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Invited Review

Regulation by Innate Immune T Lymphocytes in the Host Defense against Pulmonary Infection with *Cryptococcus neoformans*

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SUMMARY: Recently, innate immune lymphocytes, such as natural killer (NK) T cells and $\gamma\delta$ antigen receptor-bearing T ($\gamma\delta$ T) cells, have garnered much attention, and their biological significance in the tumor immunity, allergic diseases and infectious diseases is extensively exploited. We have addressed the role of these cells in the host defense using a mouse model of pulmonary infection with *Cryptococcus neoformans*, which frequently causes fatal meningoencephalitis in AIDS patients. Host defense to this fungal pathogen is largely mediated by cellular immunity, and type-1 helper T (Th1) cells play a central role in this process. This infection causes a prompt accumulation of both NKT and $\gamma\delta$ T cells in the lung tissues in a monocyte chemoattractant protein (MCP)-1-dependent or -independent manner, respectively. Genetic deletion of Vα14+ NKT cells ameliorates the Th1 response and clearance of microorganisms in the lungs, whereas these host protective responses are rather enhanced in mice lacking $\gamma\delta$ T cells. Thus, in some aspect, these innate immune lymphocytes may co-regulate the Th1-mediated response for induction of the moderate host defense. $\gamma\delta$ T cells may act to keep the balance of Th1-Th2 responses in a proper manner by suppressing the exaggerated Th1 response caused by NKT cells. In this review, I describe the recent research development in the innate immune host defense against cryptococcal infection in respiratory organs with emphasis on our data in the regulatory role of NKT cells and $\gamma\delta$ T cells.

1. Introduction

Airway is directly connected to the outer environment and always exposed to infectious agents, such as bacteria, fungi and viruses. To protect from these harmful agents, respiratory organs of hosts develop a highly sophisticated defense system. Initially, anatomical and mechanical systems trap large size particles in the inhaled air, which include nasal hairs, nasopharyngeal channels, glottis and highly divided branches of bronchi. These particles are caught by the mucous blanket lining the bronchial surface and cleared by ciliary movement into the upper airway. In contrast, small size particles less than 5 μm in diameter including most infectious pathogens reach the alveolar spaces. In order to keep the sterility in lung, additional mechanisms are equipped in these areas, which are largely divided into the two categories: innate and acquired host defense systems. The former consists of humoral components including antimicrobial proteins and complements, phagocytic cells like neutrophils and macrophages, dendritic cells and innate immune lymphocytes, while the latter is associated with antigen-specific responses mediated by antibody and cellular immunity.

Fungal infection is believed to relate with dysfunction of innate immunity. Infection of *Cryptococcus neoformans*, a yeast-like fungal pathogen with a thick polysaccharide capsule takes place by inhaling the desiccated yeast cells into the lungs. The organisms reach the subpleural area to establish the primary lesions. In normal hosts, the infection is usually self-limiting, since host defense mechanisms can eliminate the infection. In contrast, in immunocompromised

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patients with impaired cell-mediated immunity, the infection is not limited to the primary site of infection; it frequently disseminates to the central nervous system, which is often associated with a high mortality in these patients. Disseminated infection of this fungal pathogen to the brain has attracted clinical attention as a serious problem, particularly with the increased number of patients with AIDS (1,5).

In addition to conventional T cells that recognize peptide antigens in context of MHC class I or class II molecules, a number of unconventional T cell subsets have been identified (1,2). These subsets include natural killer (NK) T cells and $\gamma\delta$ antigen receptor-bearing T ($\gamma\delta$ T) cells, which are recognized as the innate immune lymphocytes. When infectious pathogens invade the tissues, these cells promptly respond by producing a variety of cytokines, which results in the promoted host protective responses. However, their overall potential is not sufficient for complete eradication of the infection, which needs more potent protective mechanisms by developing the subsequent acquired immune responses. Based on this property, the innate immune lymphocytes have been recognized merely as a "temporary protector" until the acquired immune response is established. However, recent investigations have accumulated evidences indicating that innate immune lymphocytes determine the quality of acquired immune responses (2-5), which may allow the early host protective responses mediated by these cells to be identified as more than a "temporary protector" before development of acquired immunity.

In this review, I describe the recent research development in the innate immune host defense against cryptococcal infection in respiratory organs with emphasis on our data in the regulatory role of NKT cells and $\gamma\delta$ T cells in a murine model of pulmonary infection with *C. neoformans*.

2. Basics in the host defense to cryptococcal infection

C. neoformans shows the features of intracellular parasitism within phagocyte cells, as well known in *Mycobacterium tuberculosis*, *Listeria monocytogenes* and *Salmonella typhimurium* (6). Because such pathogens resist the killing mechanisms, phagocytic cells fail to eradicate them without activation by interferon (IFN)-γ. Compatibly, the host defense against *C. neoformans* is critically regulated by cell-mediated immunity (7), and CD4+ T cells play a central role in eradicating this infection (8-10). The balance between type-1 helper T (Th1) and Th2 cytokines markedly influences the outcome of infection; the predominant synthesis of Th1 cytokines over Th2 protects mice from infection, whereas infection is exacerbated under a Th2 dominant condition (11,12). Mice depleted of Th1-type cytokines (e.g., IFN-γ and TNF-α) are highly susceptible to cryptococcal infection (13,14), while the infection is less severe in mice lacking Th2 cytokines (e.g., IL-4 and IL-10) than control mice (15,16). Differentiation of naive helper T cells into Th1 cells absolutely requires the presence of IL-12 (17), and this response is strongly potentiated by IL-18 (18). In recent investigations (15,19), targeted disruption of the gene for IL-12 or IL-18 resulted in attenuated host resistance and Th1 response to *C. neoformans*, indicating the prerequisite role for these cytokines in the development of host protective response.

3. Role of NKT cells

3-1. General features of NKT cells

NKT cell is a unique T cell subset that shares the features of NK cells. Originally, this population was discovered as a lymphocyte subset expressing both T cell receptor (TCR) $\alpha\beta$ and NK1.1 or NKR-P1 (CD161) in mice (20-22). Specific characteristics of this cell type include highly limited repertoire with an invariant Vα chain consisting of Vα14-Jα18 gene segment and highly skewed Vβ chains, Vβ8.2, 7 and 2 in mice and with Vα24-Jα18 and Vβ11 in human. By this meaning, these cells are called invariant (*i*)NKT cells. The mouse *i*NKT cells constitute CD4+ and double negative (DN) subsets. They usually do not express CD8, although CD8+ subset can be found in human. The development of *i*NKT cells is dependent on the non-classical MHC class I molecule CD1d, which is composed of non-polymorphic heavy chain and β2 microglobulin, as shown by the findings that they disappear in CD1d gene-disrupted (CD1d-KO) mice. The glycosphingolipid, α-galactosylceramide (α-GalCer) that was originally discovered from marine sponge as a novel anti-cancer agent is recognized by *i*NKT cells in context of CD1d, which results in their strong activation. These cells are found in large numbers in the liver, thymus and bone marrow and in small numbers in the spleen and lungs.

3-2. Regulatory role of NKT cells in Th1-Th2 cytokine balance

In earlier investigations (23), Yoshimoto and co-workers demonstrated that in vivo activation of NKT cells resulted in a rapid production of IL-4. Based on this observation, they speculated that this cell population may be the major source of early IL-4 production in the differentiation of Th2 cells, although it was not confirmed by subsequent studies (24,25). Recent investigations revealed that *i*NKT cells promptly secrete large amounts of both IFN-γ and IL-4 after engagement of the antigen receptor (20-22,26,27), suggesting the dual roles of this subset in the differentiation of both Th1 and Th2 cells. Vα14 TCR transgenic mice showed elevated serum levels of IgE and IL-4 (28), and activation of *i*NKT cells by α-GalCer induced T cell response to ovalbumin (OVA) polarized toward Th2-dominant condition (29). In contrast, other studies emphasized a positive role for NKT cells in the development of Th1 cells. Administration of α-GalCer led to the rapid production of IFN-γ by *i*NKT cells and other bystander cells, such as NK cells, in vitro (30) and suppressed in vivo Th2 differentiation and subsequent IgE synthesis caused by OVA immunization or infection with *Nippostrongylus brasiliensis* through the induction of IFN-γ production (31). Further evidences supporting this notion were accumulated: granuloma formation caused by mycobacterial lipid antigen (32) and IFN-γ-mediated protection of mice against infection with malaria parasites through ligand-specific activation of *i*NKT cells (33).

3-3. Significance of NKT cells in infectious diseases

There are several published studies addressing the role of NKT cells in the host defense against infectious pathogens. These studies are conducted using anti-CD1d mAb-treated and CD1d-KO mice, which manipulations abolish most of NKT cells, and Jα18 gene-disrupted (KO) mice, which lack particular NKT cell subset bearing Vα14-Jα18 antigen receptors. Based on these investigations, three roles are identified for NKT cells in the host defense to infection. First, the clinical course of *M. tuberculosis* infection in CD1d-KO mice is not much different from that in control mice (34,35) and

minimally affected by treatment with anti-CD1d mAb (36). Similarly, genetic depletion of *i*NKT cells does not result in exacerbation of infection with *M. tuberculosis*, *M. bovis* BCG and *S. choleraesuis* (34,37). Second, infection with *L. monocytogenes* or *Toxoplasma gondii* is rather improved by manipulations designed to suppress the activity of NKT cells (38,39). Administration of anti-CD1d mAb results in prolongation of *Listeria* infection in mice, which is associated with increased secretion of Th1-type cytokines and decreased TGF-β production (38). Similarly, depletion of NKT cells by anti-IL-2Rβ mAb enhances the host protection of mice from *T. gondii* infection by increasing Th1-polarized cytokine production (39). Finally, mice lacking Vα14+ NKT cells are more susceptible to *Streptococcus pneumoniae*, *Leishmania major* and *Trypanosoma cruzi* infection than control mice (40-42). Similar results are reported in CD1d-KO mice infected with *Pseudomonas aeruginosa*, *Borrelia burgdorferi* and *Plasmodium yoelii* (43-45). Thus, the significance of NKT cells in infectious diseases seems different from pathogen to pathogen.

3-4. Recruitment of NKT cells in the lung after cryptococcal infection

To understand the role of NKT cells in the host defense against pulmonary infection with *C. neoformans*, we elucidated whether these cells increased in the infected tissues (46). Inflammatory leukocytes obtained from the homogenates of infected lungs were stained with anti-TCR$\alpha\beta$ and -NK1.1 mAbs to discriminate conventional T, NK and NKT cells. The proportions of conventional T, NK and NKT cells, as indicated by TCR$\alpha\beta^+$NK1.1$^-$, TCR$\alpha\beta^-$NK1.1$^+$ and TCR$\alpha\beta^+$NK1.1$^+$ cells, respectively, started to increase on day 1, reached a peak level on day 6 and then decreased on day 10 post-infection. Interestingly, NKT cells most profoundly increased at the infected sites among these cells. We further defined the dynamics of *i*NKT cells bearing Vα14 TCR in the infected lungs by detecting cells bound to either anti-Vα14 mAb or α-GalCer-loaded CD1d tetramer. Similar kinetics was observed in this particular subset of NKT cells using both strategies for detection. Thus, *i*NKT cells as well as conventional T and NK cells were found to accumulate in the lungs after intratracheal infection with *C. neoformans*.

Migration of inflammatory leukocytes is critically regulated by a variety of chemokines, which are classified into two major subgroups, CXC- and CC-chemokines, based on the arrangement of two N-terminal cysteine residues (47). ELR$^+$ CXC-chemokines, including IL-8, are neutrophil-mediated inflammatory responses, while ELR$^-$ CXC-chemokines (e.g., IP-10 and Mig) and CC-chemokines (e.g., MCP-1, MIP-1α, -1β and RANTES) predominantly attract lymphocytes and macrophages. Many investigators have reported that resting or activated NK cells are attracted by many chemokines, including MCP-2, -3, MIP-1α, RANTES, IP-10 and lymphotactin, under various conditions (48-53). In contrast, MIP-2 had been the only chemokine that functions in trafficking NKT cells before we identified MCP-1 as a chemoattractant for this lymphocyte subset (54). In MCP-1KO mice, accumulation of NKT cells in lungs was profoundly attenuated after infection with *C. neoformans* (46). Consistent with these data, MCP-1 production preceded the kinetics of NKT cell-mediated inflammatory responses. Thus, NKT cell trafficking into the fungus-infected tissues involves at least in part the production of MCP-1, although other chemokines may contribute, as observed in NK cells.

3-5. Role of NKT cells in Th1 response and host defense to cryptococcal infection

NKT cells promptly produce a large amount of IFN-γ and IL-4 upon stimulation via their antigen receptors (20-22). Accumulating evidences indicate that NKT cells are involved in the regulation of Th1 and Th2 cell development. On the other hand, host defense to cryptococcal infection is critically regulated by the balance between Th1- and Th2-mediated immune responses (11,12). These findings suggest that NKT cells may affect the host immune responses and protection against infection with this fungal pathogen. In our study (46), Th1-mediated immune responses, as indicated by antigen-specific IFN-γ production by T cells and delayed-type hypersensitivity reaction, were significantly ameliorated in Jα18-KO mice lacking *i*NKT cells, compared with control wild-type mice. In contrast, Th2 cytokine synthesis was not influenced in these mice. Furthermore, the clearance of fungal pathogen from the infected tissues was significantly delayed in Jα18-KO mice, compared with control mice. These findings demonstrate that *i*NKT cells function not only in the innate immune phase but also in bridging to the Th1-mediated acquired immune responses, which leads to host protection against cryptococcal infection.

3-6. Natural ligands

*i*NKT cells express antigen receptors with an invariant Vα chain and highly skewed Vβ chains. Based on this property, many investigators had predicted a particular molecule to be the ligand for this lymphocyte subset. Kawano and co-workers (26) are the first group which reported that α-GalCer is a specific ligand for antigen receptors of these cells. However, the endogenous natural ligands of *i*NKT cells have not been defined, because mammals do not generate α-GalCer, originally discovered from marine sponge. Using crystal structure and mass spectrometry analyses, Joyce and co-workers (55) found glycosylphosphatidyl inositol (GPI) to be a candidate molecule that could bind to CD1d and present to NKT cell antigen receptors. In addition, it was demonstrated by Schofield et al. (56) that NKT cells regulated IgG production against GPI-anchored surface antigens of protozoans, *Plasmodium* and *Trypanosoma*. Similar results were reported by Duthie et al. (42) in mice infected with *T. cruzi*. They indicated the attenuation in chronic phase of antibody response to GPI-anchored surface antigens in NKT cell-deficient mice. From these observations, GPI is speculated as a molecule recognized by NKT cells as the endogenous and exogenous natural ligand. However, conflicting data are recently provided by other investigators (57,58). The IgG response to malarial GPI-anchored proteins is dependent on MHC class II, but not on CD1d (57). In other study, GPI-anchored mucin-like glycoproteins from *T. cruzi* bind to CD1d but do not elicit dominant innate or adaptive immune responses via the CD1d/NKT cell pathway (58). Thus, no defined ligand has so far been discovered. In order to understand the precise mechanism in which NKT cells contribute to the host defense against infection, identification of pathogen-derived ligands is desired. In this regard, Brigl and co-workers have recently reported a putative contribution of endogenous, but not pathogen-derived ligands for CD1d-dependent activation of NKT cells after infection with *S. typhimurium* and *Staphylococcus aureus* in mice (59). Further approaches will be required before understanding the mechanism of pathogen-related activation of NKT cells.

3-7. Induction of Th1 response and host defense to cryptococcal infection by ligand-specific activation

*i*NKT cells recognize α-GalCer by their antigen receptors in context of CD1d molecules expressed on DCs (20-22,27). Such engagement causes prompt secretion of both IFN-γ and IL-4 by these cells and emergence of their cytolytic activity against tumour cells. Toura et al. (60) indicated that administration of DCs pulsed with α-GalCer induced potent antitumor activity through specific activation of *i*NKT cells, and resulted in the complete suppression of melanoma metastasis in the liver.

In infectious diseases, Gonzalez-Aseguinolaza and co-workers (33) for the first time demonstrated the effectiveness of α-GalCer treatment in improving the clinical course of murine malaria. The development of liver stage, but not blood stage, malaria was strongly inhibited via induction of IFN-γ synthesis by α-GalCer. The same group recently revealed that co-administration of α-GalCer potentiated the protective effect against this infection caused by immunization with irradiated malaria parasite (61). Our group observed similar effects of this treatment in a murine model of cryptococcal infection (62). Administration of α-GalCer strongly enhanced the production of IFN-γ by NK and Th1 cells and significantly reduced the number of live colonies of *C. neoformans* in the infected organs, compared with vehicle treatment. These effects were not detected in Jα18-KO mice, indicating the involvement of *i*NKT cells. IFN-γ production induced by α-GalCer was totally mediated by IL-12, but not IL-18 (63). Similar findings are recently reported in *P. aeruginosa* and *M. tuberculosis* by other investigators (43, 64), although the contribution of *i*NKT cells to the host defense against the latter infection remains not clearly defined (34). These observations suggest that α-GalCer can be a promising immunotherapeutic agent for the treatment of certain intractable infectious diseases including cryptococcal meningitis complicated in immunodeficient patients.

4. Role of γδ T cells

4-1. General features of γδ T cells

Besides conventional T cells bearing TCRαβ, a distinct subset of T cells expressing novel antigen receptors consisting of γ and δ chains, designated as γδ T cells, was discovered approximately 20 years ago (65). In a sharp contrast to αβ T cells, which are the major population in lymphoid tissues, such as lymph node and spleen, γδ T cells are preferentially localized in non-lymphoid tissues, including epidermis, where γδ T cells are very common as dendritic epidermal T cells, and mucosal/epithelial tissues, such as intestine, lung, tongue, mammary, uterine and vaginal epithelia. Such characteristic localization suggests the role of these cells in a first line host defense against infectious agents and other antigens.

In human, TCRγ gene segments are located on chromosome 7, while TCRδ gene segments are interspersed with TCRα gene on chromosome 14. Before birth, Vγ8 and Vγ9 subsets associate with Vδ2 subset, which distribute to peripheral blood and tonsil. After birth, Vγ2, Vγ3, Vγ4, Vγ5 and Vγ8 gene segments are rearranged and associated with Vδ1 subset, which are found located preferentially in mucosal tissues, such as intestine (65,66). On the other hand, mouse TCRγ gene segments are found on chromosome 13 and TCRδ gene segments are dispersed with TCRα gene on chromosome 14 (65). Similar to human, mouse γδ T cell subsets show particular localization patterns. Vγ5, Vγ6 and Vγ4 subsets are formed during gestation and Vγ1 and Vγ7 subsets at birth and shortly thereafter. These subsets are localized in defined anatomical sites: Vγ5 in skin, Vγ6 in uterus and lung, Vγ4 in spleen, lung and tongue, Vγ1 in spleen, and Vγ7 in intestine. In an analysis with quantitative PCR technique, Vγ6 subset is the only γδ T cells found in lung when mice are born. After birth, however, Vγ4, Vγ5 and Vγ7 subsets were detected and by 2-3 months of age, Vγ4 subset becomes predominant among pulmonary γδ T cells (66-69).

4-2. Natural ligands

The number of V gene segments of γδ T cells that determine their diversity is very limited when compared with that in αβ T cells. In addition, particular subsets are localized in the defined anatomical areas and at the different developmental stages. From these features, the diversity of antigen recognition by γδ T cells is assumed to be limited in contrast to αβ T cells that recognize broad spectrum of antigens (65,66). Previous investigations have identified a variety of antigens recognized by these cells from microbial products. Human Vγ9/Vδ2+ γδ T cells react with low molecular weight nonproteinaceous antigens, such as prenyl pyrophosphate and nucleotide triphosphate from *M. tuberculosis* and alkylamine from *Proteus morganii*, in non MHC-restricted manner (70,71). In addition, protein antigens can be a ligand for the activation of γδ T cells. Human Vγ9/Vδ2+ γδ T cells recognize tetanus toxoid in context of MHC class II molecules (72). Mycobacterial heat-shock proteins stimulate both human and mouse γδ T cells (73-75). However, no ligand of these cells has so far been identified from fungal microorganisms, including *C. neoformans*.

4-3. Significance of γδ T cells in infectious diseases

From previous observations showing that γδ T cells accumulate at the sites of infection and chronic inflammation (76), their involvement in regulating the immune response has been suggested. γδ T cells secrete a variety of cytokines, including TNF-α, GM-CSF, IFN-α, IFN-β, IFN-γ, IL-2, IL-4, IL-5 and IL-10, under particular conditions, such as infection by microorganisms (77-81). Ferrick and co-workers (82) indicated that these cells produce Th1-type cytokines in mice infected with *L. monocytogenes*, while Th2 cytokines in infection with *N. brasilliensis*. Furthermore, some γδ T cells can express cytolytic activity against infected cells and tumor cells in a perforin and Fas-L-dependent manner (83).

In experimental animal models of infectious diseases, γδ T cells exert different patterns of influences on the host protection. Manipulations that result in ablation of γδ T cells, e.g., genetic disruption and treatment with a specific Ab, rendered mice susceptible to infection with *Klebsiella pneumoniae*, *Escherichia coli*, *L. monocytogenes*, *M. tuberculosis*, *L. major* and *T. gondii* (84-91). Interestingly, similar manipulations rather improved the infection caused by some microorganisms (92-94). In chlamydial infection, γδ T cells showed contrast roles at early and late stages (95). Thus, γδ T cells seem to act in a complex manner from one microbe to another and in the stage of infection.

4-4. Regulatory role in host defense to cryptococcal infection

In our recent study, the role of γδ T cells in the development of Th1 response and the host defense against pulmonary infection with *C. neoformans* has been investigated using a mouse model of pulmonary cryptococcosis (96). γδ T cells quickly increased in a similar kinetics as observed in NK and

NKT cells. Although the precise mechanism remains to be elucidated, such increase of γδ T cells in the infected lungs seemed to take place in a different manner from that in NK and NKT cells. Accumulation of NK and NKT cells in lungs after cryptococcal infection was markedly reduced in MCP-1-KO mice, while such reduction was not found in γδ T cells. At present, the precise mechanism of γδ T cell recruitment remains to be clarified.

Interestingly, clearance of *C. neoformans* in lungs was enhanced in mice receiving a manipulation that deletes γδ T cells by administration of specific antibody or targeted disruption of Cδ gene. Such increased host defense was associated with the promoted differentiation of Th1 cells and increased production of IFN-γ. These observations suggest the down-regulatory role of γδ T cells in the host defense to cryptococcal infection. This is in a sharp contrast to the role of NKT cells, which significantly contribute to the development of Th1-type immune response and host resistance to this infection (45). Earlier investigations reported anti-inflammatory γδ T cells that produced Th2 cytokines and TGF-β (97,98). These observations suggest that these cytokines mediate the down-regulatory effect observed in our study. This speculation was supported by our recent data showing the reduced production of TGF-β in the lungs of Cδ-KO mice totally lacking γδ T cells at earlier phase of cryptococcal infection, although the synthesis of Th2 cytokines, IL-4 and IL-10, was not much different from control mice. In this regard, TGF-β is known to suppress the host defense to infectious pathogens (99-102). Furthermore, other investigations revealed that γδ T cells down-regulate the host defense against infection caused by *L. monocytogenes*, *S. choleraesuis* and *Candida albicans* (92-94). Thus, our study suggests that γδ T cells may play down-regulatory roles in the host defense to pulmonary infection with *C. neoformans*.

5. Conclusions

Recently, the role of innate immunity in host defense to infectious pathogens has attracted much attention by many investigators according to the biological significance. In our series of studies on cryptococcal infection, the contribution of NKT and γδ T cells has been unveiled. Contrast roles of NKT and γδ T cells raise a possibility that these innate immune lymphocytes may co-regulate the Th1-mediated response for induction of the moderate host defense, as indicated in Fig. 1. γδ T cells may act to keep the balance of Th1-Th2 responses in a proper manner by suppressing the exaggerated Th1 response caused by NKT cells. In pulmonary infection with *C. neoformans*, number of both NKT and γδ T cells in the paratracheal lymph nodes increases in parallel with that of DCs (our unpublished data), which could be consistent with the above hypothesis. Interestingly, in toxoplasmal infection, γδ T cells appear to play a protective role in the host defense through promoting Th1-mediated immune response, while NKT cells are likely to suppress these responses (91). This is in a sharp contrast to the findings in cryptococcal infection. Thus, NKT and γδ T cells are suggested to participate in the regulation of host defense to infection by bridging from innate to antigen-specific acquired immune responses.

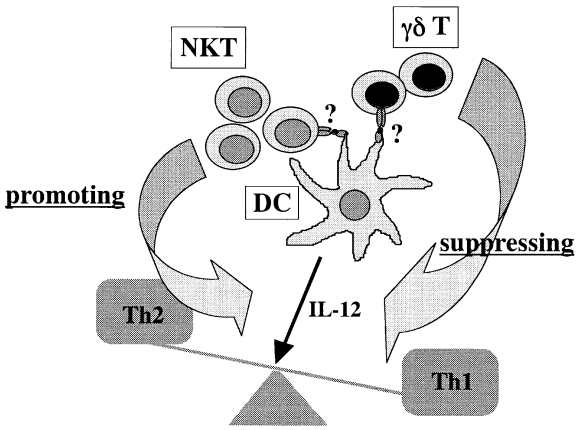

Fig. 1. Regulation of the host defense to cryptococcal infection by NKT and γδ T cells.
Host defense to cryptococcal infection is critically regulated by Th1-Th2 cytokine balance. The predominant synthesis of Th1 cytokines over Th2 protects mice from infection, whereas infection is exacerbated under a Th2-dominant condition. NKT cells regulate this balance to promote the host protection, whereas γδ T cells counter-regulate this process. Thus, these innate immune lymphocytes may act to keep the host defense in a proper manner, although the mechanism of their activation remains to be elucidated.

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REFERENCES

1. Stevens, D. A. (1990): Fungal infections in AIDS patients. Br. J. Clin. Pract., Suppl. 71, 11-22.
2. Emoto, M., Emoto. M. and Kaufmann, S. H. (2003): Liver NKT cells: an account of heterogeneity. Trends Immunol., 24, 364-369.
3. Horwitz, D. A., Gray, J. D. and Ohtsuka, K. (1999): Role of NK cells and TGF-beta in the regulation of T-cell-dependent antibody production in health and autoimmune disease. Microbes Infect., 1, 1305-1311.
4. Schaible, U. E. and Kaufmann, S. H. (2000): CD1 molecules and CD1-dependent T cells in bacterial infections: a link from innate to acquired immunity? Semin. Immunol., 12, 527-535.
5. Nishimura, T., Kitamura, H., Iwakabe, K., Yahata, T., Ohta, A., Sato, M., Takeda, K., Okumura, K., van Kaer, L., Kawano, T., Taniguchi, M., Nakui, M., Sekimoto, M. and Koda, T. (2000): The interface between innate and acquired immunity: glycolipid antigen presentation by CD1d-expressing dendritic cells to NKT cells induces the differentiation of antigen-specific cytotoxic T lymphocytes. Int. Immunol., 12, 987-994.
6. Feldmesser, M., Tucker, S. and Casadevall, A. (2001): Intracellular parasitism of macrophages by *Cryptococcus neoformans*. Trends Microbiol., 9, 273-278.
7. Lim, T. S. and Murphy, J. W. (1980): Transfer of immunity to cryptococcosis by T-enriched splenic lymphocytes from *Cryptococcus neoformans*-sensitized mice. Infect. Immun., 30, 5-11.

8. Mody, C. H., Lipscomb, M. F., Street, N. E. and Toews, G. B. (1990): Depletion of CD4+ (L3T4+) lymphocytes in vivo impairs murine host defense to *Cryptococcus neoformans*. J. Immunol., 144, 1472-1477.
9. Huffnagle, G. B., Yates, J. L. and Lipscomb, M. F. (1991): Immunity to a pulmonary *Cryptococcus neoformans* infection requires both CD4+ and CD8+ T cells. J. Exp. Med., 173, 793-800.
10. Hill, J. O. and Harmsen, A. G. (1991): Intrapulmonary growth and dissemination of an avirulent strain of *Cryptococcus neoformans* in mice depleted of CD4+ or CD8+ T cells. J. Exp. Med., 173, 755-758.
11. Kawakami, K., Tohyama, M., Qifeng, X. and Saito, A. (1997): Expression of cytokines and inducible nitric oxide synthase mRNA in the lungs of mice infected with *Cryptococcus neoformans*: effects of interleukin-12. Infect. Immun., 65, 1307-1312.
12. Koguchi, Y. and Kawakami, K. (2002): Cryptococcal infection and Th1-Th2 cytokine balance. Int. Rev. Immunol., 21, 423-438.
13. Kawakami, K., Tohyama, M., Teruya, K., Kudeken, N., Xie, Q. and Saito, A. (1996): Contribution of interferon-gamma in protecting mice during pulmonary and disseminated infection with *Cryptococcus neoformans*. FEMS Immunol. Med. Microbiol., 13, 123-130.
14. Huffnagle, G. B., Toews, G. B., Burdick, M. D., Boyd, M. B., McAllister, K. S., McDonald, R. A., Kunkel, S. L. and Strieter, R. M. (1996): Afferent phase production of TNF-alpha is required for the development of protective T cell immunity to *Cryptococcus neoformans*. J. Immunol., 157, 4529-4536.
15. Decken, K., Kohler, G., Palmer-Lehmann, K., Wunderlin, A., Mattner, F., Magram, J., Gately, M. K. and Alber, G. (1998): Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. Infect. Immun., 66, 4994-5000.
16. Blackstock, R., Buchanan, K. L., Adesina, A. M. and Murphy, J. W. (1999): Differential regulation of immune responses by highly and weakly virulent *Cryptococcus neoformans* isolates. Infect. Immun., 67, 3601-3609.
17. Trinchieri, G. (1995): Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu. Rev. Immunol., 13, 251-276.
18. Robinson, D., Shibuya, K., Mui, A., Zonin, F., Murphy, E., Sana, T., Hartley, S. B., Menon, S., Kastelein, R., Bazan, F. and O'Garra, A. (1997): IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity, 7, 571-581.
19. Kawakami, K., Koguchi, Y., Qureshi, M. H., Miyazato, A., Yara, S., Kinjo, Y., Iwakura, Y., Takeda, K., Akira, S., Kurimoto, M. and Saito, A. (2000): IL-18 contributes to host resistance against infection with *Cryptococcus neoformans* in mice with defective IL-12 synthesis through induction of IFN-gamma production by NK cells. J. Immunol., 165, 941-947.
20. Taniguchi, M. and Nakayama, T. (2000): Recognition and function of Valpha14 NKT cells. Semin. Immunol., 12, 543-550.
21. Godfrey, D. I., Hammond, K. J. L., Poulton, L. D., Smyth, M. J. and Baxter, A. G. (2000): NKT cells: facts, functions and fallacies. Immunol. Today, 21, 573-583.
22. Kronenberg, M. and Gapin, L. (2002): The unconventional lifestyle of NKT cells. Nat. Rev. Immunol., 2, 557-568.
23. Yoshimoto, T., Bendelac, A., Watson, C., Hu-Li, J. and Paul, W. E. (1995): Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. Science, 270, 1845-1847.
24. Brown, D. R., Fowell, D. J., Corry, D. B., Wynn, T. A., Moskowitz, N. H., Cheever, A. W., Locksley, R. M. and Reiner, S. L. (1996): beta2-microglobulin-dependent NK1.1+T cells are not essential for T helper cell 2 immune responses. J. Exp. Med., 184, 1295-1304.
25. Chen, Y.-H., Chiu, N. M., Mandal, M., Wang, N. and Wang, C. R. (1997): Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice. Immunity, 6, 459-467.
26. Kawano, T., Cui, J., Koezuka, Y., Toura, I., Kaneko, Y., Motoki, K., Ueno, H., Nakagawa, R., Sato, H., Kondo, E., Koseki, H. and Taniguchi, M. (1997): CD1d-restricted and TCR-mediated activation of Valpha14 NKT cells by glycosylceramides. Science, 278, 1626-1629.
27. Burdin, N., Brossay, L., Koezuka, Y., Smiley, S. T., Grusby, M. J., Taniguchi, M., Hayakawa, K. and Kronenburg, M. (1998): Selective ability of mouse CD1 to present glycolipids: alpha-galactosylceramide specifically stimulates Valpha14 NK T lymphocytes. J. Immunol., 161, 3271-3281.
28. Lehuen, A., Lantz, O., Beaudoin, L., Laloux, V., Carnaud, C., Bendelac, A., Bach, J. F. and Monteiro, R. C. (1998): Overexpression of natural killer T cells protects Valpha14- Jalpha281 transgenic nonobese diabetic mice against diabetes. J. Exp. Med., 188, 1831-1839.
29. Singh, N., Hong, S., Scherer, D. C., Serizawa, I., Burdin, N., Kronenberg, M., Koezuka, Y. and van Kaer, L. (1999): Activation of NK T cells by CD1d and α-galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype. J. Immunol., 163, 2373-2377.
30. Carnaud, C., Lee, D., Donnars, O., Park, S. H., Beavis, A., Koezuka, Y. and Bendelac, A. (1999): Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J. Immunol., 163, 4647-4650.
31. Cui, J., Watanabe, N., Kawano, T., Yamashita, M., Kamata, T., Shimizu, C., Kimura, M., Shimizu, E., Oike, J., Koseki, H., Tanaka, Y., Taniguchi, M. and Nakayama, T. (1999): Inhibition of T helper cell type 2 cell differentiation and immunoglobulin E response by ligand-activated Valpha14 natural killer cells. J. Exp. Med., 190, 783-792.
32. Apostolou, I., Takahama, Y., Belmant, C., Kawano, T., Huerre, M., Marchal, G., Cui, J., Taniguchi, M., Nakauchi, H., Fournie, J.-J., Kourilsky, P. and Gachelin, G. (1999): Murine natural killer cells contribute to the granulomatous reaction caused by mycobacterial cell walls. Proc. Natl. Acad. Sci. USA, 96, 5141-5146.
33. Gonzalez-Aseguinolaza, G., de Oliveira, C., Tomsaka, M., Hong, S., Bruna-Romero, O., Nakayama, T., Taniguchi, M., Bendelac, A., van Kaer, L., Koezuka, Y. and Tsuji, M. (2000): alpha-galactosylceramide-activated Valpha14 natural killer T cells mediate protection against murine malaria. Proc. Natl. Acad. Sci. USA, 97, 8461-8466.
34. Kawakami, K., Kinjo, Y., Uezu, K., Yara, S., Miyagi, K., Koguchi, Y., Nakayama, T., Taniguchi, M. and Saito,

A. (2002): Minimal contribution of Valpha14 natural killer T cells to Th1 response and host resistance against mycobacterial infection in mice. Microbiol. Immunol., 46, 207-210.
35. Sousa, A. O., Mazzaccaro, R. J., Russell, R. G., Lee, F. K., Turner, O. C., Hong, S., van Kaer, L. and Bloom, B. R. (2000): Relative contributions of distinct MHC class 1-dependent cell populations in protection to tuberculosis infection in mice. Proc. Natl. Acad. Sci. USA, 97, 4204-4208.
36. Szalay, G., Zugel, U., Ladel, C. H. and Kaufmann, S. H. E. (1999): Participation of group 2 CD1 molecules in the control of murine tuberculosis. Microb. Infect., 1, 1153-1157.
37. Ishigami, M., Nishimura, H., Naiki, Y., Yoshioka, K., Kawano, T., Tanaka, Y., Taniguchi, M., Kakumu, S. and Yoshikai, Y. (1999): The roles of intrahepatic Valpha14+ NK1.1+ T cells for liver injury induced by *Salmonella* infection in mice. Hepatology, 29, 1799-1808.
38. Szalay, G., Ladel, C. H., Blum, C., Brossay, L., Kronenberg, M. and Kaufmann, S. H. E. (1999): Anti-CD1 monoclonal antibody treatment reverses the production patterns of TGF-beta2 and Th1 cytokines and ameliorates listeriosis in mice. J. Immunol., 162, 6955-6958.
39. Nakano, Y., Hisaeda, H., Sakai, T., Zhang, M., Maekawa, Y., Zhang, T. and Himeno, K. (2001): Role of innate immune cells in protection against *Toxoplasma gondii* at inflamed site. J. Med. Invest., 48, 73-80.
40. Kawakami, K., Yamamoto, N., Kinjo, Y., Miyagi, K., Nakasone, C., Uezu, K., Kinjo, T., Nakayama, T., Taniguchi, M. and Saito, A. (2003): Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against *Streptococcus pneumoniae* infection. Eur. J. Immunol., 33, 3322-3330.
41. Ishikawa, H., Hisaeda, H., Taniguchi, M., Nakayama, T., Sakai, T., Maekawa, Y., Nakano, Y., Zhang, M., Zhang, T., Nishitani, M., Takashima, M. and Himeno, K. (2000): CD4+ Valpha14 NKT cells play a crucial role in an early stage of protective immunity against infection with *Leishmania major*. Int. Immunol., 12, 1267-1274.
42. Duthie, M. S., Wleklinski-Lee, M., Smith, S., Nakayama, T., Taniguchi, M. and Kahn, S. J. (2002): During *Trypanosoma cruzi* infection CD1d-restricted NK T cells limit parasitemia and augment the antibody response to a glycophosphoinositol-modified surface protein. Infect. Immun., 70, 36-48.
43. Nieuwenhuis, E. E., Matsumoto, T., Exley, M., Schleipman, R. A., Glickman, J., Bailey, D. T., Corazza, N., Colgan, S. P., Onderdonk, A. B. and Blumberg, R. S. (2002): CD1d-dependent macrophage-mediated clearance of *Pseudomonas aeruginosa* from lung. Nat. Med., 8, 588-593.
44. Kumar, H., Belperron, A., Barthold, S. W. and Bockenstedt, L. K. (2000): CD1d deficiency impairs murine host defense against the spirochete, *Borrelia burgdorferi*. J. Immunol., 165, 4797-4801.
45. Mannoor, M. K., Weerasinfhe, A., Halder, R. C., Reza, S., Morshed, M., Ariyasinghe, A., Watanabe, H., Sekikawa, H. and Abo, T. (2001): Resistance to malarial infection is achieved by the cooperation of $NK1.1^+$ and $NK1.1^-$ subsets of intermediate TCR cells which are constituents of innate immunity. Cell. Immunol., 211, 96-104.
46. Kawakami, K., Kinjo, Y., Uezu, K., Yara, S., Miyagi, K., Koguchi, Y., Nakayama, T., Taniguchi, M. and Saito, A. (2001): Monocyte chemoattractant protein-1-dependent increase of Valpha 14 NKT cells in lungs and their roles in Th1 response and host defense in cryptococcal infection. J. Immunol., 167, 6525-6532.
47. Rossi, D. and Zlotnik, A. (2000): The biology of chemokines and their receptors. Annu. Rev. Immunol., 217, 217-242.
48. Maghazachi, A. A., Skalhegg, B. S., Rolstad, B. and Al-Aoukaty, A. (1997): Interferon-inducible protei-10 and lymphotactin induce the chemotaxis and mobilization of intracellular calcium in natural killer cells through pertussis toxin-sensitive and -insensitive heterotrimeric G-proteins. FASEB J., 11, 765-774.
49. Taub, D. D., Sayers, T. J., Carter, C. R. and Ortaldo, J. R. (1995): Alpha and beta chemokines induce NK cell migration and enhance NK-mediated cytolysis. J. Immunol., 155, 3877-3888.
50. Allavena, P., Bianchi, G., Zhou, D., van Damme, J., Jilek, P., Sozzani, S. and Mantovani, A. (1994): Induction of natural killer cell migration by monocyte chemotactic protein-1, -2 and -3. Eur. J. Immunol., 24, 3233-3236.
51. Maghazachi, A. A., Al-Aoukaty, A. and Schall, T. J. (1994): C-C chemokine induce the chemotaxis of NK and IL-2-activated NK cells. Role for G-proteins. J. Immunol., 153, 4969-4977.
52. Loetscher, P., Seitz, M., Clark-Lewis, I., Baggiolini, M. and Moser, B. (1996): Activation of NK cells by CC chemokines. Chemotaxis, Ca^{2+} mobilization, and enzyme release. J. Immunol., 156, 322-327.
53. Giancarlo, B., Silvano, S., Albert, Z., Mantovani, A. and Allavena, P. (1996): Migratory response of human natural killer cells to lymphotactin. Eur. J. Immunol., 26, 3238-3241.
54. Faunce, D. E., Sonoda, K. and Stein-Streilein, J. (2001): MIP-2 recruits NKT cells to the spleen during tolerance induction. J. Immunol., 166, 313-321.
55. Joyce, S., Woods, A. S., Yewdell, J. W., Bennink, J. R., de Silva, A. D., Boesteanu, A., Balk, S. P., Cotter, R. J. and Brutkiewicz, R. R. (1998): Natural ligand of mouse CD1d: cellular glycosylphosphatidyl-inositol. Science, 279, 1541-1544.
56. Schofield, L., McConville, M. J., Hansen, D., Campbell, A. S., Fraser-Reid, B., Grusby, M. J. and Tachado, S. D. (1999): CD1d-restricted immunoglobulin G formation to GPI-anchored antigens mediated by NKT cells. Science, 283, 225-229.
57. Molano, A., Park, S. H., Chiu, Y. H., Nosseir, S., Bendelac, A. and Tsuji, M. (2000): Cutting edge: the IgG response to the circumsporozoite protein is MHC class II-dependent and CD1d-independent: exploring the role of GPIs in NK T cell activation and antimalarial responses. J. Immunol., 164, 5005-5009.
58. Procopio, D. O., Almeida, I. C., Torrecilhas, A. C., Cardoso, J. E., Teyton, L., Travassos, L. R., Bendelac, A. and Gazzinelli, R. T. (2002): Glycosylphosphatidylinositol-anchored mucin-like glycoproteins from *Trypanosoma cruzi* bind to CD1d but do not elicit dominant innate or adaptive immune responses via the CD1d/NKT cell pathway. J. Immunol., 169, 3926-3933.
59. Brigl, M., Bry, L., Kent, S. C., Gumperz, J. E. and

Brenner, M. B. (2003): Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. Nat. Immunol., 4, 1230-1237.
60. Toura, I., Kawano, T., Akutsu, Y., Nakayama, T., Ochiai, T. and Taniguchi, M. (1999): Inhibition of experimental tumor metastasis by dendritic cells pulsed with alpha-galactosylceramide. J. Immunol., 163, 2387-2391.
61. Gonzalez-Aseguinolaza, G., Van Kaer, L., Bergmann, C. C., Wilson, J. M., Schmieg, J., Kronenberg, M., Nakayama, T., Taniguchi, M., Koezuka, Y. and Tsuji, M. (2002): Natural killer T cell ligand alpha-galactosylceramide enhances protective immunity induced by malaria vaccines. J. Exp. Med., 195, 617-624.
62. Kawakami, K., Kinjo, Y., Yara, S., Koguchi, Y., Uezu, K., Nakayama, T., Taniguchi, M. and Saito, A. (2001): Activation of Valpha14(+) natural killer T cells by alpha-galactosylceramide results in development of Th1 response and local host resistance in mice infected with *Cryptococcus neoformans*. Infect. Immun., 69, 213-220.
63. Kawakami, K., Kinjo, Y., Yara, S., Uezu, K., Koguchi, Y., Tohyama, M., Azuma, M., Takeda, K., Akira, S. and Saito, A. (2001): Enhanced gamma interferon production through activation of Valpha14(+) natural killer T cells by alpha-galactosylceramide in interleukin-18-deficient mice with systemic cryptococcosis. Infect. Immun., 69, 6643-6650.
64. Chackerian, A., Alt, J., Perera, V. and Behar, S. M. (2002): Activation of NKT cells protects mice from tuberculosis. Infect. Immun., 70, 6302-6309.
65. Hayday, A. C. (2000): Gamma/delta cells: a right time and a right place for a conserved third way of protection. Annu. Rev. Immunol., 18, 975-1026.
66. Lahn, M. (2000): The role of gamma/delta T cells in the airways. J. Mol. Med., 78, 409-425.
67. Augustin, A., Kubo, R. T. and Sim, G. K. (1989): Resident pulmonary lymphocytes expressing the gamma/delta T-cell receptor. Nature, 340, 239-241.
68. Hayes, S. M., Sirr, A., Jacob, S., Sim, G. K. and Augustin, A. (1996): Role of IL-7 in the shaping of the pulmonary gamma delta T cell repertoire. J. Immunol., 156, 2723-2729.
69. Sim, G. K., Rajaserkar, R., Dessing, M. and Augustin, A. (1994): Homing and in situ differentiation of resident pulmonary lymphocytes. Int. Immunol., 6, 1287-1295.
70. Tanaka, Y., Morita, C. T., Tanaka, Y., Nieves, E., Brenner, M. B. and Bloom, B. R. (1995): Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. Nature, 375, 155-158.
71. Bukowski, J. F., Morita, C. T. and Brenner, M. B. (1999): Human gamma delta T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. Immunity, 11, 57-65.
72. Holoshitz, J., Vila, L. M., Keroack, B. J., McKinley, D. R. and Bayne, N. K. (1992): Dual antigenic recognition by cloned human gamma delta T cells. J. Clin. Invest., 89, 308-314.
73. O'Brien, R. L., Happ, M. P., Dallas, A., Palmer, E., Kubo, R. and 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, 667-674.
74. Born, W., Hall, L., Dallas, A., Boymel, J., Shinnick, T., Young, D., Brennan, P. and O'Brien, R. (1990): Recognition of a peptide antigen by heat shock-reactive gamma delta T lymphocytes. Science, 249, 67-69.
75. O'Brien, R. L., Fu, Y. X., Cranfill, R., Dallas, A., Ellis, C., Reardon, C., Lang, J., Carding, S. R., Kubo, R. and Born, W. (1992): Heat shock protein Hsp60-reactive gamma delta cells: a large, diversified T-lymphocyte subset with highly focused specificity. Proc. Natl. Acad. Sci. USA, 89, 4348-4352.
76. Janeway, C. A. Jr., Jones, B. and Hayday, A. (1988): Specificity and function of T cells bearing gamma delta receptors. Immunol. Today, 9, 73-76.
77. Porcelli, S., Brenner, M. B. and Band, H. (1991): Biology of the human gamma delta T-cell receptor. Immunol. Rev., 120, 137-183.
78. Morita, C. T., Verma, S., Aparicio, P., Martinez, C., Spits, H. and Brenner. M. B. (1991): Functionally distinct subsets of human gamma/delta T cells. Eur. J. Immunol., 21, 2999-3007.
79. Eichelberger, M., Allan, W., Carding, S. R., Bottomly, K. and Doherty, P. C. (1991): Activation status of the $CD4^{-}8^{-}$ gamma delta-T cells recovered from mice with influenza pneumonia. J. Immunol., 147, 2069-2074.
80. Taguchi, T., Aicher, W. K., Fujihashi, K., Yamamoto, M., McGhee, J. R., Bluestone, J. A. and Kiyono, H. (1991): Novel function for intestinal intraepithelial lymphocytes. Murine CD3+, gamma/delta TCR+ T cells produce IFN-gamma and IL-5. J. Immunol., 147, 3736-3744.
81. Bluestone, J. A., Cron, R. Q., Barrett, T. A., Houlden, B., Sperling, A. I., Dent, A., Hedrick, S., Rellahan, B. and Matis, L. A. (1991): Repertoire development and ligand specificity of murine TCR gamma delta cells. Immunol. Rev., 120, 5-33.
82. Ferrick, D. A., Schrenzel, M. D., Mulvania, T., Hsieh, B., Ferlin, W. G. and Lepper, H. (1995): Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. Nature, 373, 255-257.
83. Roessner, K., Wolfe, J., Shi, C., Sigal, L. H., Huber, S. and Budd, R. C. (2003): High expression of Fas ligand by synovial fluid-derived gamma delta T cells in Lyme arthritis. J. Immunol., 170, 2702-2710.
84. Moore, T. A., Moore, B. B., Newstead, M. W. and Standiford, T. J. (2000): Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine *Klebsiella pneumoniae*. J. Immunol., 165, 2643-2650.
85. Takano, M., Nishimura, H., Kimura, Y., Mokuno, Y., Washizu, J., Itohara, S., Nimura, Y. and Yoshikai, Y. (1998): Protective roles of gamma delta T cells and interleukin-15 in *Escherichia coli* infection in mice. Infect. Immun., 66, 3270-3278.
86. Hiromatsu, K., Yoshikai, Y., Matsuzaki, G., Ohga, S., Muramori, K., Matsumoto, K., Bluestone, J. A. and Nomoto, K. (1992): A protective role of gamma/delta T cells in primary infection with *Listeria monocytogenes* in mice. J. Exp. Med., 175, 49-56.
87. Ladel, C. H., Blum, C., Dreher, A., Reifenberg, K. and Kaufmann, S. H. (1995): Protective role of gamma/delta T cells and alpha/beta T cells in tuberculosis. Eur. J. Immunol., 25, 2877-2881.
88. King, D. P., Hyde, D. M., Jackson, K. A., Novosad, D. M., Ellis, T. N., Putney, L., Stovall, M. Y., van Winkle, L. S., Beaman, B. L. and Ferrick, D.A. (1999): Cutting

edge: protective response to pulmonary injury requires gamma delta T lymphocytes. J. Immunol., 162, 5033-5036.
89. Jones-Carson, J., Vazquez-Torres, A., van der Heyde, H. C., Warner, T., Wagner, R. D. and Balish, E. (1995): Gamma delta T cell-induced nitric oxide production enhances resistance to mucosal candidiasis. Nat. Med., 1, 552-557.
90. Rosat, J. P., MacDonald, H. R. and Louis, J. A. (1993): A role for gamma delta + T cells during experimental infection of mice with *Leishmania major*. J. Immunol., 150, 550-555.
91. Hisaeda, H., Nagasawa, H., Maeda, K., Maekawa, Y., Ishikawa, H., Ito, Y., Good, R. A. and Himeno, K. (1995): Gamma delta T cells play an important role in hsp65 expression and in acquiring protective immune responses against infection with *Toxoplasma gondii*. J. Immunol., 155, 244-251.
92. O'Brien, R. L., Yin, X., Huber, S. A., Ikuta, K. and Born, W. K. (2000): Depletion of a gamma delta T cell subset can increase host resistance to a bacterial infection. J. Immunol., 165, 6472-6479.
93. Emoto, M., Nishimura, H., Sakai, T., Hiromatsu, K., Gomi, H., Itohara, S. and Yoshikai, Y. (1995): Mice deficient in gamma delta T cells are resistant to lethal infection with *Salmonella choleraesuis*. Infect. Immun., 63, 3736-3738.
94. Wormley, F. L. Jr., Steele, C., Wozniak, K., Fujihashi, K., McGhee, J. R. and Fidel, P. L. Jr. (2001): Resistance of T-cell receptor delta-chain-deficient mice to experimental *Candida albicans* vaginitis. Infect. Immun., 69, 7162-7164.
95. Williams, D. M., Grubbs, B. G., Kelly, K., Pack, E. and Rank, R. G. (1996): Role of gamma-delta T cells in murine *Chlamydia trachomatis* infection. Infect. Immun., 64, 3916-3919.
96. Uezu, K., Kawakami, K., Miyagi, K., Kinjo, Y., Kinjo, T., Ishikawa, H. and Saito, A. (2004): Accumulation of gammadelta T cells in the lungs and their regulatory roles in Th1 response and host defense against pulmonary infection with *Cryptococcus neoformans*. J. Immunol., 172, 7629-7634.
97. Wesch, D., Glatzel, A. and Kabelitz, D. (2001): Differentiation of resting human peripheral blood gamma delta T cells toward Th1- or Th2-phenotype. Cell. Immunol., 212, 110-117.
98. Nagaeva, O., Jonsson, L. and Mincheva-Nilsson, L. (2002): Dominant IL-10 and TGF-beta mRNA expression in gamma delta T cells of human early pregnancy decidua suggests immunoregulatory potential. Am. J. Reprod. Immunol., 48, 9-17.
99. Reed, S. G. (1999): TGF-beta in infections and infectious diseases. Microbes Infect., 1, 1313-1325.
100. Letterio, J. J. and Roberts, A. B. (1998): Regulation of immune responses by TGF-beta. Annu. Rev. Immunol., 16, 137-161.
101. Hirsch, C. S., Ellner, J. J., Blinkhorn, R. and Toossi, Z. (1997): In vitro restoration of T cell responses in tuberculosis and augmentation of monocyte effector function against *Mycobacterium tuberculosis* by natural inhibitors of transforming growth factor beta. Proc. Natl. Acad .Sci. USA, 94, 3926-3931.
102. Li, J., Hunter, C. A. and Farrell, J. P. (1999): Anti-TGF-beta treatment promotes rapid healing of *Leishmania major* infection in mice by enhancing in vivo nitric oxide production. J. Immunol., 162, 974-979.