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Innate Immunity in the Lungs to Cryptococcal Infection

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1. Introduction

Humans breathe about 10,000 liters of air per day. Therefore, the airway is always exposed to infectious agents, such as bacteria, fungi, and viruses, and environmental hazards, including cigarette smoke, pollutants, and allergens. To protect against these harmful stresses, highly sophisticated immune systems are developed in the lung. These systems are largely divided into two distinct categories: innate and acquired immunity. The former consists of humoral antimicrobial molecules, complements, phagocytic cells, such as neutrophils and alveolar macrophages, and other innate immune cells, including dendritic cells (DCs), natural killer (NK) cells, NKT cells, antigen receptor γδ-bearing T (γδ T) cells, and B1-B cells. On the other hand, acquired immunity is characterized by antigen-specific cell-mediated and antibody-mediated immune responses. Recent development in research of the innate immune system includes the identification of Toll-like receptors (TLRs) that specifically recognize various microbial components called pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan, lipoarabinomannan (LAM), and lipopeptides. In addition, other cell surface molecules recognizing PAMPs have been identified, which include receptors for complement components, mannose, and β-glucan. These discoveries accelerate the understanding of innate immune mechanisms operating against infectious pathogens.

Cryptococcus neoformans is a yeast-like fungal pathogen with a thick polysaccharide capsule. Infection 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 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 microbe to the brain has attracted attention as a serious problem, particularly with the increased number of patients with acquired immunodeficiency syndrome (AIDS).

The host defense against *C. neoformans* is critically regulated by cell-mediated immunity (Lim and Murphy, 1980), and CD4+ T cells play a central role in eradicating this infection (Mody et al., 1990; Hill and Harmsen, 1991; Huffnagle et al., 1991). 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 (Kawakami et al., 1997; Koguchi and Kawakami, 2002). Mice depleted of Th1-type cytokines (e.g., IFN-γ and TNF-α) are highly susceptible to cryptococcal infection (Huffnagle et al., 1996; Kawakami et al., 1996), while the infection is less severe in mice lacking Th2 cytokines (e.g., IL-4 and IL-10) than control mice (Decken et al., 1998; Blackstock et al., 1999). Differentiation of

naïve helper T cells into Th1 cells absolutely requires the presence of IL-12 (Trinchieri, 1995), and this response is strongly potentiated by IL-18 (Robinson et al., 1997). In recent investigations (Decken et al., 1998: Kawakami et al., 2000a), targeted disruption of the gene for IL-12 or IL-18 resulted in attenuated host resistance and Th1 response to *C. neoformans*, indicating the role of these cytokines in the development of host protective response.

In this chapter, recent research developments in innate immune defense against cryptococcal infection in the respiratory system are highlighted with particular emphasis on the role of innate immune lymphocytes, such as NKT cells and γδ T cells, based on our data using a murine model of pulmonary cryptococcal infection.

2. General Characteristics of Host Defense in the Lung

Host defense mechanisms against infectious microbial pathogens develop in the upper and lower respiratory tracts. Initially, anatomical and mechanical host defense systems trap large-sized particles in the inhaled air. These systems include nasal hairs, nasopharyngeal channels, glottis, and highly divided branches of bronchi. The particles are caught by the mucous blanket lining the bronchial surface that contains viscid glycoproteins called mucins and cleared by ciliary movement and coughing up to the oropharynx. In contrast, small-sized particles less than 5 μm in diameter, including most infectious pathogens, reach the alveolar spaces where they can cause pulmonary infection. *C. neoformans* in the inhaled air from environment is usually in an acapsular form, the size of which is 1 to 5 μm in diameter (Powell et al., 1972), suggesting the ability of this fungal pathogen to penetrate into the terminal air spaces. In order to keep lung sterility, additional host defense mechanisms are found in these areas, which are largely divided into 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 antibody-mediated and cell-mediated immune responses. Furthermore, in the airway, specific mucosal immune systems composed of nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) play important roles as the mucosal barriers in the acquired phase of host defense (Pabst, 1992; Wu and Russell, 1997). AQ1

3. Humoral Components Operate in Innate Host Defense

In the respiratory tract, many antimicrobial molecules are secreted by various immune and non-immune cells, which include lysozyme, fibronectin, lactoferin, transferin, defensins, cathelicidin, collectins, and complements (Zhang et al., 2000). I will discuss here the role of defensins, collectins, and complements in conjunction with host defense against cryptococcal infection.

3.1. Defensins

Defensins are antimicrobial cationic peptides of a small molecular weight (2 to 6 kDa) that contain six cysteines and three intramolecular disulfide bonds. Based on their size and pattern of disulfide bonds, these molecules are largely classified into two categories, α- and β-defensins (Lehrer et al., 1993). In human, so far eight defensins have been identified, including six α-defensins [human defensins (HD)-1 to 6] and two β-defensins [human β-defensins (HBD)-1 and 2]. HD-1, -2, -3, and -4 constitute 30 to 50% of the total protein in azurophilic granules of neutrophils and are also released from these cells into the airway lining fluids, while

HBD-1 and -2 are synthesized by the airway epithelial cells (Zhang et al., 2000). Defensins exert a broad-spectrum antimicrobial activity by permeabilizing the membrane of microorganisms, including bacteria, fungi, and viruses (Lehrer et al., 1993; Zhang et al., 2000). *C. neoformans* is directly killed by α-defensin from rabbit and rhesus macaque in a dose-dependent manner (Alcouloumre et al., 1993; Tang et al., 1999).

In addition, recent studies have identified a variety of immunomodulatory roles for defensins (Yang et al., 2002; Ganz, 2003). These include the activation of complement system, induction of IL-8 production by epithelial cells, augmentation of expression of adhesion molecules on neutrophils and their adhesion responses, induction of chemotaxis of leukocytes, and enhancement of proliferation and cytokine production of CD4+ T cells. Thus, defensins may be involved in the innate and acquired phase host defense against cryptococcal infection through their immunoregulatory as well as antimicrobial activities.

3.2. Collectins

Collectins, collagenous C-type lectins, are oligomeric proteins composed of carbohydrate-recognition domains (CRDs) attached to collagenous regions and recognize PAMPs through calcium-dependent binding to mannose-specific carbohydrates (Clark et al., 2000). In addition to mannose-binding lectin (MBL) and conglutinin in plasma, the surfactant protein A and D (SP-A and SP-D), present in the surface layer of alveolar spaces, belong to the collectin superfamily. SP-A and SP-D bind a variety of infectious pathogens including Gram-positive and -negative bacteria, mycobacteria, fungi, and viruses, mainly via their CRD domains (Clark et al., 2000; McCormack and Whitsett, 2002). Such binding results in enhanced phagocytosis of microorganisms by alveolar macrophages and neutrophils (McCormack and Whitsett, 2002). SP-A binds to both acapsular and encapsulated forms of *C. neoformans* in a concentration-dependent manner, although three-fold better binding is observed with the acapsular form. Despite this feature, SP-A fails to function as an opsonin in the phagocytosis of this fungal pathogen by macrophages (Walenkamp et al., 1999). On the other hand, SP-D binds to the acapsular form, but either does not bind to the encapsulated form of *C. neoformans*, or does bind to a lesser extent. In contrast to SP-A, SP-D causes aggregation of acapsular yeast cells, which may stimulate their removal by enhancing the mucociliary clearance (Schelenz et al., 1995; van de Wetering et al., 2004).

3.3. Complement

The complement system is activated via three distinct pathways: classical, alternative, and lectin pathways. Whereas the activation of the classical pathway occurs from C1q subcomponent only in the presence of antigen–antibody complex, the alternative pathway is directly activated by interaction of C3 component with microbial surfaces and does not require the existence of antibody for its activation. The activation of recently identified lectin pathway is initiated by the binding of MBL with a structure similar to that of C1q, to carbohydrate (Fujita, 2002). The activated components of the complement pathways play various roles in the host defense. The deposition of C3b and iC3b fragments on the surface of microorganisms facilitates their phagocytosis by neutrophils and macrophages through interaction with complement receptor type 1 (CR1) and type 3 (CR3), respectively. In addition, C3a and C5a fragments initiate the inflammatory responses by attracting the recruitment of neutrophils into the sites of inflammation.

C. neoformans is a potent activator of the complement system. Previous studies showed that encapsulated *C. neoformans* directly activates the alternative pathway, which results in the deposition of C3 fragments on the capsular surface of this fungal microbe (Kozel et al., 1989, et al 1991). Similar deposition was

detected on the surface of encapsulated cryptococci derived from the infected tissues in mice (Truelsen et al., 1992). In non-immune hosts, this pathway is considered dominant at the innate phase of infection, although the classical pathway may be triggered through binding to naturally occurring antibodies to the cell wall polysaccharide components. In contrast, further studies will be required for understanding the contribution of lectin pathway.

Early studies showed that fresh serum enhanced the engulfment of *C. neoformans* by phagocytes, and that such activity was lost by heating at 56°C (Mitchell and Friedman, 1972; Diamond et al., 1974; Davies et al., 1982;). The opsonic potential of serum was attributed to the activation of complement system in in vitro experiments showing the blocking by treatment with antibodies specific for complement receptors (Levitz and Tabuni, 1991; Collins and Bancroft, 1992). The importance of the complement system in the host defense to cryptococcal infection was also documented in vivo in animals depleted of C3 by treatment with cobra venom factor or neutralizing antibody. These animals died of this infection earlier than did untreated animals (Graybill and Ahrens, 1981; Cross et al., 1997). Similar results are reported using congenitally C5-deficient mice, which were associated with increased susceptibility to the infection and with attenuated recruitment of neutrophils (Rhodes, 1985; Lovchik and Lipscomb, 1993). Thus, the complement system appears to play an important role in the innate phase resistance against cryptococcal infection by operating as opsonins and chemotactic factors in the lung.

4. Recognition of Cryptococcus

4.1. Nonopsonic Phagocytosis

Upon entry into alveolar spaces, *C. neoformans* are first recognized and then phagocytosed by macrophages. In this process, complement acts as opsonins via interaction with particular receptors, such as CR1, CR3, and CR4 (Levitz and Tabuni, 1991; Collins and Bancroft, 1992). Recently, however, nonopsonic phagocytosis by macrophages has been reported by several investigators. In earlier studies, the ingestion of acapsular *C. neoformans* was thought to be independent of complement, as indicated by the failure of anti-CR3 mAb to inhibit this response and efficient phagocytosis without prior opsonization (Cross and Bancroft, 1995). For nonopsonic engulfment of cryptococci, several cell surface receptors on phagocytic cells appear to be involved. CR3 mediates nonopsonic binding of *Mycobacterium tuberculosis* via a binding site distinct from the complement-binding site, which leads to the entrance of this bacterium into macrophages (Cywes et al., 1996). In the case of *C. neoformans*, a direct interaction between glucuronoxylomannan (GXM) and CR3 may facilitate phagocytosis by macrophages (Zaragoza et al., 2003). In previous investigations, receptors for mannose and β-glucan were suggested to mediate nonopsonic ingestion of the acapsular strain of *C. neoformans* and synthesis of proinflammatory cytokines by macrophages (Cross and Bancroft, 1995). Mannose receptor is also likely to participate in the phagocytosis of this fungal microbe for antigen presentation to T cells by dendritic cells (Syme et al., 2002). Recently, dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) and Dectin-1 have been identified as the receptors for mannose and β-glucan, respectively (Feinberg et al., 2001; Brown et al., 2002), which are found to act in the nonopsonic recognition of *Candida albicans* and zymosan by macrophages (Cambi et al., 2003; Taylor et al., 2004). The role of these receptors in phagocytosis of cryptococci remains to be elucidated.

4.2. Toll-like Receptors (TLRs)

The TLRs are evolutionally conserved from *Drosophila* to mammalian. *Drosophila* Toll are originally discovered as molecules that deliver

signals for the expression of antifungal peptides (Lemaitre et al., 1996). TLRs are expressed on macrophages and dendritic cells and involved in the recognition of PAMPs from various infectious pathogens, followed by signaling to nuclei via NFκB and MAP-kinases for the expression of cytokines and cell surface molecules (Akira et al., 2001; Takeda et al., 2003). TLR2 is activated by peptidoglycan, bacterial lipoproteins, and mycobacterial lipoarabinomannan, TLR3 by double-stranded RNA, TLR4 by LPS, TLR5 by flagellin, and TLR9 by bacterial CpG-DNA (Takeda et al., 2003). Upon ingestion by macrophages, *C. neoformans* secrete polysaccharides, including GXM, galactoxylomannan and mannoproteins, into phagosomes, in which various sets of TLRs are expressed. Recently, GXM was shown to interact with TLR4 and to activate NFκB in phagocytic cells, although such interaction did not result in the activation of MAP-kinases and production of TNF-α (Shohaml et al., 2001). Thus, further effort is still required to uncover the contribution of these receptors in the innate immune response against this fungal microbe.

5. Innate Cellular Host Defense

In addition to activation of the cellular response, the invading pathogens also activate the humoral components of the innate immune mechanisms. In the airways, alveolar-resident macrophages, which permanently reside in the alveolar spaces, first encounter these pathogens, followed by recruitment of other cellular components including phagocytic cells and innate immune lymphocytes. Here, I discuss the role of alveolar macrophages, neutrophils, and dendritic cells (DCs) in the local host defense against pulmonary cryptococcal infection.

5.1. Alveolar Macrophages

In the lung, alveolar macrophages (AMϕ) play a key role in the local host defense mechanism by exerting both phagocytic and immunoregulatory functions. After invading the terminal respiratory tract, AMϕ phagocytize infectious pathogens opsonized by complement fragments through complement receptors (CR1, CR3, and CR4). The microorganisms are also recognized via interaction with other PAMP receptors present on the surface of AMϕ. AMϕ express TLR2, TLR4, DC-SIGN, and Dectin-1 (Soilleux et al., 2002; Oshikawa and Sugiyama, 2003; Steele et al., 2004), all of which are considered important in the recognition of fungal PAMPs (Shohaml et al., 2001; Cambi et al., 2003; Steele et al., 2004; Roeder et al., 2004; Taylor et al., 2004). AQ2
Following such recognition, AMϕ release proinflammatory cytokines and chemokines and express co-stimulatory molecules, which results in the recruitment of neutrophils, macrophages, DCs, and innate immune lymphocytes, from the peripheral circulation into alveolar spaces and in the activation of these inflammatory cells.

In vitro studies indicate that AMϕ can phagocytize, kill, and present antigens in *C. neoformans* infection. AMϕ efficiently phagocytize the fungal microorganisms opsonized by serum (Bulmer and Tacker, 1975). The fungicidal activity against encapsulated *C. neoformans* in unstimulated human AMϕ is limited compared with other natural effector cells, peripheral blood monocytes, and neutrophils, although the acapsular strain is killed to a higher degree (Vecchiarelli et al., 1994a). Cryptococcus-laden AMϕ show a potent antigen-presenting activity to CD4+ T cells in context of HLA class II DR molecules, as indicated by their proliferative response and IFN-γ production (Vecchiarelli et al., 1994b). In addition, culture of AMϕ with this fungal microbe causes the production of proinflammatory cytokines, including IL-1β, TNF-α, IL-6, IL-12, and IL-18, and chemokines, such as monocyte chemoattractant protein-1 (MCP-1) (Vecchiarelli et al., 1994b; Li and Mitchell, 1997; He et al., 2003; our unpublished data), which may contribute to the regulation of host immune responses that takes place in the bron-

choalveolar spaces. Interestingly, these activities in human AMϕ are down-regulated by encapsulation of *C. neoformans* (Vecchiarelli et al., 1994b). In contrast, the in vivo role of AMϕ in the local protection against pulmonary cryptococcosis remains to be fully understood, although earlier investigations demonstrated that depletion of macrophages by systemic administration of silica markedly decreased the host resistance to cryptococcal infection (Monga, 1981).

Thus, AMϕ play a key role in the initiation of host protective response against *C. neoformans* at the gateway of the airway tract. In addition to the phagocytic and fungicidal functions, AMϕ exert immunoregulatory activities through the production of various proinflammatory cytokines and chemokines, which could provide a great influence on the quality of subsequent immune responses, recruitment of innate immune cells, and the activation and development of acquired immune responses.

5.2. Neutrophils

Although the development of cryptococcosis is not clinically associated with neutropenia, neutrophils are highly active in engulfing and killing *C. neoformans* in vitro (Miller and Mitchell, 1991). Neutrophils are thought to contribute to the innate defenses against cryptococcosis, particularly early in the course of infection before the development of acquired immune responses. In animal models, an influx of neutrophils into tissues is observed soon after cryptococcal challenge and is associated with rapid, but partial, clearance of the organisms (Gadebusch and Johnson, 1966; Perfect et al., 1980). Neutropenia caused by administration of cyclophosphamide is associated with exacerbation of this infection, although the same treatment also affects lymphocyte function (Graybill and Mitchell, 1978). In mouse models of cryptococcosis, boosting neutrophil-mediated host defenses by administration of granulocyte colony-stimulating factor (G-CSF) resulted in reduced brain tissue burden and prolonged survival of mice treated with fluconazole (Graybill et al., 1977). In human, neutrophils are frequently found in pathology specimens taken from patients with cryptococcosis (Baker and Haugen, 1955; Lee et al., 1996).

Recently, accumulating evidence highlights the immunoregulatory role of neutrophils in the host defense against infectious pathogens. In pulmonary infection with *Legionella pneumophila*, neutrophils play an important role in protection through polarizing the immune response toward a Th1-dominant condition (Tateda et al., 2001). Similarly, neutropenic mice are more susceptible to *M. tuberculosis* infection than mice bearing normal counts of neutrophils, which is associated with reduced expression of IFN-γ and iNOS (Pedrosa et al., 2000). Interestingly, Mednick and co-workers (2003) reported opposite results, indicating the enhanced survival of mice infected with a lethal dose of *C. neoformans* probably by coordinating inflammatory responses via modulation of cytokine synthesis in the lung.

In our previous studies, accumulation of neutrophils was not associated with protective host responses against lethal pulmonary infection with *C. neoformans* (Kawakami, 1999). The infected mice showed little or no expression of CC- and ELR^- CXC-chemokines that attract lymphocytes and macrophages, but ELR^+ CXC-chemokines that recruit neutrophils. These observations were consistent with poor infiltration of mononuclear leukocytes, but accumulation of neutrophils at the site of infection. Administration of IL-12 that rescued mice from lethal infection polarized the chemokine and cellular inflammatory responses toward the preferential accumulation of lymphocytes and macrophages, but not of neutrophils. These findings may provide evidence arguing against the protective role of neutrophils in infection with *C. neoformans*.

Thus, the contribution of neutrophils to the host defense against cryptococcal infection remains controversial. However, any such role is not likely pronounced according to the previous observations reported.

5.3. Dendritic Cells

DC is an efficient antigen-presenting cell for naïve T lymphocytes. Immature DCs that develop from $CD34^+$ progenitors in the bone marrow are characterized by their ability to capture antigens by endocytosis and phagocytosis. After capturing antigens, DCs undergo maturation that is associated with the expression of processed antigens in context of MHC class II and upregulation of co-stimulatory molecules, such as CD40, CD80, and CD86, expressed on their surface (Banchereau et al., 2000). During these changes, DCs migrate from the infected tissues into the T cell area of draining lymph nodes, where they encounter naïve antigen-specific CD4+ T cells (Guermonprez et al., 2002). DCs have an important role in determining the profile of cytokine production by T cells, i.e., Th1 or Th2 cells. In human, monocyte-derived DCs seem to promote the differentiation of Th1 cells by producing IL-12, while Th2 cells are likely induced by plasmacytoid-derived DCs (Moser and Murphy, 2000). DCs express many receptors for PAMPs, including TLRs, mannose receptors, and β-glucan receptors. Interaction of these receptors with the PAMPs leads to the production of proinflammatory cytokines and increased expression of co-stimulatory molecules by DCs. IL-12 and IL-18 released from these cells strongly promote the production of IFN-γ by innate immune lymphocytes, such as NK, NKT, and γδ T cells, as well as the differentiation of Th1 cells (Trinchieri, 1995; Robinson et al., 1997; Okamura et al., 1998; Qureshi et al., 1999; Baxevanis et al., 2003).

The cell-mediated immune responses are essential for the host defense against cryptococcal infection. The involvement of DCs in the development of cell-mediated immunity against this fungal microbe is not well understood. Bauman and co-workers (2000) examined the kinetics of DC accumulation in the draining lymph nodes after subcutaneous immunization with cryptococcal antigens. The protective anticryptococcal immune responses are associated with preferential accumulation of myeloid DCs in the draining lymph nodes, while lymphoid DC is the major subset in the unprotected mice. Accumulation of DCs in the lymph nodes is regulated by TNF-α (Bauman et al., 2003). In our unpublished data, DCs, identified as cells expressing both CD11c and class II MHC molecules, migrate into paratracheal lymph nodes after pulmonary infection with *C. neoformans*, although further investigation is required for understanding the role of these cells in the local host defense in the lung.

6. Innate Immune Lymphocytes

C. neoformans show the features of intracellular parasitism within phagocyte cells, as is well known in *M. tuberculosis*, *Listeria monocytogenes*, and *Salmonella typhimurium* (Feldmesser et al., 2001). Because such pathogens resist the killing mechanisms, phagocytes fail to eradicate them without any activation. The innate immune lymphocytes, such as NK, NKT, and γδ T cells, can enhance their killing activity through the production of IFN-γ, although the overall potential is not sufficient for complete eradication of the infection, which needs more potent protective mechanisms by developing 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 disagree with this concept. In this respect, accumulating evidences suggest that innate immune lymphocytes are the cells that determine the quality of acquired immune responses (Horwitz et al., 1999; Nishimura et al., 2000; Schaible and Kaufmann, 2000). Thus, the early host protective responses mediated by these cells is more than a "temporary protector" before development of acquired immunity.

6.1. NK Cells

NK cells play a role in the innate cellular host defense mechanisms to eliminate virus-infected cells and tumor cells (Trinchieri, 1989). NK cells express their killing activity through a non-phagocytic mechanism, which is mediated by several killing molecules including perforin and granzyme B (Kagi et al., 1996). In host defense against infectious pathogens, NK cells regulate the innate defense mechanisms through the production of cytokines such as IFN-γ (Dunn and North, 1991; Laskay et al., 1993; Scharton and Scott, 1993). This process operates especially in the early phase of infection before the establishment of a specific immune response mediated by T cells, through the enhancement of the antimicrobial activity of phagocytic cells. IL-12 and IL-18 potentiate the tumoricidal activity as well as production of IFN-γ by NK cells and act synergistically when these cytokines are administered in combination (Okamura et al., 1995, 1998; Trinchieri, 1995; Zhang et al., 1997; Hyodo et al., 1999).

Earlier studies also indicated the role of NK cells in eliminating *C. neoformans* from the host. In a series of studies, Murphy and coworkers demonstrated that NK cells inhibited the growth of fungal microorganisms by directly binding to them (Murphy and MacDaniel, 1982; Nabavi and Murphy, 1985; Hidore and Murphy, 1989; Hidrore et al., 1990, et al 1991a, et al b; Murphy et al., 1991, et al 1993). Other studies by various investigators indicated that mice defective in NK cell activity were more susceptible to intravenous challenge with *C. neoformans* than control animals (Hidore and Murphy, 1986; Lipscomb et al., 1991; Scharton and Scott, 1993). These early observations emphasized the role of NK cells in eliminating *C. neoformans* from infected organs through a direct fungicidal activity. In contrast, our studies showed that SCID mouse-derived splenic NK cells, which were stimulated with a combination of IL-12 and IL-18, did not show any direct cryptococcocidal effect, although the production of IFN-γ and cytolytic activity to NK-sensitive tumor cells were markedly induced by the same treatment. NK cells rather upregulated the nitric oxide (NO)-mediated antifungal activity against *C. neoformans* through the production of IFN-γ (Kawakami et al., 2000b). Thus, NK cells contribute to the host defense against cryptococcal infection by regulating the immune response as well as by directly killing this fungal microbe.

The in vivo role of NK cells, which form approximately 5 to 6% of the lymphocyte population in lung (Kawakami et al., 2001a), in the local host defense against cryptococcal infection in airway tissues remains to be fully understood. In earlier studies, lung infection was not aggravated in mice depleted of NK cells by administration of anti-NK1.1 mAb (Lipscomb et al., 1987), although the same treatment deleted not only NK cells but also NKT cells. However, NK cells appear to be the source of IFN-γ to control *C. neoformans* infection in mice receiving a combined treatment with IL-12 and IL-18 (Qureshi et al., 1999) or in mice with a genetic disruption of IL-12p40 gene (Kawakami et al., 2000a). In contrast, IFN-γ production and host protection from cryptococcal infection caused by administration of unmethylated synthetic DNA-containing CpG-motif do not involve NK cells (our unpublished data). Thus, the in vivo role of NK cells in anticryptococcal host response appears to vary in different settings; further studies will be necessary to better understand this role.

6.2. NKT Cells

6.2.1. Characteristics

NKT cell is a unique T cell subset sharing some features with NK cells. Originally, this population was identified as a lymphocyte subset that expresses both T cell receptor (TCR) αβ and NK1.1 or NKR-P1 (CD161) in mice (Godfrey et al., 2000; Taniguchi and Nakayama, 2000). Specific characteristics of

this cell type include highly limited repertoire with an invariant Vα chain consisting of Vα14-Jα18 (formerly Jα281) gene segment and highly skewed Vβ chains, Vβ8.2, 7, and 2 in mice and with Vα24Jα18 and Vβ11 in human. Accordingly, these cells are called invariant (*i*)NKT cells. The mouse *i*NKT cells are either CD4+ or double negative (DN) and usually do not express CD8, while 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 because this population disappears in CD1d gene-disrupted (CD1d-KO) mice. The glycosphingolipid, α-galactosylceramide (α-GalCer) that was originally discovered in marine sponge as a novel anticancer agent, is recognized by *i*NKT cells in context with 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.

6.2.2. Role in Host Defense to Infection

NKT cells contribute to development of both Th1 and Th2 responses under different experimental conditions (Lehuen et al., 1998; Carnaud et al., 1999; Cui et al., 1999; Singh et al., 1999). Although the significance of NKT cells in infectious diseases remains to be fully elucidated, to date there are several published studies on this issue. Three roles are identified for these cells in host defense against infectious pathogens. First, the clinical course of *M. tuberculosis* and *Salmonella choleraesuis* infection is not much affected by manipulations designed to suppress the activity of NKT cells (Behar et al., 1999; Ishigami et al., 1999). Second, infection with *Listeria monocytogenes* or *Toxoplasma gondii* is rather improved by the similar manipulations (Szalay et al., 1999; Nakano et al., 2001). Finally, mice lacking NKT cells are more susceptible to infection caused by *Leishmania major*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Borrelia burgdorferi*, and *Plasmodium yoelii* than control mice (Ishikawa et al., 2000; Kumar et al., 2000; Mannoor et al., 2001; Nieuwnhuis et al., 2002; Kawakami et al., 2003). Thus, the role of NKT cells seems different among infectious pathogens.

6.2.3. Accumulation of iNKT Cells in Lung after Cryptococcal Infection

Recently, we reported the increase of NKT cells in lungs after intratracheal infection with *C. neoformans* (Kawakami et al., 2001a). Inflammatory leukocytes obtained from the homogenates of infected lungs were stained with anti-TCRαβ 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 peak values 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, $V\alpha14^{+}$ NKT cells as well as conventional T and NK cells were found to increase in the lungs after intratracheal infection with *C. neoformans*.

Migration of inflammatory leukocytes from the peripheral circulation to the site of infection 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 (Rossi and Zlotnik, 2000). 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 to the site of infection by many chemokines, including MCP-2, -3, MIP-1α, RANTES, IP-10, and lymphotactin, under various conditions (Allavena et al., 1994; Maghazachi et al., 1994, 1997; Taub et al., 1995; Giancarlo et al., 1996; Loetscher et al., 1996 et al). In contrast, MIP-2 was the only chemokine known to function in trafficking NKT cells until MCP-1 was identified as a chemoattractant for these cells (Faunce et al., 2001). In MCP-1KO mice, accumulation of NKT cells in lungs was not observed after infection with *C. neoformans* (Kawakami et al., 2001a). Consistent with these data, MCP-1 production preceded the kinetics of NKT cell-mediated inflammatory responses. Thus, NKT cell trafficking into the fungus-infected sites involves at least in part the production of MCP-1, although other chemokines may contribute, as observed in NK cells.

6.2.4. Role of iNKT cells in Th1 Response and Host Defense to Cryptococcal Infection

A remarkable feature of NKT cells is the expeditious and abundant production of IFN-γ and IL-4 upon stimulation via their antigen receptors (Godfrey et al., 2000; Taniguchi and Nakayama, 2000). Accumulating evidence suggests that NKT cells are involved in the regulation of Th1 and Th2 cell development. On the other hand, host defense against cryptococcal infection is critically regulated by the balance between Th1- and Th2-mediated immune responses (Kawakami et al., 1997; Koguchi and Kawakami, 2002). These findings suggest that NKT cells may affect the host immune responses and protection against infection with this fungal microorganism. In our study (Kawakami et al., 2001), the Th1-mediated immune responses, as indicated by antigen-specific IFN-γ production by T cells and delayed-type hypersensitivity reaction, were significantly ameliorated in Jα281-KO mice lacking *i*NKT cells, compared with control wild-type mice. In contrast, Th2 cytokine synthesis was not altered in these mice. Furthermore, the clearance of fungal microorganisms from the infected sites was significantly delayed in Jα281-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 the establishment of Th1-mediated acquired immune responses, which leads to host protection against cryptococcal infection.

6.2.5. Induction of Th1 Response and Protection against Cryptococcal Infection by Ligand-Specific Activation of iNKT Cells

*i*NKT cells recognize α-GalCer by their antigen receptors in the context of CD1d molecules expressed on DCs (Kawano et al., 1997; Godfrey et al., 2000; Taniguchi and Nakayama, 2000). Such engagement causes prompt secretion of both IFN-γ and IL-4 by these cells and emergence of their cytolytic activity against tumor cells. Toura et al. (1999) 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 et al. (2000) were the first group to demonstrate 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 (Gonzalez-Aseguinolaza et al., 2002). Our group observed similar effects for this treatment in a murine model of cryptococcal infection (Kawakami et al., 2001b). 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 Jα281-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 (Kawakami et al., 2001c). The protective effects by the ligand-specific activation of *i*NKT cells against *P. aeruginosa* and *M. tuberculosis* are recently reported by other investigators (Chackerian et al., 2002; Nieuwenhuis et al., 2002), although their contribution to the host defense against the latter infection is not clearly defined (Behar et al., 1999). These observations suggest that α-GalCer can be a promising immunotherapeutic agent for the treatment of certain intractable infectious diseases including cryptococcal meningitis in immunodeficient patients.

6.3. γδ T cells

In addition to 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 (Hayday, 2000). In 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 they are known as dendritic epidermal T cells (DETC), and mucosal/epithelial tissues, such as intestine, lung, tongue, mammary, uterine, and vaginal epithelia, although some cells exist in lymphoid tissues. Such characteristic localization suggests the role of these cells in first line host defense against infectious agents and other antigens.

6.3.1. Natural Ligands

The number of V gene segments of γδ T cells that determine their diversity is very limited when compared with that of αβ T cells. In addition, particular subsets are localized in the defined anatomical areas and at different developmental stages. Based on 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 (Hayday, 2000; Lahn, 2000). 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 (Tanaka et al., 1995; Bukowski et al., 1999). In addition, protein antigens can be ligands for the activation of γδ T cells. Human Vγ9/Vδ2+ γδ T cells recognize tetanus toxoid in the context of MHC class II molecules (Holoshitz et al., 1992). Mycobacterial heat-shock proteins stimulate both human and mouse γδ T cells (O'Brien et al., 1989, 1992; Born et al., 1990et al). However, no ligand of these cells has so far been identified from fungal microorganisms, including *C. neoformans*.

6.3.2. Regulatory Role in Host Defense to Cryptococcal Infection

In our recent study, we investigated the role of γδ T cells in the development of Th1 response and the host defense against pulmonary infection with *C. neoformans* using a mouse model of pulmonary cryptococcosis (Uezu et al., 2004). γδ T cells rapidly 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 was likely to take place in a manner different from that of NK and NKT cells. Accumulation of NK and NKT cells in lungs after cryptococcal infection was markedly reduced in MCP-1KO 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 lacking γδ T cells, induced by administration of a 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 suppressive role of γδ T cells in the host defense against cryptococcal infection. This is in a sharp contrast to the role of NKT cells, which contribute significantly to the development of Th1-type immune response and host resistance to this infection (Kawakami et al., 2001a). Earlier investigations reported anti-inflammatory γδ T cells that produced Th2 cytokines and TGF-β (Wesch et al., 2001; Nagaeva et al., 2002). These observations suggest that these cytokines mediate the down-regulatory effect observed in our study. This speculation was supported by our recent data showing low 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 (Hirsch et al., 1997; Letterio and Roberts, 1998; Li et al., 1999; Reed, 1999). Furthermore, other investigations revealed that γδ T cells down-regulate the host defense against infection caused by *L. monocytogenes*, *S. choleraesuis*, and *C. albicans* (Emoto et al., 1995; O'Brien et al., 2000; Wormley et al., 2001). Thus, our study suggests that γδ T cells may suppress the host defense to pulmonary infection with *C. neoformans* via a TGF-β-mediated mechanism.

6.4. Regulation of Host Defense against Cryptococcal Infection by NKT and γδ T Cells

The distinct roles of *i*NKT and γδ T cells in the host resistance against cryptococcal infection suggest that these innate immune lymphocytes co-regulate Th1-mediated response for induction of a moderate host defense. γδ T cells may act to keep the balance of Th1–Th2 response in a proper manner by suppressing the exaggerated Th1 response caused by NKT cells, as speculated in Fig. 7.1. In pulmonary infection with *C. neoformans*, the 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 by promoting Th1-mediated immune response, while NKT cells are likely to suppress these responses (Hisaeda et al., 1995). This is in sharp contrast to the findings in cryptococcal infection. Although the precise mechanism of such difference remains to be clarified, the role of NKT and γδ T cells in the host protective response seems to vary from one microbe to another.

7. Concluding Remarks

Acquired immunity, an antigen-specific host defense mechanism, had been the central dogma of previous studies on immunological response to infection. Recently, however, the role of innate immune mechanisms, mediated by soluble antimicrobial components, complements, phagocytes, and innate immune lymphocytes, have garnered much attention by many investigators and the biological significance of these cellular components is being

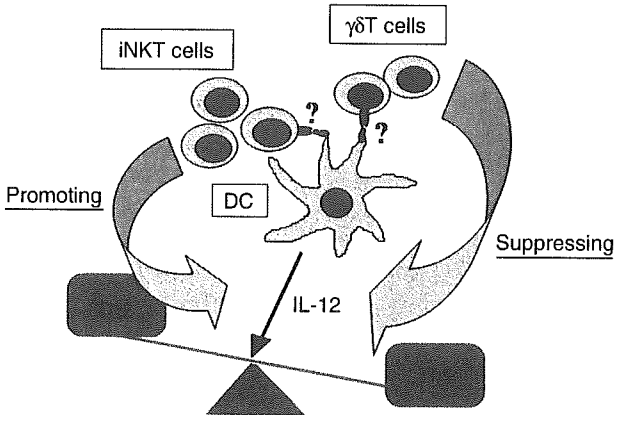

Figure 7.1. *Regulation of Th1–Th2 cytokine balance by iNKT and γδ T cells in cryptococcal infection.* Host defense to cryptococcal infection is critically regulated by Th1–Th2 cytokine balance. The predominant synthesis of Th1 cytokines over Th2 protects mice from infection, whereas infection is exacerbated under a Th2-dominant condition. *i*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.

extensively explored. Furthermore, the discovery of PAMP receptors has accelerated research in this area. In the host immune response to infectious pathogens, as demonstrated in our series of investigations on cryptococcal infection, the important roles of innate immunity in respiratory tissues, mediated especially by NKT and γδ T cells, have been unveiled. Furthermore, the results of several studies support the involvement of these particular lymphocyte subsets in determining the balance of Th1–Th2 immune responses. Thus, both NKT and γδ T cells seem to participate in bridging early host protection, by innate immune mechanism, to antigen-specific acquired immune responses in pulmonary cryptococcosis.

References

Akira, S., Takeda, K., and Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* 2: 675–680.

Alcouloumre, M.S., Ghannoum, M.A., Ibrahim, A.S., Selsted, M.E., and Edwards, J.E. Jr. (1993). Fungicidal properties of defensin NP-1 and activity against *Cryptococcus neoformans* in vitro. *Antimicrob. Agents Chemother.* 37: 2628–2632.

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.

Baker, R.D. and Haugen, R.K. (1955). Tissue changes and tissue diagnosis in cryptococcosis: A study of 26 cases. *Am. J. Clin. Pathol.* 25: 14–24.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.J., Pulendran, B., and Palucka K. (2000). Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18: 767–811.

Bauman, S.K., Nichols, K.L., and Murphy, J.W. (2000). Dendritic cells in the induction of protective and nonprotective anticryptococcal cell-mediated immune responses. *J. Immunol.* 165: 158–167.

Bauman, S.K., Huffnagle, G.B., and Murphy, J.W. (2003). Effects of tumor necrosis factor alpha on dendritic cell accumulation in lymph nodes draining the immunization site and the impact on the anticryptococcal cell-mediated immune response. *Infect. Immun.* 71: 68–74.

Baxevanis, C.N., Gritzapis, A.D., and Papamichail, M. (2003). In vivo antitumor activity of NKT cells

activated by the combination of IL-12 and IL-18. *J. Immunol.* 171: 2953–2959.

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.

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.

Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., Wong, S.Y., and Gordon, S. (2002). Dectin-1 is a major beta-glucan receptor on macrophages. *J. Exp. Med.* 196: 407–412.

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.

Bulmer, G.S. and Tacker, J.R. (1975). Phagocytosis of *Cryptococcus neoformans* by alveolar macrophages. *Infect. Immun.* 11: 73–79.

Cambi, A., Gijzen, K., de Vries, J.M., Torensma, R., Joosten, B., Adema, G.J., Netea, M.G., Kullberg, B.J., Romani, L., and Figdor, C.G. (2003). The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol.* 33: 532–538.

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.

Chackerian, A., Alt, J., Perera, V., and Behar, S.M. (2002). Activation of NKT cells protects mice from tuberculosis. *Infect. Immun.* 70: 6302–6309.

Clark, H.W., Reid, K.B., and Sim, R.B. (2000). Collectins and innate immunity in the lung. *Microbes Infect.* 2: 273–278.

Collins, H.L. and Bancroft, G.J. (1992). Cytokine enhancement of complement-dependent phagocytosis by macrophages: Synergy of tumor necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor for phagocytosis of *Cryptococcus neoformans. Eur. J. Immunol.* 22: 1447–1454.

Cross, C.E. and Bancroft, G.J. (1995). Ingestion of acapsular *Cryptococcus neoformans* occurs via mannose and beta-glucan receptors, resulting in cytokine production and increased phagocytosis of the encapsulated form. *Infect. Immun.* 63: 2604–2611.

Cross, C.E., Collins, H.L., and Bancroft, G.J. (1997). CR3-dependent phagocytosis by murine macrophages: Different cytokines regulate ingestion of a defined CR3 ligand and complement-opsonized *Cryptococcus neoformans. Immunology* 91: 289–296.

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 Vα14 natural killer cells. *J. Exp. Med.* 190: 783–792.

Cywes, C., Godenir, N.L., Hoppe, H.C., Scholle, R.R., Steyn, L.M., Kirsch, R.E., and Ehlers, M.R. (1996). Nonopsonic binding of *Mycobacterium tuberculosis* to human complement receptor type 3 expressed in Chinese hamster ovary cells. *Infect. Immun.* 64: 5373–5383.

Davies, S.F., Clifford, D.P., Hoidal, J.R., and Repine, J.E. (1982). Opsonic requirements for the uptake of *Cryptococcus neoformans* by human polymorphonuclear leukocytes and monocytes. *J. Infect. Dis.* 145: 870–874.

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.

Diamond, R.D., May, J.E., Kane, M.A., Frank, M.M., and Bennett, J.E. (1974). The role of the classical and alternate complement pathways in host defenses against *Cryptococcus neoformans* infection. *J. Immunol.* 112: 2260–2270.

Dunn, P.L. and North, R.J. (1991). Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect. Immun.* 59: 2892–2900.

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.

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.

Feinberg, H., Mitchell, D.A., Drickamer, K., and Weis, W.I. (2001). Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294: 2163–2166.

Feldmesser, M., Tucker, S., and Casadevall, A. (2001). Intracellular parasitism of macrophages by *Cryptococcus neoformans*. *Trends Microbiol.* 9: 273–278.

Fujita, T. (2002). Evolution of the lectin-complement pathway and its role in innate immunity. *Nat. Rev. Immunol.* 2: 346–353.

Gadebusch, H.H. and Johnson, A.G. (1966). Natural host resistance to infection with *Cryptococcus neoformans*. IV. The effect of some cationic proteins on the experimental disease. *J. Infect. Dis.* 116: 551–565.

Ganz, T. (2003). Defensins: Antimicrobial peptides of innate immunity. *Nat. Rev. Immunol.* 3: 710–720.

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.

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.

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). α-Galactosylceramide-activated Vα14 natural killer T cells mediate protection against murine malaria. *Proc. Natl. Acad. Sci. USA* 97: 8461–8466.

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.

Graybill, J.R. and Ahrens, J. (1981). Immunization and complement interaction in host defense against murine Cryptococcosis. *J. Reticuloendothel. Soc.* 30: 347–357.

Graybill, J.R. and Mitchell, L. (1978). Cyclophosphamide effects on murine cryptococcosis. *Infect. Immun.* 21: 674–677.

Graybill, J.R., Bocanegra, R., Lambros, C., and Luther, M.F. (1977). Granulocyte colony stimulating factor therapy of experimental cryptococcal meningitis. *J. Med. Vet. Mycol.* 35: 243–247.

Guermonprez, P., Valladeau, J., Zitvogel, L., Thery, C., and Amigorena, S. (2002). Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol.* 20: 621–667.

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.

He, W., Casadevall, A., Lee, S.C., and Goldman, D.L. (2003). Phagocytic activity and monocyte chemotactic protein expression by pulmonary macrophages in persistent pulmonary cryptococcosis. *Infect. Immun.* 71: 930–936.

Hidore, M.R. and Murphy, J.W. (1986). Natural cellular resistance of beige mice against *Cryptococcus neoformans*. *J. Immunol.* 137: 3624–3631.

Hidore, M.R. and Murphy, J.W. (1989). Murine natural killer cell interactions with a fungal target, *Cryptococcus neoformans*. *Infect. Immun.* 57: 1990–1997.

Hidore, M.R., Nabavi, N., Reynolds, C.W., Henkart, P.A., and Murphy, J.W. (1990). Cytoplasmic components of natural killer cells limit the growth of *Cryptococcus neoformans*. *J. Leukoc. Biol.* 48: 15–26.

Hidore, M.R., Mislan, T.W., and Murphy, J.W. (1991a). Responses of murine natural killer cells to binding of the fungal target *Cryptococcus neoformans*. *Infect. Immun.* 59: 1489–1499.

Hidore, M.R., Nabavi, N., Sonleitner, F., and Murphy, J.W. (1991b). Murine natural killer cells are fungicidal to *Cryptococcus neoformans*. *Infect. Immun.* 59: 1747–1754.

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.

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.

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.

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.

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.

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.

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.

Hyodo, Y., Matsui, K., Hayashi, N., Tsutsui, H., Kashiwamura, S., Yamauchi, H., Hiroishi, K., Takeda, K., Tagawa, Y., Iwakura, Y., Kayagaki, N., Kurimoto, M., Okamura, H., Hada, T., Yagita, H., Akira, S., Nakanishi, K., and Higashino, K. (1999). IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. *J. Immunol.* 162: 1662–1668.

Ishigami, M., Nishimura, H., Naiki, Y., Yoshioka, K., Kawano, T., Tanaka, Y., Taniguchi, M., Kakumu, S., and Yoshikai, Y. (1999). The roles of intrahepatic Vα14+ NK1.1+ T cells for liver injury induced by *Salmonella* infection in mice. *Hepatology* 29: 1799–1808.

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+ Vα14 NKT cells play a crucial role in an early stage of protective immunity against infection with *Leishmania major*. *Int. Immunol.* 12: 1267–1274.

Kagi, D., Ledermann, B., Burki, K., Zinkernagel, R.M., and Hengartner, H. (1996). Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis in vivo. *Ann. Rev. Immunol.* 14: 207–232.

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.

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.

Kawakami, K., Shibuya, K., Qureshi, M.H., Zhang, T., Koguchi, Y., Tohyama, M., Xie, Q., Naoe, S., and Saito, A. (1999). Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent *Cryptococcus neoformans*: effects of interleukin-12. *FEMS Immunol. Med. Microbiol.* 25: 391–402.

Kawakami, K., Koguchi, Y., Qureshi, M.H., Miyazato, A., Yara, S., Kinjo, Y., Iwakura, Y., Takeda, K., Akira, S., Kurimoto, M., and Saito, A. (2000a). 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.

Kawakami, K., Koguchi, Y., Qureshi, M.H., Yara, S., Kinjo, Y., Uezu, K., and Saito, A. (2000b). NK cells eliminate *Cryptococcus neoformans* by potentiating the fungicidal activity of macrophages rather than by directly killing them upon stimulation with IL-12 and IL-18. *Microbiol. Immunol.* 44: 1043–1050.

Kawakami, K., Kinjo, Y., Uezu, K., Yara, S., Miyagi, K., Koguchi, Y., Nakayama, T., Taniguchi, M., and Saito, A. (2001a). 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.

Kawakami, K., Kinjo, Y., Yara, S., Koguchi, Y., Uezu, K., Nakayama, T., Taniguchi, M., and Saito, A. (2001b). 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.

Kawakami, K., Kinjo, Y., Yara, S., Uezu, K., Koguchi, Y., Tohyama, M., Azuma, M., Takeda, K., Akira, S., and Saito, A. (2001c). 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.

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.

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 Vα14 NKT cells by glycosylceramides. *Science* 278: 1626–1629.

Koguchi, Y. and Kawakami, K. (2002). Cryptococcal infection and Th1–Th2 cytokine balance. *Int. Rev. Immunol.* 21: 423–438.

Kozel, T.R., Wilson, M.A., Pfrommer, G.S., and Schlageter, A.M. (1989). Activation and binding of opsonic fragments of C3 on encapsulated *Cryptococcus neoformans* by using an alternative complement pathway reconstituted from six isolated proteins. *Infect. Immun.* 57: 1922–1927.

Kozel, T.R., Wilson, M.A., and Murphy, J.W. (1991). Early events in initiation of alternative complement pathway activation by the capsule of *Cryptococcus neoformans*. *Infect. Immun.* 59: 3101–3110.

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.

Lahn, M. (2000). The role of gamma/delta T cells in the airways. *J. Mol. Med.* 78: 409–425.

Laskay, T., Rolinghoff, M., and Solbach, W. (1993). Natural killer cells participate in the early defense against *Leishmania major* infection in mice. *Eur. J. Immunol.* 23: 2237–2241.

Lee, S.C., Dickson, D.W., and Casadevall, A. (1996). Pathology of cryptococcal meningoencephalitis: Analysis of 27 patients with pathogenetic implications. *Hum. Pathol.* 27: 839–847.

Lehrer, R.I., Lichtenstein, A.K., and Ganz, T. (1993). Defensins: Antimicrobial and cytotoxic peptides of mammalian cells. *Annu. Rev. Immunol.* 11: 105–128.

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.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996). The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 86: 973–983.

Letterio, J.J. and Roberts, A.B. (1998). Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol.* 16: 137–161.

Levitz, S.M. and Tabuni, A. (1991). Binding of *Cryptococcus neoformans* by human cultured macrophages. Requirements for multiple complement receptors and actin. *J. Clin. Invest.* 87: 528–535.

Li, R.K. and Mitchell, T.G. (1997). Induction of interleukin-6 mRNA in rat alveolar macrophages by in vitro exposure to both *Cryptococcus neoformans* and anti-*C. neoformans* antiserum. *J. Med. Vet. Mycol.* 35: 327–334.

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.

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.

Lipscomb, M.F., Alvarellos, T., Toews, G.B., Tompkins, R., Evans, Z., Koo, G., and Kumar, V. (1987). Role of natural killer cells in resistance to *Cryptococcus neoformans* infections in mice. *Am. J. Pathol.* 128: 354–361.

Lipscomb, M.F., Alvarellos, T., Toews, G.B., Tompkins, R., Evans, Z., Koo, G., and Kumar, V. (1991). Role of natural killer cells in resistance to *Cryptococcus neoformans* infections in mice. *Am. J. Pathol.* 128: 354–361.

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.

Lovchik, J.A. and Lipscomb, M.F. (1993). Role for C5 and neutrophils in the pulmonary intravascular clearance of circulating *Cryptococcus neoformans*. *Am. J. Respir. Cell. Mol. Biol.* 9: 617–627.

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.

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.

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.

McCormack, F.X. and Whitsett, J.A. (2002). The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung. *J. Clin. Invest.* 109: 707–712.

Mednick, A.J., Feldmesser, M., Rivera, J., and Casadevall, A. (2003). Neutropenia alters lung cytokine production in mice and reduces their susceptibility to pulmonary cryptococcosis. *Eur. J. Immunol.* 33: 1744–1753.

Miller, M.F. and Mitchell, T.G. (1991). Killing of *Cryptococcus neoformans* strains by human neutrophils and monocytes. *Infect. Immun.* 59: 24–28.

Mitchell, T.G. and Friedman, L. (1972). In vitro phagocytosis and intracellular fate of variously encapsulated strains of *Cryptococcus neoformans*. *Infect. Immun.* 5: 491–498.

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.

Monga, D.P. (1981). Role of macrophages in resistance of mice to experimental cryptococcosis. *Infect. Immun.* 32: 975–978.

Moser, M. and Murphy, K.M. (2000). Dendritic cell regulation of TH1–TH2 development. *Nat. Immunol.* 1: 199–205.

Murphy, J.W. and MacDaniel, D.O. (1982). In vitro reactivity of natural killer (NK) cells on *Cryptococcus neoformans*. *J. Immunol.* 128: 1577–1583.

Murphy, J.W., Hidore, M.R., and Nabavi, N. (1991). Binding interactions of murine natural killer cells with the fungal target *Cryptococcus neoformans*. *Infect. Immun.* 59: 1476–1488.

Murphy, J.W., Hidore, M.R., and Wong, S.C. (1993). Direct interactions of human lymphocytes with the yeast-like organism, *Cryptococcus neoformans*. *J. Clin. Invest.* 91: 1553–1566.

Nabavi, N. and Murphy, J.W. (1985). In vitro binding of natural killer cells to *Cryptococcus neoformans* targets. *Infect. Immun.* 50: 50–57.

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.

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.

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.

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.

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.

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.

Okamura, H., Tsutsui, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., Akita, K., Namba, M., Tanabe, F., Konishi, K., Fukuda, S., and Kurimoto, M. (1995). Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378: 88–91.

Okamura, H., Tsutsui, H., Kashiwamura, S., Yoshimoto, T., and Nakanishi, K. (1998). Interleukin-18: A novel cytokine that augments both innate and acquired immunity. *Adv. Immunol.* 70: 281–312.

Oshikawa, K. and Sugiyama, Y. (2003). Gene expression of Toll-like receptors and associated molecules induced by inflammatory stimuli in the primary alveolar macrophage. *Biochem. Biophys. Res. Commun.* 305: 649–655.

Pabst, R. (1992). Is BALT a major component of the human lung immune system? *Immunol. Today* 13: 119–122.

Pedrosa, J., Saunders, B.M., Appelberg, R., Orme, I.M., Silva, M.T., and Cooper, A.M. (2000). Neutrophils play a protective nonphagocytic role in systemic *Mycobacterium tuberculosis* infection of mice. *Infect. Immun.* 68: 577–583.

Perfect, J.R., Lang, S.D., and Durack, D.T. (1980). Chronic cryptococcal meningitis: A new experimental model in rabbits. *Am. J. Pathol.* 101: 177–194.

Powell, K.E., Dahl, B.A., Weeks, R.J., and Tosh, F.E. (1972). Airborne *Cryptococcus neoformans*:

Particles from pigeon excreta compatible with alveolar deposition. *J. Infect. Dis.* 125: 412–415.

Qureshi, M.H., Zhang, T., Koguchi, Y., Nakashima, K., Okamura, H., Kurimoto, M., and Kawakami, K. (1999). Combined effects of IL-12 and IL-18 on the clinical course and local cytokine production in murine pulmonary infection with *Cryptococcus neoformans*. *Eur. J. Immunol.* 29: 643–649.

Reed, S.G. (1999). TGF-beta in infections and infectious diseases. *Microbes Infect.* 1: 1313–1325.

Rhodes, J.C. (1985). Contribution of complement component C5 to the pathogenesis of experimental murine cryptococcosis. *Sabouraudia* 23: 225–234.

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.

Rossi, D. and Zlotnik, A. (2000). The biology of chemokines and their receptors. *Annu. Rev. Immunol.* 217: 217–242.

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.

Scharton, T.M. and Scott, P. (1993). Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to *Leishmania major* in mice. *J. Exp. Med.* 178: 567–577.

Schelenz, S., Malhotra, R., Sim, R.B., Holmskov, U., and Bancroft, G.J. (1995). Binding of host collectins to the pathogenic yeast *Cryptococcus neoformans*: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect. Immun.* 63: 3360–3366.

Shellito, J.E. and Kolls, J.K. (2003). Alveolar macrophage-mediated killing of *Pneumocystis carinii* f. sp. muris involves molecular recognition by the Dectin-1 beta-glucan receptor. *J. Exp. Med.* 198: 1677–1688.

Shohaml, S., Huang, C., Chen, J.M., Golenbock, D.T., and Levitz, S.M. (2001). Toll-like receptor 4 mediates intracellular signaling without TNF-alpha release in response to *Cryptococcus neoformans* polysaccharide capsule. *J. Immunol.* 166: 4620–4626.

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.

Soilleux, E.J., Morris, L.S., Leslie, G., Chehimi, J., Luo, Q., Levroney, E., Trowsdale, J., Montaner, L.J., Doms, R.W., Weissman, D., Coleman, N., and Lee, B. (2002). Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations in situ and in vitro. *J. Leukoc. Biol.* 71: 445–457.

Steele, C., Marrero, L., Swain, S., Harmsen, A.G., Zheng, M., Brown, G.D., Gordon, S., Roeder, A., Kirschning, C.J., Rupec, R.A., Schaller, M., and Korting, H.C. (2004). Toll-like receptors and innate antifungal responses. *Trends Microbiol.* 12: 44–49.

Syme, R.M., Spurrell, J.C., Amankwah, E.K., Green, F.H., and Mody, C.H. (2002). Primary dendritic cells phagocytose *Cryptococcus neoformans* via mannose receptors and Fcgamma receptor II for presentation to T lymphocytes. *Infect. Immun.* 70: 5972–5981.

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-β2 and Th1 cytokines and ameliorates listeriosis in mice. *J. Immunol.* 162: 6955–6958.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-like receptors. *Annu. Rev. Immunol.* 21: 335–376.

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.

Tang, Y.Q., Yuan, J., Miller, C.J., and Selsted, M.E. (1999). Isolation, characterization, cDNA cloning, and antimicrobial properties of two distinct subfamilies of alpha-defensins from rhesus macaque leukocytes. *Infect. Immun.* 67: 6139–6144.

Taniguchi, M. and Nakayama, T. (2000). Recognition and function of Vα14NKT cells. *Semin. Immunol.* 12: 543–550.

Tateda, K., Moore, T.A., Deng, J.C., Newstead, M.W., Zeng, X., Matsukawa, A., Swanson, M.S., Yamaguchi, K., and Standiford, T.J. (2001). Early recruitment of neutrophils determines subsequent T1/T2 host responses in a murine model of *Legionella pneumophila* pneumonia. *J. Immunol.* 166: 3355–3361.

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.