Bacteria

Cell-mediated immunity to mycobacteria: a double-sided sword?

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Summary. Mycobacteria are intracellular pathogens capable of replicating in resting macrophages. Specific helper T lymphocytes which activate antimycobacterial capacities in infected macrophages represent an important constituent of acquired resistance. In addition, cytolytic T lymphocytes may contribute to resistance. On the other hand, lysis of infected host cells may also comprise autoaggressive consequences. Recent evidence suggest that T cells with specificity for mycobacterial heat shock proteins are involved in the antimycobacterial immune response. Heat shock proteins are evolutionarily highly conserved and cross-reactivity between microbial and mammalian molecules may occur on the B-cell and T-cell level. Thus, T cells directed against shared epitopes of mycobacterial and autologous origin could initiate autoimmune reactions.

Key words: Autoimmunity – Heat shock proteins – Mycobacteria – T cells

Introduction

Intracellular bacteria including the etiologic agents of tuberculosis, *Mycobacterium tuberculosis* and *Mycobacterium bovis* are capable of surviving inside professional phagocytes [1]. In this way they are well shielded from humoral defence mechanisms. During their intracellular life, mycobacteria produce molecules which are then processed and presented in the context of major histocompatibility complex (MHC) products on the surface of infected macrophages. In this way host cells infected with mycobacteria become recognizable for T lymphocytes which set into motion protective effector mechanisms. Since mycobacteria reside in host cells, these protective mechanisms must be directed against the infected cells which may suffer from this

response. Furthermore, certain mycobacterial antigens share epitopes with host cell components and hence have the potential to induce autoimmune responses. Thus, in mycobacterial infections, protection and autoaggression are closely intertwined and cannot be separated into independent entities. In this paper evidences from an experimental mouse model will be discussed which support this notion.

Function

The peripheral T lymphocyte system segregates into two major sets of effector cells with distinct phenotype and antigen recognition pattern [2]. CD4 T cells see foreign peptides together with MHC class II molecules whereas CD8 T cells respond to antigenic peptides in the context of MHC class I molecules. According to current thinking only newly synthesized polypeptides (so-called endogenous antigens) are capable of associating with class I molecules, while antigens taken up by presenting cells (so-called exogenous antigens) exclusively associate with class II molecules. Polypeptides from intracellular bacteria should fall into the latter class of antigens. Indeed, interleukin secretion – the predominant function of CD4 T cells – is thought to be of major relevance to protection against intracellular bacteria. In contrast, target cell lysis, the prime function of CD8 T lymphocytes is thought to be important for defense against viral rather than bacterial infections.

Evidence that not only CD4 but also CD8 T lymphocytes are relevant to protection against tubercle bacilli

Mice were thymectomized and afterwards treated with monoclonal antibodies directed against the CD4 or the CD8 molecule, respectively [3]. In this way, mice deficient of CD4, CD8, or both T cell

sets could be generated. After infection with *M. tuberculosis*, not only CD4- but also CD8-deficient mice showed increased numbers of bacteria and depletion of both subsets did not exacerbate the disease. These findings suggest a role for both CD4 and CD8 T cells in acquired resistance to tuberculosis.

Interferon-γ (IFN-γ) production by *M. tuberculosis* reactive CD4 T lymphocytes and activation of tuberculostatic macrophage functions by IFN-γ

Mice were immunized with killed *M. tuberculosis* in incomplete Freund's adjuvant and cloned T-cell lines established. These T cell lines were *M. tuberculosis* reactive, CD4$^+$, and class II restricted [4]. After restimulation with mycobacterial antigens and accessory cells, the T cells produced IFN-γ. Macrophages were derived from bone marrow cells by in vitro culture in conditioned medium for 9 days. These cells expressed the Mac-1 marker and class I, but not class II, molecules. After stimulation with recombinant (r) IFN-γ they also became Ia$^+$. Bone marrow derived macrophages (BMMΦ) were stimulated with factors produced by mycobacteria reactive T cells or by r-IFN-γ and subsequently were infected with viable *M. bovis* [4]. After 4 days, survival of *M. bovis* was assessed by counting colony forming units or by measuring ^{3}H-uracil uptake. BMMΦ activated with IFN-γ (either T-cell derived or recombinant) markedly inhibited growth of *M. bovis* [4, 5]. These findings show that *M. tuberculosis* reactive CD4 T cells produce IFN-γ which activates tuberculostatic functions in BMMΦ. Recently we have obtained evidence that *M. bovis* infected BMMΦ can be stimulated by the B-cell stimulatory factors, interleukin 4 and interleukin 6 to inhibit mycobacterial growth (I.E.A. Flesch and S.H.E. Kaufmann submitted).

Induction of necrotic reactions by r-tumor necrosis factor (r-TNF-α) in combination with mycobacterial products

The previous section provided evidence for a protective function of activated macrophages. At the same time activated macrophages secrete reactive molecules potentially harmful for the host. TNF-α, is a molecule of this type. Nu/nu mice were injected (subcutaneously) with r-TNF-α killed mycobacteria or both into the root of the tail. Mice receiving either material alone showed only marginal and transient reactions. In contrast, mice injected with both r-TNF-α and killed mycobacteria developed

marked necrosis at the site of injection [6]. Similarly, in a tuberculous granuloma secretion of TNF-α by activated macrophages in the presence of mycobacterial debris could induce tissue necrosis. Thus, macrophage activation seems to comprise both beneficial and harmful sequelae.

Generation of mycobacteria reactive CD8 T lymphocytes with cytolytic activity

Mice were immunized with viable *M. bovis* or killed *M. tuberculosis* in incomplete Freund's adjuvant and cloned T-cell lines established. These T-cell lines were *M. tuberculosis* reactive, CD8$^+$, and some of them could be shown to be class I restricted [7]. These T cells were capable of lysing BMMΦ primed with *M. tuberculosis* leaving unprimed BMMΦ unaffected. Thus, CD8 T cells with cytolytic activity participate in the immune response to tubercle bacilli. BMMΦ were infected with viable *M. bovis* and subsequently cytolytic T cells were added. In these cultures growth of *M. bovis* was markedly inhibited, suggesting that target cell lysis is paralleled by tuberculostasis [7]. Thus, cytolytic T cells might directly contribute to protection. Mycobacteria residing in host cells of low antibacterial potential could be released by target cell lysis and – in this way – become accessible to more potent phagocytes, e.g., monocytes. On the other hand, target cell lysis could result in bacterial dissemination and tissue destruction and in this way harm the host.

IFN-γ secretion by CD8 T lymphocytes and target cell lysis by CD4 T cells

CD8 T cells stimulated with mycobacterial antigens and accessory cells failed to secrete IFN-γ. However, after addition of interleukin 2 to these cultures, many CD8 T-cell lines secreted IFN-γ [7]. CD4 T-cell lines failed to lyse BMMΦ primed with mycobacterial antigens. However, after prestimulation of BMMΦ with IFN-γ, antigen primed BMMΦ were lysed by several CD4 T-cell lines probably because IFN-γ had induced expression of class II molecules on BMMΦ as the relevant restriction element for CD4 T lymphocytes [8]. Thus, under appropriate conditions, cytolysis and IFN-γ secretion are expressed by T cells of either phenotype.

Antigen reactivity

Recently, the genome of *M. tuberculosis* has been cloned in the λgt11 expression system and in this

way several defined mycobacterial proteins became available for analysis by immunologists [9]. One molecule of 65 kD molecular mass has been particularly well characterized. Its sequence has been identified and it has been shown that this molecule is identical in *M. bovis* and *M. tuberculosis* and shares significant homology with the groEL heat shock protein (hsp) of *E. coli* and other bacteria [10]. Recently, the human homologue of the mycobacterial r-65 kDa protein has been cloned and sequenced and it was found that the two molecules share a remarkable degree of sequence homology [10a].

T-cell responses to the r-65 kDa hsp of tubercle bacilli

Mice were immunized with viable *M. bovis* and after 14 days challenged with a highly purified r-65 kDa antigen. This antigen elicited delayed-type hypersensitivity responses indicating that T cells with reactivity to the 65 kDa protein are induced during tuberculosis. Mice were immunized with killed *M. tuberculosis* in incomplete Freund's adjuvant or with the r-65 kDa hsp in the Ribi adjuvant [11]. Limiting numbers of T lymphocytes were then cultured in the presence of excess feeder cells and antigens and the frequency of antigen reactive T cells estimated according to Poisson distribution [11]. Approximately 1/3 000 T cells from mice immunized with mycobacterial organisms reacted with *M. tuberculosis* and of these about 20% were specific for the 65 kDa hsp. When T cells from mice immunized with the 65 kDa hsp were analyzed, an equal number of T cells was found to react with whole mycobacteria and with the 65 kDa hsp. Thus, T cells with specificity to the 65 kDa hsp seem to comprise a dominant fraction of *M. tuberculosis* reactive T cells and immunization with the 65 kDa hsp induces a high number of T cells which respond to *M. tuberculosis*.

Identification of a cross-reactive 65 kDa hsp in BMMΦ and its surface expression by IFN-γ activated BMMΦ

BMMΦ were lysed and subjected to Western blot analysis. In parallel, the mycobacterial 65 kDa hsp was applied. An antibody was identified which cross-reacted with protein bands of an apparent molecular weight of 65 kDa both of murine and mycobacterial origin [11a]. BMMΦ were stimulated with r-IFN-γ or left untreated and afterwards incubated with anti-hsp antibodies and with fluoresceine isothiocyanate labelled goat-anti-mouse antiserum.

Analysis by flow cytometry revealed that BMMΦ expressed a cross-reactive epitope on their surface and it appears that IFN-γ increases its expression (S. H. E. Kaufmann, unpublished). This recognition could result in autoaggressive sequelae.

Recognition of IFN-γ activated BMMΦ by T cells with specificity to the r-65 kDa hsp of *M. tuberculosis*

Mice were immunized (subcutaneously) with the r-65 kDa hsp in Ribi adjuvant or left untreated and after 8 days lymph node cells were collected. T cells were restimulated in vitro with *M. tuberculosis* organisms, the r-65 kDa hsp, unstimulated BMMΦ or r-IFN-γ stimulated BMMΦ, respectively, in the presence of accessory cells. After 5 days, proliferative responses were determined by ^{3}H-thymidine incorporation. T cells from untreated mice responded to neither stimulus. T cells from mice immunized with the r-65 kDa polypeptide not only responded to *M. tuberculosis* organisms and the 65 kDa hsp but also to r-IFN-γ activated BMMΦ in the absence of the mycobacterial antigen (S. H. E. Kaufmann, unpublished). In contrast, unstimulated BMMΦ were not recognized. The responsive T cells were of CD4 phenotype.

The r-65 kDa hsp was digested with trypsin and T cells from naive mice were stimulated by tryptic fragments of the r-65 kDa hsp according to Carbone et al. [12]. After 5 days, cytolytic T cells of CD8 phenotype had developed. These T cells not only lysed BMMΦ primed with peptide fragments of the 65 kDa hsp but also IFN-γ stimulated BMMΦ in the absence of antigen [11 a]. Similarly, virus-infected BMMΦ were killed by these T cells. In contrast, unstimulated BMMΦ were only marginally lysed. Thus, cross-reactive epitope(s) ot the r-65 kDa hsp is (are) presented by stressed macrophages to cytolytic T cells with reactivity to the mycobacterial r-65 kDa hsp. These findings suggest that both CD4 and CD8 T cells can recognize cross-reactive epitope(s) of the 65 kDa hsp and hence that recognition of this epitope by mycobacteria reactive T cells could set into motion an autoaggressive response.

Discussion

The data discussed in this paper shed some light on the double-sided nature of the immune response to mycobacteria. On the one hand, this response is required to protect the infected host; on the other hand, harmful reactions for the host seem to be intimately associated with this response.
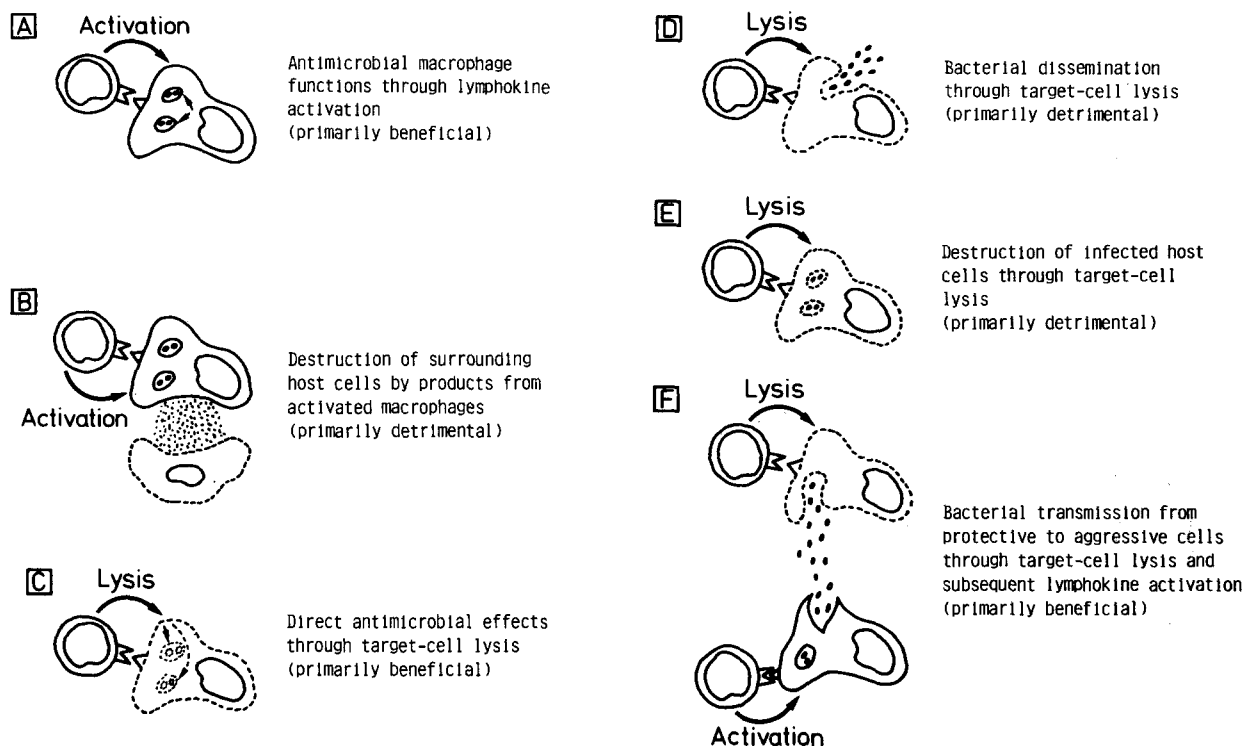
A Activation

Antimicrobial macrophage functions through lymphokine activation (primarily beneficial)

B Activation

Destruction of surrounding host cells by products from activated macrophages (primarily detrimental)

C Lysis

Direct antimicrobial effects through target-cell lysis (primarily beneficial)

D Lysis

Bacterial dissemination through target-cell lysis (primarily detrimental)

E Lysis

Destruction of infected host cells through target-cell lysis (primarily detrimental)

F Lysis

Bacterial transmission from protective to aggressive cells through target-cell lysis and subsequent lymphokine activation (primarily beneficial)

Activation

Fig. 1. Possible consequences of T cell-macrophage interactions in intracellular bacterial infections

The activation of antimycobacterial macrophage functions is well appreciated as being of outstanding importance for resistance to tubercle bacilli [13]. Activation is primarily a function of CD4 helper T cells capable of secreting macrophage-activating interleukins. The data presented here strongly support this notion by showing (a) that depletion of CD4 T cells worsens tuberculosis in infected mice; (b) that *M. tuberculosis* reactive CD4 T cells secrete macrophage activating interleukins, and (c) that IFN-γ activates tuberculostasis.

The data discussed, however, also warrant modification of the notion that protective immunity is a function solely of CD4 T cells since they show that CD8 T cells are also involved in protection. Under appropriate conditions many of these CD8 T cells secrete IFN-γ and hence may contribute to protection through activation as do CD4 T cells. In addition, these CD8 T cells can lyse BMMφ presenting mycobacterial antigens and – in vitro – target cell lysis is paralleled by mycobacterial growth inhibition. Under appropriate conditions also CD4 T cells were found to be cytolytic. One is, therefore, forced to consider lysis of infected host cells as an additional mechanism of antibacterial immunity.

In fact, in a highly organized micromilieu synergy between help and kill could improve the protective immune response. A productive granuloma could provide the morphological substrate for protective interactions. In tuberculoid leprosy lesions CD8 T cells with the phenotype of killer cells have been found to surround a nucleus of CD4 T cells and macrophages [14]. In mycobacteria-induced granulomas aged macrophages can be found which harbor numerous mycobacteria but apparently are unable to kill and degrade their intracellular parasites. Furthermore, mycobacteria can also inhabit nonprofessional phagocytes. These cells are insufficiently equipped for the killing and destruction of their intracellular parasites and hence serve as Trojan horses. Lysis of these cells, therefore, may be an unavoidable step towards eradication of the pathogen since release of bacteria would allow for engulfment by phagocytes with higher antimicrobial potential. Immigrant blood monocytes after activation by appropriate interleukins could represent such cells.

Activation of macrophages not only results in tuberculostasis but also in the secretion of harmful molecules. The data described here show that TNF-α, a typical secretory product of activated macrophages, in combination with mycobacterial compounds induces marked necrosis. At the site of host defense such reactions could participate in tissue destruction. In addition, lysis of target cells

nearly by definition should also contribute to tissue damage. This is summarized in Fig. 1.

As long as such harmful reactions are confined to the site of microbial multiplication, protective effects may dominate. Under certain conditions, however, harmful events could gain importance. Two situations can be envisaged: (1) Cells of particular importance for the host are infected. (2) Host cells share cross-reactive epitopes with mycobacteria. In both cases host cells which are not directly related to the protective immune response would become targets of the response. A example for (1) could be found in leprosy. The etiologic agent, *M. leprae*, inhabits Schwann cells and it has been shown recently that Schwann cells presenting *M. leprae* antigen are targets of cytolytic T cells [14]. In natural infection, such an event could contribute to the nerve-cell damage seen in leprosy.

An example for (2) could be seen in the hsp. As described above, a large proportion of mycobacteria reactive T cells recognize a protein of 65 kDa which has been shown to belong to the hsp family. Hsp are produced under a variety of insults including conditions that occur in granulomatous lesions such as bombardment with reactive oxygen metabolites, low oxygen pressure, as well as depletion of nutrients and essential ions [15]. These hsp are produced by the cell in an attempt to protect itself from injury. It is conceivable that in a tuberculous granuloma both pathogens and host cells are stressed and produce hsp.

Hsp are evolutionarily highly conserved and for the 65 kDa protein marked homology has been found in different species [10, 16, 17]. This not only holds true for a variety of microorganisms but even for mammals [10a]. The data described here show that cross-reactive antibodies to the mycobacterial 65 kDa hsp recognize a protein of similar size in murine macrophages. After activation (or stress) this protein appears on the surface and it is recognized by CD4 and CD8 T cells specific for the mycobacterial 65 kDa hsp. These data strongly suggest that T cells recognize shared epitope(s) of mycobacterial and self origin after they had been processed and presented in the context of MHC products. Thus, T cells directed against the 65 kDa hsp of mycobacteria could interact with activated or stressed host cells and this interaction could result in the destruction of the latter. These interactions could take place even after the pathogen had been eradicated and the infectious disease had been overcome. Hence, they represent a paradigmatic model for an autoimmune disease caused by an infectious agent. This is summarized in Fig. 2.
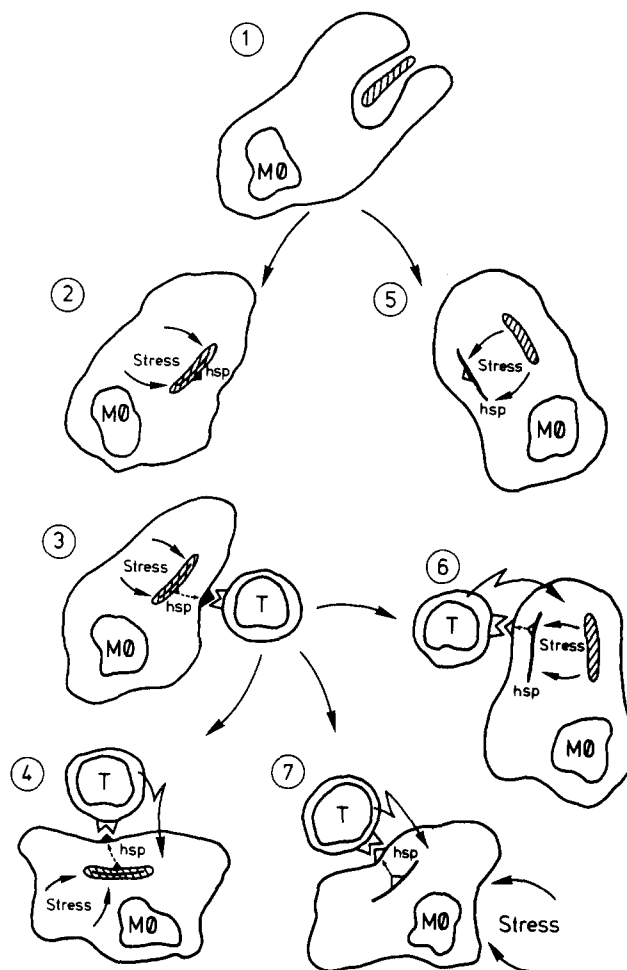

Fig. 2. Possible functions of hsp-specific T cells. (1) Hsp-specific T cells are stimulted by MΦ infected with a microbial pathogen. Because hsp of different microorganisms are similar, hsp-specific T cells not only recognize MΦ infected with the same agent (2) but also those harboring other pathogens (3). This could lead to cross-protection. Because pro- and eukaryotic hsp share sequence homology, host cells presenting autologous hsp can also be recognized by these T cells (4). This could lead to autoimmunity

Evidence has been presented recently that in patients [19] and in an experimental rat model [20] rheumatoid arthritis is associated with T-cell immunity to the mycobacterial 65 kDa hsp. Because this protein is shared by many if not all microbes [10, 18], it may be a challenging task to direct the search for etiologic agents of certain autoimmune diseases from species to molecules.

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