte line cells migrate to the thymus and persist there. *Nature* 300: 262–3
16. Cohen, I. R. 1986. Regulation of autoimmune disease: Physiological and therapeutic. *Immunol. Rev.* 94: 5–21
17. Holoshitz, J., Naparstek, Y., Ben-Nun, A., Cohen, I. R. 1983. Lines of T lymphocytes mediate or vaccinate against autoimmune arthritis. *Science* 219: 56–58
18. Mor, F., Lohse, A., Karin, N., Cohen, I. R. 1990. Clinical modeling of T cell vaccination against autoimmune disease in rats. Selection of antigen-specific T cells using a mitogen. *J. Clin. Invest.* 85: 1594–98
19. Holoshitz, J., Matitau, A., Cohen, I. R. 1984. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J. Clin. Invest.* 73: 211–15
20. Maron, R., Zarubavel, R., Friedman, A., Cohen, I. R. 1983. T lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice. *J. Immunol.* 131: 2316–22
21. van Eden, W., Holoshitz, J., Nevo, Z., Frenkel, A., Klajman, A., Cohen, I. R. 1985. Arthritis induced by a T lymphocyte clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans . *Proc. Natl. Acad. Sci. USA* 82: 5117–20
22. Cohen, I. R., Holoshitz, J., van Eden, W., Frenkel, A. 1985. T lymphocyte clones illuminate pathogenesis and effect therapy of experimental arthritis. *Arthritis Rheum.* 28: 841–45
23. van Eden, W., Thole, J., van der Zee, R., Noordzij, A., Embden, J. D. A., Hensen, E. J., Cohen, I. R. 1988. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* 331: 171–73
24. Res, P. C. M., Schaar, C. G., Breedveld, F. C., van Eden, W., van Embden, J. D. A., Cohen, I. R., de Vries, R. R. P. 1988. Synovial fluid T cell reactivity against 65KD heat shock protein of mycobacteria in early chronic arthritis. *Lancet* II: 478–80
25. Holoshitz, I., Klajman, A., Druker, I., Lapidot, Z., Yaretzky, A., Frenkel, A., van Eden, W., Cohen, I. R. 1986. T lymphocytes of rheumatoid arthritis patients show augmented reactivity to a fraction of Mycobacteria cross-reactive with cartilage. *Lancet* II: 305–9
26. Cohen, I. R. 1988. The self, the world and autoimmunity. *Sci. Am.* 258: 52–60
27. Karlsson-Parra, A., Soderstrom, K., Ivanyi, F. J., Kiessling, R., Klareskog, L. 1990. Presence of human 65kD heat shock protein in inflamed joints and subcutaneous nodules of RA patients. *Scand. J. Immunol.* 31: 283–88
28. Palacios-Boix, A. A., Estrada-G, I., Colston, J., Panayi, G. S. 1988. HLA-Dr4 restricted lymphocyte proliferation to Mycobacterium tuberculosis extract in

rheumatoid arthritis and healthy subjects. *J. Immunol.* 140: 1844–50
29. Gaston, J. S. H., Life, P. F., Bailey, L. C., Bacon, P. A. 1989. *In vitro* responses to a 65-kilodalton mycobacterial protein by synovial T cells from inflammatory arthritis patients. *J. Immunol.* 143: 2494–2500
30. Gaston, J. S. H., Life, P. F., Jenner, P. J., Colston, M. J., Bacon, P. A. 1990. Recognition of a mycobacteria-specific epitope in the 65-kD heat-shock protein by synovial fluid-derived T cell clones. *J. Exp. Med.* 171: 831–41
31. Danieli, M. G., Markovits, D., Gabrielli, A., Corvetta, A., Giorgi, P. L., van der Zee, R., van Embden, J. D. A., Danieli, G., Cohen, I. R. 1990. Juvenile rheumatoid arthritis patients manifest immune reactivity to the mycobacterial 65kD heat shock protein, to its 180–188 peptide and to a partially homologous peptide of the proteoglycan link protein. Submitted for publication
32. Holoshitz, J., Koning, F., Coligan, J. E., de Bruyn, J., Strober, S. 1989. Isolation of CD4-CD8- mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature* 339: 226–29
33. O'Brien, R. L., Happ, M. P., Dallas, A., Palmer, E., Kubo, R., Born, W. K. 1989. Stimulation of a major subset of lymphocytes expressing T cell receptor gamma-delta by an antigen derived from *Mycobacterium tuberculosis*. *Cell* 57: 667–74
34. Haregewoin, A., Soman, G., Hom, R. C., Finberg, R. W. 1989. Human gamma-delta T cells respond to mycobacterial heat-shock protein. *Nature* 340: 309–12
35. Raulet, D. H. 1989. The structure, function, and molecular genetics of the $\gamma\delta$ T cell receptor. *Annu. Rev. Immunol.* 7: 175–207
36. Billingham, M. E. J., Carney, S., Butter, R., Colston, M. J. 1990. A mycobacterial 65-kDa heat shock protein induces antigen-specific suppression of adjuvant arthritis, but is not itself arthritogenic. *J. Exp. Med.* 171: 339–44
37. van den Broek, M. F., Hogervorst, E. J. M., Bruggen, M. C. J., van Eden, W., van der Zee, R., van den Berg, W. B. 1989. Protection against streptococcal cell wall-induced arthritis by pretreatment with the 65-kD mycobacterial heat shock protein. *J. Exp. Med.* 170: 449–66
38. Chang, Y-H., Pearson, C. M., Chedid, L. 1981. Adjuvant polyarthritis. V. Induction by N-acetylmuramyl-L-alany-D-isoglutamine the smallest peptide subunit of bacteria peptidoglycan. *J. Exp. Med.* 153: 1021–26
39. Chang, Y-H., Pearson, C. H., Abe, C. 1980. Adjuvant polyarthritis IV. induction by a synthetic adjuvant: immunologic, histopathological, and other studies. *Arthritis Rheum.* 23: 62–71
40. Zhang, Z. J., Lee, C. S. Y., Lider, O., Weiner, H. L. 1990. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. *J. Immunol.* In press
41. Elias, D., Markovits, D., Reshef, T., van der Zee, R., Cohen, I. R. 1990. Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/LT) mouse by a 65-kDa heat shock protein. *Proc. Natl. Acad. Sci. USA* 87: 1576–80
42. Castano, L., Eisenbarth, G. S. 1990. Type-1 diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu. Rev. Immunol.* 8: 647–79
43. Cohen, I. R. 1989. Type 1 insulin dependent diabetes mellitus. *Curr. Opinion Immunol.* 1: 727–32
44. Bendelac, A., Carnaud, C., Boitard, C., Bach, J. F. 1987. Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both $L3T4^+$ and $Lyt\text{-}2^+$ T cells. *J. Exp. Med.* 166: 823–32
45. Elias, D., Reshef, T., Birk, O. S., van der Zee, R., Walker, M. O., Cohen, I. R. 1990. Vaccination against autoimmune mouse diabetes using a T cell epitope of human hsp65. Submitted
46. Cohen, I. R. 1989. T cell vaccination and suppression of autoimmune disease. In *Progress in Immunology VII, Proceedings of the 7th Internatinal Congress of Immunology*, ed. F. Melchers, et al. pp. 867–73. Berlin: Springer-Verlag
47. Cohen, I. R. 1989. The physiological basis of T cell vaccination against autoimmune diseases. *Cold Spring Harbor Symposia on Quantitative Biology* LIV: 879–84
48. de Jong, W. W., Hendriks, W., Mulders, J. W. M., Bloemendal, H. 1989. Evolution of eye lens crystallins: the stress connection. *Trends Biochem. Sci.* 14: 365–68
49. Munk, M. E., Schoel, B., Modrow, S., Karr, R. W., Young, R. A., Kaufmann, S. H. E. 1989. T lymphocytes from healthy individuals with specificity to self epitopes shared by the mycobacterial and human 65kDa heat shock protein. *J. Immunol.* 143: 2844–49
50. Koga, T., Wand-Wurttenberger, A., de Bruyn, J., Munk, M. E., Schoel, B., Kaufmann, S. H. E. 1989. T cells against

a bacterial heat shock protein recognize stressed macrophages. *Science* 246: 1112–15
51. Cohen, I. R., Altmann, D., Friedman, A. 1985. The advantage of being a low responder. *Immunol. Today* 6: 147–48
52. Jackett, P. S., Bothamley, G. H., Batra, H. V., Mistry, A., Young, D. B., Ivanyi, J. 1988. Specificity of antibodies to immunodominant mycobacterial antigen in pulmonary tuberculosis. *J. Clin. Microbiol.* 26: 2313–18
53. Young, D., Lathigra, R., Hendrix, R., Sweetser, D., Young, R. A. 1988. Stress proteins are immune targets in leprosy and tuberculosis. *Proc. Natl. Acad. Sci. USA* 85: 4267–70
54. Hindersson, P., Peterson, C. S., Pederson, N. S., Hoiby, N., Axelson, N. H. 1984. Immunological cross-reaction between antigen Tp-4 of *Treponema pallidum* and an antigen common to a wide range of bacteria. *Acta Path. Microbiol. Immunol. Scand. Sect. B* 92: 183–88
55. Vodkin, M. H., Williams, J. C. 1988. A heat shock operon in *Coxiella burnetii* produces a major antigen homologous to a protein in both mycobacteria and *Escherichia coli*. *J. Bacteriol.* 170: 1227–34
56. Plikaytis, B. B., Carlone, G. M., Pau, C. P., Wilkinson, H. W. 1987. Purified 60-kilodalton *Legionella* protein antigen with *Legionella*-specific and nonspecific epitopes. *J. Clin. Microbiol.* 25: 2080–84
57. Hansen, K., Bangsborg, J. M., Fjordvang, H., Pederson, N. S., Hindersson, P. 1988. Immunochemical characterization of, and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60kDa antigen common to a wide range of bacteria. *Infect. Immun.* 56: 2047–53
58. Morrisin, R. P., Belland, R. J., Lyng, K., Caldwell, H. D. 1989. Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J. Exp. Med.* 170: 1271–83
59. Young, D. B., Ivanyi, J., Cox, J. H., Lamb, J. R. 1987. The 65kD antigen of mycobacteria—a common bacterial protein? *Immunol. Today* 8: 215–19
60. Young, D. B. 1990. Chaperonins and the immune response. *Semin. Cell Biol.* 1: 27–35
61. Kaufmann, S. H. E., Vath, U., Thole, J. E. R., van Embden, J. D. A., Emmrich, F. 1987. Enumeration of T cells reactive with Mycobacterium tuberculosis organisms and specific for the recombinant mycobacterial 64 kilodalton protein. *Eur. J. Immunol.* 17: 351–57
62. Davis, M. M., Bjorkman, P. J. 1988. T cell antigen receptor genes and T cell recognition. *Nature* 334: 395–402
63. Polla, B. S. 1988. A role for heat shock proteins in inflammation? *Immunol. Today* 9: 134–37
64. Buchmeier, N. A., Heffron, F. 1990. Induction of Salmonella stress proteins upon infection of macrophages. *Science* 248: 739–42
65. Pierce, S. K., DeNagel, D. C., Van Buskirk, A. M. 1990. A role for heat shock proteins in antigen processing and presentation. *Curr. Topics Micro. Immunol.* In press
66. Naparstek, Y., Cohen, I. R., Fuks, Z., Vlodavsky, I. 1984. Activated T lymphocytes produce a matrix degrading heparan sulphate endoglycosidase. *Nature* 310: 241–44
67. van Boehmer, H. 1988. The developmental biology of T lymphocytes. *Annu. Rev. Immunol.* 6: 309–26
68. Beraud, E., Reshef, T., Vandenbark, A., Offner, H., Fritz, R., Chou, C.-H. J., Bernard, D., Cohen, I. R. 1986. Experimental autoimmune encephalomyelitis mediated by T lymphocyte lines: Genotype of antigen presenting cells influences immunodominant epitope of basic protein. *J. Immunol.* 136: 511–15
69. Cohen, I. R., Atlan, H. 1989. Network regulation of autoimmunity: An automaton model. *J. Autoimmun.* 2: 613–25
70. Cohen, I. R. 1989. Natural id-anti-id networks and the immunological homunculus. In *Theories of Immune Networks*, ed. H. Atlan, I. R. Cohen, pp. 6–12. Berlin: Springer-Verlag
71. Holoshitz, J., Frenkel, A., Ben-Nun, A., Cohen, I. R. 1983. Autoimmune EAE mediated or prevented by T lymphocyte lines directed against diverse antigenic determinants of myelin basic protein. Vaccination is determinant specific. *J. Immunol.* 131: 2810–13
72. Lider, O., Reshef, T., Beraud, E., Ben-Nun, A., Cohen, I. R. 1986. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science* 239: 181–83
73. Lider, O., Karin, N., Shinitzky, M., Cohen, I. R. 1987. Therapeutic vaccination against adjuvant arthritis using autoimmune T lymphocytes treated with hydrostatic pressure. *Proc. Natl. Acad. Sci. USA* 84: 4577–80
74. Lider, O., Beraud, E., Reshef, T., Friedman, A., Cohen, I. R. 1989. Vaccination against experimental autoimmune encephalomyelitis using a subencephalito-

genic dose of autoimmune effector T cells. II. Induction of a protective anti-idiotypic response. *J. Autoimmun.* 2: 87–99
75. Sun, D., Qin, Y., Chluba, J., Epplen, J. T., Wekerle, H. 1988. Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T interactions. *Nature* 332: 843–45
76. Foulis, A. K., Farquharson, M. A., Hardman, R. 1987. Aberrant expression of class-II major histocompatibility complex molecules by B-cells and hyperexpression of class-I major histocompatibility molecules by insulin containing islets in type-I (insulin dependent) diabetes mellitus. *Diabetologia* 30: 333–43
77. Sadelain, M. W. J., Qin, H-Y., Lauzon, J., Singh, B. 1990. Prevention of type I diabetes in NOD mice by adjuvant therapy. *Diabetes* 39: 583–89
78. Parham, P. 1990. A diversity of diabetes. *Nature* 345: 662–64
79. Baekkeskov, S., Nielsen, J. H., Marner, B., Bilde, T., Ludvigsson, J., Lernmark, A. 1982. Autoantibodies in newly diagnosed diabetic children immunoprecipitate specific human islet cell proteins. *Nature* 298: 167–69
80. Cohen, I. R., Young, D. B. 1990. The immune system's view of invading microorganisms, autoimmunity and the immunological homunculus. Submitted
81. Shoenfeld, Y., Isenberg, D. 1989. *The Mosaic of Autoimmunity*. Amsterdam: Elsevier