Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. V, 91-94, 1992

IMMUNITY TO INTRACELLULAR BACTERIA

STEFAN H. E. KAUFMANN/+; GEORGE A. FOLLOWS & MARTIN E. MUNK

Department of Immunology, University of Ulm, Albert-Einstein-Allee 11, D-7900 Ulm, F. R. Germany

Immunity to intracellular bacteria including Mycobacterium tuberculosis, Mycobacterium leprae, *and* Listeria monocytogenes *depends on specific T cells. Evidence to be described suggests that CD4* α/β *T cells, CD8* α/β *T cells and* γ/δ *T cells which interact with each other and with macrophages contribute to aquired resistance against as well as pathogenesis of intracellular bacterial infections.*

Key words: intracellular bacteria – tuberculosis – leprosy – T cell – macrophage – interleukin

Intracellular bacteria have decided to abuse mononuclear phagocytes as preferred habitat (Hahn & Kaufmann, 1981). This group of pathogens includes *Mycobacterium tuberculosis, Mycobacterium leprae, Listeria monocytogenes, Legionella pneumophila* and others. The medically most important member of this group, the etiologic agent of tuberculosis, afflicts more than 60 million people worldwide (Kaufmann & Young, 1992). Three million people die of this disease annually and yearly more than 10 million people become diseased. According to current estimates one third to one half of the world population is infected with this pathogen. Despite the severity of this medical problem, therefore, many people must possess potent immune mechanisms capable of controlling infection.

Immunity rests on the cellular arm of the immune response. T lymphocytes are able to recognize infected macrophages and then to set into motion protective mechanisms. These are potent in that they cause microbial containment in discrete foci where replication is markedly reduced. On the other hand, the immune response is impotent in that it often fails to totally eradicate the intracellular pathogens. Protective immunity, therefore, may be viewed as a labile balance, the regulation of which depends on may factors both on the side of the host and on the side of the pathogen. Such a labile balance is subject to changes that may cause transition to clinical disease at a later time. In tuberculosis, therefore, reactivation of persistent foci after weakening of the immune system is the typical reason for adult tuberculosis. In this short treatise we will discuss findings, mostly from our laboratory, which may help to better understand the mechanisms that contribute to protection against tuberculosis and probably other intracellular bacterial infections.

THE MURINE MODEL

Although the mouse is relatively resistant against tuberculous bacilli, it provides a helpful model for experimental analyses because its immune system is extemely well understood. In the murine model, both CD4 T cells and CD8 T cells seem to contribute to protection against tuberculosis (Müller et al., 1987). This notion is based on findings in which mice were treated with monoclonal antibodies against CD4 or CD8 T cells to deplete the relevant T cell populations. Subsequently, bacterial numbers in spleens were determined. Treatment with either anti-CD4 or with anti-CD8 antibodies significantly increased bacterial counts. Treatment with both antibodies did not lead to further exacerbation. These findings suggest that both CD4 and CD8 T cells are involved in the acquisition of protective immunity against tuberculosis in a murine model. However, it may also be noted that the increase in bacterial counts was less than 10-fold in antibody-treated mice. These data may be taken as evidence that additional, α/β T-cell-independent mechanisms contribute to acquired resistance against tuberculosis. Evidence in favour of this notion will be discussed below.

+Corresponding author.

To further characterize the T cells involved in acquired resistance, cloned T cells of CD4 and CD8 phenotype were established from mice immunized with mycobacteria. CD4 T cell clones were class-II-restricted and they produced interferon-γ (IFN-γ) and interleukin 2 (IL-2) after restimulation with mycobacterial antigens plus accessory cells (Kaufmann & Flesch, 1986). Therefore, they have the characteristic features of T_{H1} cells (Mosmann & Coffman, 1989). Importantly, the supernatants produced by these T cells were capable of activating tuberculostatic activities in macrophages. Using a variety of recombinant interleukins, it could be shown that activation of mycobacterial growth inhibition in murine macrophages is primarily a function of IFN-γ (Flesch & Kaufmann, 1987). CD4 T cell clones with specificity for mycobacterial antigens also express cytolytic activity: they are capable of lysing macrophages presenting mycobacterial antigens (Kaufmann, 1988). In these studies, bone marrow derived macrophages were used which express only negligible amounts of class II molecules. Therefore, in addition to antigen pulsing these macrophages had to be prestimulated with IFN-γ to cause class II expression.

CD8 T cell clones with specificity for mycobacteria were also isolated (Chiplunkar et al., 1986; DeLibero et al., 1988). Several clones were class-I-restricted although clones were identified which were apparently non-restricted. These T cells lysed macrophages pulsed with mycobacterial antigens even without prior IFN-γ stimulation. In addition, CD8 T cells with specificity for mycobacteria secreted IFN-γ after costimulation with mycobacterial antigens, accessory cells and IL-2.

In order to assess whether cytolytic T cells affect intracellular growth of mycobacteria the following experiment was performed: Macrophages were infected with viable mycobacteria and afterwards cytolytic CD8 T cells added. Not only were the mycobacteria-infected macrophages lysed. Cytolysis was also paralleled by mycobacterial growth inhibition as assessed by 3H-uracil uptake (DeLibero et al., 1988). These data may be taken as circumstantial evidence that macrophage destruction is associated with inhibition of mycobacterial growth.

In summary, the functional activities of murine CD4 and CD8 T cells with specificity for mycobacteria do not appear to differ markedly. Rather, these cells seem to differ in their genetic restriction with CD8 T cells having a much broader target spectrum. Since *M. tuberculosis* primarily infects macrophages, this broad spectrum may be of minor relevance for the control of tuberculosis. However, other intracellular bacteria also inhabit nonprofessional phagocytes, as well. Thus, hepatocytes represent a major habitat for *L. monocytogenes* and Schwann cells are often abused by *M. leprae*. These cells are constitutively class II-negative and may only be recognizeable for CD8 T cells. In fact, in the murine model Schwann cells are even constitutively class-I-negative. However, stimulation with IFN-γ caused class I expression. Accordingly, we found that Schwann cells pulsed with *M. leprae* and stimulated with IFN-γ are lysed by mycobacteria-specific CD8 T cells (Steinhoff & Kaufmann, 1988).

THE HUMAN SYSTEM

The role of CD4 and CD8 T cells in antibacterial immunity as analyzed in the murine model, in principle, also holds true for the human system. T cells with specificity for mycobacterial antigens have been isolated both from patients and from healthy individuals without clinical signs of disease (Emmrich et al., 1986; Munk et al., 1988, 1989). These CD4 T cells produce IL-2 and IFN-γ upon appropriate stimulation and express cytolytic activity. These biological features ascribe mycobacteria-reactive CD4 T cells to the T_{H1} set (Romagnani, 1991). In contrast to the murine system, CD8 T cells have been isolated only occasionally and it appears that both, cytolytic effector function and interleukin secretion, primarily rest in the CD4 T cell population. Therefore, further studies will be required to definitely define the relevance of CD8 T cells in the control of human tuberculosis.

Several *in vitro* studies performed during recent years suggest that an additional T cell population contributes to immunity against tuberculosis and probably other bacterial infections: these T cells express a different T cell receptor composed of a γ and a δ chain and, accordingly, are termed γ/δ T cells. The γ/δ T lymphocytes represent a minor fraction among peripheral blood T cells and in normal healthy individuals they make up less than 10% of the peripheral lymphocyte pool. However,

in vitro activation with mycobacterial or other bacterial preparations causes a marked expansion of the γ/δ T cell set. Often, after cultivation for 7-10 days, the relative percentage increases to up to 50%. Besides *M. tuberculosis*, also *M. leprae*, *L. monocytogenes*, *S. aureus*, and group A streptococcus organisms caused a significant expansion of γ/δ T cells in certain individuals (Munk et al., 1990). Different individuals showed distinct response patterns indicating some specificity of the response. On the other hand, limiting dilution analyses indicate that frequencies of mycobacteria-reactive γ/δ T cells often range in the order of 1/10 (Kabelitz et al., 1990). These findings would support oligoclonal activation and expansion of γ/δ T cells, perhaps caused by superantigen-like entities. Although the biochemical nature of the responsible moieties is as yet elusive, evidence has been presented that protease-resistant low molecular weight fractions of *M. tuberculosis* stimulate γ/δ T cells (Pfeffer et al., 1990). This finding would argue for involvement of nonproteinacious entities very different from the antigenic peptides and protein superantigens responsible for α/β T cell activation. Protease-sensitive, high molecular weight material (probably proteins) also stimulates human γ/δ T cells.

Mycobacteria-activated γ/δ T cells are capable of lysing macrophages pulsed with mycobacteria leaving unpulsed macrophages virtually unaffected (Munk et al., 1990). In addition, selected γ/δ T cells after restimulation with *M. tuberculosis* preparations produced interleukins (Follows et al., 1992). Thus far, appreciable concentrations of IFN-γ and marginal levels of IL-2 have been identified. Perhaps also tumor necrosis factor β (TNF-β) is secreted. In contrast, interleukin 4 activities were not detected in cultures of mycobacteria-activated γ/δ T cells. Hence, both γ/δ and α/β T cells show remarkable functional similarities. Whether this is mere redundancy or whether α/β and γ/δ T cells differ in other important parameters remains to be established.

CONCLUDING REMARKS

The γ/δ T cells accumulate in reactive lesions of leprosy patients (Modlin et al., 1989); they are rapidly activated by *in vivo* immunization of mice with complete Freund's adjuvant (Janis et al., 1989); and appear early at the site of *M. bovis* BCG replication (Inoue et al., 1991). Perhaps γ/δ T cells precede α/β T cells in bacterial infections because of a lower activation threshold. Another important difference may be the differential tissue distribution of α/β and γ/δ T cells because γ/δ T cells preponderate in epithelial layers which represent the first barrier of entry for many microorganisms. Taken together, γ/δ T cells seem to provide a first line of anti-bacterial defence characterized by less stringent specificity and activation requirements which are succeeded by the more tightly controlled and more specific α/β T lymphocytes.

The functional similarities of activated α/β and γ/δ T cells, on the other hand, support the notion that both, target cell lysis as well as mobilization and activation of macrophages represent the essential steps in the acquisition of protective immunity. There is no doubt that attraction of mononuclear phagocytes to and macrophage activation at tuberculous granulomas represent essential mechanisms of protection. Tuberculous granulomas are composed of multinucleated giant cells and epitheloid cells which appear to be of low antimycobacterial potential. These cells confine mycobacteria to distinct foci and in this way contribute to the control of infection. Complete eradication of pathogens (which often does not occur in the human host) may, however, require lysis of such cells to allow uptake by more potent effector cells such as monocytes. As long as such mechanisms function in a coordinate way, protective corollae will preponderate. Once lytic effects caused by T lymphocytes and other mechanisms dominate, harmful consequences may become prominent, including the risk of bacterial dissemination and tissue damage.

ACKNOWLEDGEMENT

S. H. E. K. gratefully acknowledges financial support from UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases; Sonderforschungsbereich 322; German Leprosy Relief Association; EC-India Science and Technology Cooperation Program; and Landesschwerpunkt 30. G. A. F. was supported by a scholarship from the British Chamber of Commerce in Germany and a grant from Worcester College, Oxford. Many thanks to R. Mahmoudi.

REFERENCES

CHIPLUNKAR, S.; DELIBERO, G. & KAUFMANN, S. H. E., 1986. *Mycobacterium leprae*-specific Lyt2+

T lymphocytes with cytolytic activity. *Infect. Immun., 54:* 793-797.

DELIBERO, G.; FLESCH, I. & KAUFMANN, S. H. E., 1988. Mycobacteria reactive Lyt2+ T cell lines. *Eur. J. Immunol., 18:* 59-66.

EMMRICH, F.; THOLE, J.; VAN EMBDEN, J. & KAUFMANN, S. H. E., 1986. A recombinant 64 kiloDalton protein of *Mycobacterium bovis* BCG specifically stimulates human T4 clones reactive to mycobacterial antigens. *J. Exp. Med., 163:* 1024-1029.

FLESCH, I. & KAUFMANN, S. H. E., 1987. Mycobacterial growth inhibition by interferon-γ-activated bone marrow macrophages and differential susceptibility among strains of *Mycobacterium tuberculosis. J. Immunol., 138:* 4408-4413.

FOLLOWS, G. A.; MUNK, M. E.; GATRILL, A. J.; CONRADT, P. & KAUFMANN, S. H. E., 1992. Interferon-gamma but no detectable interleukin 4 production by γ/δ T cells after activation with bacteria. *Infect. Immun., 60:* 1229-1231.

HAHN, H. & KAUFMANN, S. H. E., 1981. Role of cell mediated immunity in bacterial infections. *Rev. Infect. Dis., 3:* 1221-1250.

INOUE, T.; YOSUNOBU, Y.; MATSUZAKI, G. & NOMOTO, K., 1991. Early appearing γ/δ-bearing T cells during infection with Calmette Guérin Bacillus. *J. Immunol., 146:* 2754-2762.

JANIS, E. M.; KAUFMANN, S. H. E.; SCHWARTZ, R. H. & PARDOLL, D. M., 1989. Activation of γ/δ T cells in the primary immune response to *Mycobacterium tuberculosis. Science, 244:* 713-716.

KABELITZ, D.; BENDER, A.; SCHONDELMAIER, S.; SCHOEL, B. & KAUFMANN, S. H. E., 1990. A large fraction of human peripheral blood γ/δ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. *J. Exp. Med., 171:* 667-679.

KAUFMANN, S. H. E., 1988. CD8+ T lymphocytes in intracellular microbial infections. *Immunol. Today, 9:* 168-174.

KAUFMANN, S. H. E. & FLESCH, I., 1986. Function and antigen recognition pattern of L3T4+ T cell clones from *Mycobacterium tuberculosis*-immune mice. *Infect. Immun., 54:* 291-296.

KAUFMANN, S. H. E. & YOUNG, D. B., 1992. Vaccination against tuberculosis and leprosy. *Immunobiology,* in press.

MODLIN, R. L.; PIRMEZ, C.; HOFMANN, F. M.; TORIGIAN, V.; UYEMURA, K.; REA, T. H.; BLOOM, B. R. & BRENNER, M. B., 1989. Lymphocytes bearing antigen-specific γ/δ T cell receptors accumulate in human infectious disease lesions. *Nature, 339:* 544-548.

MOSMANN, T. R. & COFFMAN, R. L., 1989. T_{H1} and T_{H2} cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol., 7:* 145-174.

MÜLLER, I.; COBBOLD, S. P.; WALDMANN, H. & KAUFMANN, S. H. E., 1987. Impaired resistance against *Mycobacterium tuberculosis* infection after selective in-vivo depletion of L3T4+ and Lyt2+ T cells. *Infect. Immun., 55:* 2037-2041.

MUNK, M. E.; GATRILL, A. & KAUFMANN, S. H. E., 1990. Antigen specific target cell lysis and interleukin-2 secretion by *Mycobacterium tuberculosis* activated γ/δ T cells. *J. Immunol., 145:* 2434-2439.

MUNK, M. E.; SCHOEL, B. & KAUFMANN, S. H. E., 1988. T cell responses of normal individuals towards recombinant protein antigens of *Mycobacterium tuberculosis. Eur. J. Immunol., 18:* 1835-1838.

MUNK, M. E.; SCHOEL, B.; MODROW, S.; KARR, R. W.; YOUNG, R. A. & KAUFMANN, S. H. E., 1989. Cytolytic T lymphocytes from healthy individuals with specificity to self epitopes shared by the mycobacterial and human 65 kDa heat shock protein. *J. Immunol., 143:* 2844-2849.

PFEFFER, K.; SCHOEL, B.; GULLE, H.; KAUFMANN, S. H. E. & WAGNER, H., 1990. Primary responses of human T cells to mycobacteria: a frequent set of γ/δ T cells are stimulated by protease-resistant ligands. *Eur. J. Immunol., 20:* 1175-1179.

ROMAGNANI, S., 1991. Human T_{H1} and T_{H2} subsets: doubt no more. *Immunol. Today, 12:* 256-257.

STEINHOFF, U. & KAUFMANN, S. H. E., 1988. Specific lysis by CD8+ T cells of Schwann cells expressing *Mycobacterium leprae* antigens. *Eur. J. Immunol., 18:* 973-976.