CHAPTER 1

General Introduction

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1. CRYPTOCOCCOSIS

1.1 History of Cryptococcus neoformans

*Cryptococcus neoformans* is an encapsulated yeast-like fungus, which can cause a systemic infection (cryptococcosis), mainly in patients with impaired cell mediated immunity. The organism was identified as a human pathogen in 1894 by the pathologist Busse, and the surgeon Buschke (1). They independently cultured the same fungus from lesions of the tibia and cutaneous lesions, respectively, of a 31-year old woman. In the same year, *C. neoformans* was isolated from peach juice by the Italian Sanfelice, who later proved its pathogenicity in laboratory animals (2).

Until 1950 many case reports were published on clinical infection with *C. neoformans*, including animal experiments with the organism isolated from these patients (3). Infection in the brain, lungs, kidneys and many other organs were described. Pathologist reported gelatinous masses and chronic granulomatous and tumor-like lesions (4) as well as relative absence of polymorphonuclear infiltrates (5). These different appearances of the yeast caused an on-going confusion with regard to the correct nomenclature. For example, *Saccharomyces hominis*, *Cryptococcus hominis*, *Torula histolytica*, *Debaryomyces hominis* were all early names for the pathogen. Around 1950 the name *C. neoformans* was established and two varieties were recognized: var. *neoformans* and var. *gattii*. Currently we recognize 3 varieties: *grubii* (serotype A), *gattii* (serotype B, C) and *neoformans* (serotype D). Most likely, var. *gattii* will be renamed as a novel species and called *C. bacillisporus* (6). The name of the teleomorphic form of *C. neoformans* var. *neoformans* is *Filobasidiella neoformans* (7).

From 1981 on the incidence of *C. neoformans*-infected patients has increased dramatically as a result of the ongoing AIDS epidemic. An estimated 36 million people worldwide are currently living with HIV, and some 20 million people have already died, giving a cumulative total number of HIV infections of 56 million (8). There were 5.3 million new HIV infections globally in 2000, and there is a clear potential for further massive spread. In most western countries 5-10% of HIV infected patients will develop cryptococcosis (9). In 1991 over 1200 cases of cryptococcal meningitis were diagnosed in New York City (10). Recently a decrease in incidence in these countries has been described, probably due to the introduction of HAART (Highly Active Antiretroviral Therapy) for treatment of HIV-infection (11). In sub-Saharan Africa, 10-15% of HIV infected patients will develop cryptococcosis (12). In Zimbabwe, however, cryptococcosis constitutes in 88% of HIV-infected patients the AIDS-defining illness, where it currently represents the most important cause of meningitis in adults (13;14).

Before 1950 untreated cryptococcal meningitis was, with some sporadic exceptions, in 100% of cases fatal (15). With the introduction of Amphotericin B in the late
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1950’s the first effective therapy against cryptococcosis was discovered (16) and death rates dropped to 10-25% of patients during treatment or in the immediate follow-up period (17;18). Due to the high prices of currently available antimycotics, we have unfortunately been able to observe the natural history of untreated HIV and cryptococcal meningitis in sub-Saharan countries. In Zimbabwe, for example median survival time from diagnosis to death is 14 days and only 22% of patients survived for more than 30 days without treatment (13). In Malawi, a median survival time after diagnosis is 4 days (19).

1.2 Clinical manifestation

Cryptococcosis, a systemic infection with *C. neoformans*, is mostly seen in patients who have a compromised immune system. Most important predisposing factors for cryptococcosis are HIV infection, use of immunosuppressive drugs, organ and bone marrow transplantation, chronic leukemias and lymphomas. The severity of clinical manifestation depends on the immune status of the host. In patients with AIDS, as compared to immunocompetent patients, the fungal burden is usually higher, and more extraneural sites of infection are present. In addition, relapse rates are higher. Time between first presentation and diagnosis is significantly longer in non-HIV infected patients (described in chapter 2). Cryptococcal meningitis (infection of the subarachnoid space) and meningoencephalitis (infection of both the subarachnoid space and the brain parenchyma) are the most frequently encountered life-threatening manifestations of cryptococcosis. Patients usually present with symptoms like headache, fever, nausea/vomiting, seizures, altered mentation, vision changes, and/or cranial nerve paresis which may have been already existing for 2 to 4 weeks before presentation. Physical examination can reveal signs such as papilledema and cranial nerve paresis. Nuchal rigidity does occur in 28% of HIV infected patients, and in 14% of non-HIV infected patients (chapter 2).

Pulmonary cryptococcosis is the second most relevant site of infection for *C. neoformans*. Clinical course depends on immune status of the host. *C. neoformans* can cause pneumonia in immunocompetent host. Symptoms include cough (54%), chest pain (46%), increased sputum production (32%), weight loss and fever (26%) (20). Immunocompromised (either HIV or non-HIV infected) patients with cryptococcal pneumonia generally have a more rapid clinical course and a greater tendency to disseminate their infection then immunocompetent patients (21;22).

Radiographically, cryptococcal pneumonia in the normal host may present with well-defined, non-calcified single or multiple lung nodules (23). Pulmonary cryptococcosis is diagnosed through antigen detection and culture of expectorated sputum, BAL (broncho alveolar lavage), transbronchial lung biopsy or needle aspiration. Serum and cerebrospinal fluid (CSF) analysis for antigen and cryptococcal culture should be performed to assess dissemination.
*C. neoformans* involvement of skin, eyes, genitourinary tract, bone and joints, muscle, heart, gastrointestinal tract, breast, lymph nodes, thyroid, adrenal gland head and neck has been described. Most of these cases should be considered as manifestations of disseminated cryptococcosis. It is currently an accepted practice to exclude presence of cryptococci in the brain by examination the CSF of all patients in whom the yeast has been isolated from another body site and who have defined risk factors for dissemination (see before) even if they are asymptomatic. Investigation of disseminated cryptococcosis should include CSF and blood culture as well as serological tests.

Microscopically, encapsulated *C. neoformans* can be detected in specimens of CSF or other host fluids dissolved in Indian ink preparations (24). Indian ink examination is a rapid test that can often deliver an immediate diagnosis within minutes after lumbar puncture. *C. neoformans* can be cultured from CSF, blood, sputum, urine and other specimens. Furthermore, latex agglutination tests for cryptococcal antigen (capsule) in body fluids are available (25). These tests are approximately 95% sensitive and 95% specific for identification of invasive cryptococcosis (26). Histopathologically, *C. neoformans* can be relatively easy identified in tissue because of its prominent capsule.

### 1.3 Management of cryptococcal disease

The choice of treatment for disease caused by *C. neoformans* depends on both the anatomic sites of involvement and the host’s immune status. Recently, guidelines for the management of cryptococcal disease were published by M.S. Saag et al. (27). For immunocompetent hosts with isolated pulmonary disease, careful observation may be warranted; in case of symptomatic infection, the indicated treatment is fluconazole, 200-400 mg/day for 3-6 months. This treatment is also recommended for individuals with non-CNS-isolated cryptococccemia, a positive serum cryptococcal antigen titer >1:8, urinary tract or cutaneous disease. For patients with more severe disease, treatment with amphotericin B (0.5-1 mg/kg/d) may be necessary for 6-10 weeks. For otherwise healthy hosts with CNS disease, standard therapy consists of amphotericin B 0.7-1 mg/kg/d, plus flucytosine, 100 mg/kg/d, for 6-10 weeks. Fluconazole "consolidation" therapy may be continued for as long as 6-12 months, depending on the clinical status of the patient. HIV-negative, immunocompromised hosts should be treated in the same fashion as those with CNS disease, regardless of the site of involvement.

For those patients with HIV who present with isolated pulmonary or urinary tract disease, fluconazole at 200-400 mg/d is indicated, lifelong (see later). For patients with more severe disease, a combination of fluconazole (400 mg/d) plus flucytosine, (100-150 mg/kg/d) may be used for 10 weeks, followed by fluconazole maintenance therapy. Among patients with HIV infection and cryptococal meningitis, induction
therapy with amphotericin B 0.7-1 mg/kg/d, plus flucytosine, 100 mg/kg/d, for 2 weeks followed by fluconazole (400 mg/d) for a minimum of 10 weeks is the treatment of choice. After 10 weeks of therapy, the fluconazole dosage may be reduced to 200 mg/d, depending on the patient’s clinical status.

Secondary prophylaxis against \textit{C. neoformans} used to be indicated in all HIV infected patients with a history of cryptococcal disease. However, the introduction of HAART has dramatically changed the course of HIV infection (28). The improvements in immunological function, as the result of HAART, made it possible to stop primary and secondary prophylaxes against various opportunistic infections (29;30). Very recently, two studies suggesting that it is safe to stop secondary prophylaxis against \textit{C. neoformans} in patients responding to HAART (increase in CD4 cell count to >100 cells/mm³), without relapse of their cryptococcal disease have been published (31;32).

2. \textbf{C. NEOFORMANS AND HOST RESPONSE}

2.1 Innate host defense in the lung

\textit{C. neoformans} is a worldwide occurring free-living organism that can survive in a variety of environmental niches. It has been isolated from pigeon and other avian excreta, fruits and soils (33). In contrast to bacteria, eukaryotic pathogens are generally not passed from person to person, and their evolution has not necessarily attained specific abilities to infect or invade the human host. A human fungal infection is generally the result of an accidental encounter during the life cycle of the fungus. Virulence genes and factors, in nature necessary for the organism to survive under various circumstances, are used to overcome the natural defenses of the host. A number of genes that allow the organism to grow and survive in the mammalian host have been defined.

Several lines of evidence suggest that human cryptococcosis result from inhalation of either desiccated, poorly encapsulated yeast forms, or basidiospores (34-36). These poorly encapsulated strains have a diameter of about 2 to 5 µm (37) and can penetrate the alveoli if not expelled through respiratory epithelia. In the alveolar spaces \textit{C. neoformans} is initially confronted by alveolar macrophages (38), which play a central role in host defense against \textit{C. neoformans}. Macrophages are able to bind, ingest and, with appropriate stimulation, kill yeast cells (39;40). Macrophage phagocytosis of \textit{C. neoformans} can occur through either antibody (41), and complement receptors (42) (serum-dependent-phagocytosis) or mannose (39), and β-glucan (43) receptors (serum-independent-phagocytosis). In the alveoli primitive opsonins, termed collectins, are present which contribute to innate resistance against inhaled microorganisms (44-47).
Collectins are humoral lectins found in mammals and birds. They belong to the animal C-type lectin superfamily characterized by a carbohydrate recognition domain (CRD), which binds ligands in a Ca\(^{2+}\)-dependent manner, plus a collagen tail involved in their biological function (48). They are related structurally and functionally to the first component of the classical complement pathway, C1q, and serve important roles in innate immunity through opsonization and complement activation. The lectin domain binds carbohydrates on microorganisms, while the collagenous regions are ligands for the collectin receptor (C1q receptor) on phagocytes and also mediate C1q-independent activation of the classical complement pathway (49). The pulmonary surfactant proteins A (SP-A) and SP-D, as well as the serum collectins mannose-binding lectin, conglutinen and CL-43 have been identified (50). The serum collectin human mannose binding lectin is involved in TNF-\(\alpha\) production by human monocytes stimulated with mannoprotein, one of the capsule components of \textit{C. neoformans} (51). One of the pulmonary collectins, SP-D, has been described to agglutinate acapsular \textit{C. neoformans} (52). The role of SP-D and SP-A in serum-independent phagocytosis of \textit{C. neoformans} is described in chapter 4. Serum-free phagocytosis of acapsular \textit{C. neoformans} by macrophages induces a range of proinflammatory cytokines, which may stimulate an effective cell mediated immune response before the yeasts are able to grow and synthesize appreciable quantities of capsule, which is known to undermine host defense (see later). Also, human alveolar macrophages containing phagocytosed \textit{C. neoformans} can induce proliferation of autologous T lymphocytes, suggesting their role as antigen-presenting cells in cryptococcal infection (53).

\textbf{2.2 Cell mediated immunity}

\textit{2.2.1 Delayed type hypersensitivity}

Full protection against \textit{C. neoformans} is dependent on multiple factors, which include innate and adaptive immune defenses. The factors that stand out as being crucial are the functional CD\(^{4+}\) T helper cells and the ability of the host to mount a cell mediated immune response. The importance of cell-mediated immunity (CMI) in protection against \textit{C. neoformans} has been firmly established in both animal experiments and patients (54-56). In absence of CD\(^{4+}\) T cells, patients with disseminated cryptococcosis are not able to clear the organism completely even with the best available antifungal therapy, and must be put on maintenance antifungal therapy for life (38) (see before). Evidence that CMI response induced by low dose infection with \textit{C. neoformans} is protective has come from studies with nude mice (54). After infection, heterozygous nu\(^{+}/\) mice developed an anticytococcal delayed type hypersensitivity (DTH) response and concomitantly the numbers of cryptococcal Colony Forming Units (CFU) in tissues were reduced. T-cell-deficient nude (nu/nu) mice did not develop an anticytococcal CMI response but did make high levels of anticytococcal antibodies and showed no signs of controlling the infection (54).
Positive DTH responses not only indicate the presence in the body of activated T cells, but also confirm that other components in the CMI cascade such as cytokines, chemokines, adhesion molecules, chemokine receptors and leukocyte migration are functioning.

The leukocyte infiltrate after intratracheal inoculation of *C. neoformans* into susceptible mice (like CBA/J mice) consists of macrophages, lymphocytes (CD4+ T cells, CD8+ T cells, B cells and NK cells), neutrophils and eosinophils (57;58). Activated macrophages appear to be the most important fungicidal effector cell (40;59). Thus, the recruitment and activation of phagocytes is an important component of CMI during cryptococcal infection. Mechanisms by which the host defense cells are attracted to the site of infection are complex. They involve local production of chemotactic factors that promote directed migration of phagocytes.

### 2.2.2 Chemokines

Specific substances termed chemotactic factors trigger directed locomotion of phagocytes towards the actual site of infection (chemokinesis). The phagocytes carry several receptors on their cell surface by which they are able to recognize these chemotactic factors. Chemoattractants are either secreted by activated host cells, or produced by complement activation or by the invading microorganism itself. They form gradients, which determine the direction of phagocyte migration. Well known chemoattractants for phagocytes are platelet-activating factor (PAF), arachidonate metabolite leukotrine B4 (LTB4), complement fragment C5a, bacterial derived formylated peptides like N-formyl-methionyl-leucyl-phenylalanine (fMLP) and chemokines like IL-8 and MCP. The two most important chemokine families are subdivided based on the position of the first two conserved cysteine residues: C-C chemokines (two adjacent cysteine residues) and the C-X-C chemokines (two cysteine residues separated by one aminoacid) (60;61). The C-C chemokines include MCP-1, MCP-2, MCP-3, RANTES, MIP-1α and MIP-1β, and are predominantly chemotactic for mononuclear cells. The C-X-C chemokines include IL-8, MIP2, and PF4, attract mainly neutrophils. Binding of chemotactic factors to their specific receptors activates multiple intracellular signaling pathways that regulate the intracellular machinery necessary to propel the cell in its chosen direction. The C-C chemokine MCP-1 is involved in clearance of pulmonary cryptococcal infection, and plays a critical role in the T cell-dependent immune response to *C. neoformans* (62). MIP-1α production is required during the efferent phase of pulmonary CMI for the recruitment of macrophages and neutrophils to the site of *C. neoformans* infection (63). Data of Huffnagle et al. (62;63) support the hypothesis that MCP-1 plays an important role in the initial recruitment of leukocytes (T lymphocytes and a small number of monocytes and neutrophils) that produce MIP-1α, which, in turn, mediates the bulk of monocytes
and neutrophil recruitment into the lungs. Furthermore, neutralization of MIP-1α reduces both TNF-α and IL-6 levels in C. neoformans infected mice (63).

2.2.3 Cytokines

Peripheral blood mononuclear cells (PBMC) and neutrophils have been shown to produce proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 when stimulated with C. neoformans or their cell wall components (51;64;65). The presence of certain cytokines during the induction phase of a CMI response also determines whether Th1 cells or Th2 cells predominate as activated T cells (66). Typically, the presence of IL-12, IL-18 or IFN-γ ensures that Th1 cells develop, whereas the presence of IL-10 and/or IL-4 directs the response to a Th2 response. Production of TNF-α is required for the development of protective T cell immunity to C. neoformans (67). The development of a protective Th1 type CMI and the production of Th1- and macrophage-activating cytokines TNF-α, IL1β, IL-12, IFN-γ, and GM-CSF are required to eradicate the infection (68), control cryptococcal dissemination from the lungs, and eliminate subsequent invasion in the brain (69). Phagocytes like macrophages, monocytes, and PBMC stimulated with pro-inflammatory cytokines show in general enhanced phagocytosis and fungicidal activity (70). In chapter 5 cytokines, which are produced by PBMC of healthy donors stimulated with C. neoformans and its capsular components, have been studied.

2.3 Dissemination to the brain compartment

In cases of dissemination from the lungs, cryptococci are able to escape local (impaired) innate and adaptive immunity and gain access to the bloodstream. In the vascular compartment, polymorphonuclear cells and monocytes are present. These cells have demonstrated effective in vitro killing, especially in the presence of opsonins, like complement (71;72). C. neoformans has a unique, partly unexplained, predilection for the brain. The yeast produces a unique phenol oxidase that converts a variety of hydroxybenzoic substrates, including catecholamines (e.g. norepinephrine and dopamine), into melanin, which impart a dark color to colonies (73). Melanin can function as an antioxidant, which may protect C. neoformans from oxidative host defenses (74). The wide availability of catecholamines in the brain could be a factor for the neurotropic features of the yeast.

In order to produce infection in the brain yeast cells must cross the blood brain barrier. Endothelial cells are able to phagocyte acapsular C. neoformans through a serum-dependent process (75), however no direct killing activity has been demonstrated in vitro (76). These observations suggest that endothelial cells may take up poorly encapsulated forms and that this phenomenon may contribute to C. neoformans dissemination to the brain compartment (75). After arriving in the brain, biosynthesis routes leading to capsule formation are strongly up-regulated. Brain
cryptococci are characterized by the presence of huge capsules and high levels of capsular products are shed into the vascular compartment.

In an autopsy series of 27 patients with cryptococcal meningoencephalitis significant differences in neuropathologic inflammatory response between AIDS and non-AIDS infected patients were found (77). Most non-AIDS patients had granulomas, supporting a role for CMI, whereas AIDS patients did not show granulomatous inflammation. The principal reactive cells in cryptococcal meningoencephalitis in AIDS patients were brain macrophages and microglia. In order to investigate if chemokines play a role in the recruitment of these cells, the induction of MIP-1α, MIP1-β and RANTES by HIV and non-HIV infected macrophages stimulated by \textit{C. neoformans} was investigated in chapter 6.

In HIV-infected patients with cryptococcal meningitis cytokine profiles in CSF were analyzed and showed high levels of the C-X-C chemokine, interleukin 8 (IL-8) (78). \textit{C. neoformans} products such as GXM (see later) can directly induce IL-8 production by isolated microglial cells in culture (79). IL-8 is a chemotactic cytokine, which partially mediates the adhesion of neutrophils to the endothelium and their migration through the vascular wall. Despite the high levels of IL-8 in CSF of infected patients, no leucocytosis was observed (78). Cryptococcal polysaccharides are generally considered to mediate inhibition of neutrophil extravasation (80).

3. POLYSACCHARIDE CAPSULE

3.1 Chemical composition

A distinctive feature of \textit{C. neoformans} relative to other medically important yeasts is the ability to produce an extracellular polysaccharide capsule. In nature it may protect the yeast from desiccation or reduce its ability to be ingested and destroyed by soil amoebae (81). In the pathogenesis of cryptococcosis the capsule has been shown to be a virulence factor (82;83). Several genes necessary for capsule formation CAP59, CAP64, CAP60, and CAP10 have been identified (82;84). In experimental cryptococcosis, capsule-free isolates are less virulent than their encapsulated wild-type cells (85). An encapsulated strain created by complementation of the CAP64 mutation produced fatal infection of mice within 25 days, while the CAP64 acapsular strain was avirulent (82). A number of effects of cryptococcal capsule polysaccharides, which may contribute to enhanced virulence, have been described. The capsule inhibits phagocytosis of \textit{C. neoformans} by macrophages, monocytes, and neutrophils (86). The capsule does not directly modulate phagocytic function, but instead, presents a surface that is not recognized by phagocytes (87). Furthermore, interference with antigen presentation by the cryptococcal polysaccharides has been described (88).
The capsule can vary in size between <1 \( \mu \text{m} \) and >50 \( \mu \text{m} \), depending on growing conditions. The capsule consists for 88% of glucuronoxylomannan (GXM), for 10% of galactoxylomannan (GalXM) and for 2% of mannoproteins (MP) (89). GXM is composed of a \( \alpha \)-1,3-linked polymannose backbone with \( \beta \)-linked monomeric branches of xylose and glucuronic acid. The four described serotypes A, B, C and D are discriminated on the base of differences in GXM structure. The different serotypes have a common core-repeating unit, but differ in the degree of mannosyl substitution and the molar ratios of mannose, xylose, and glucuronic acid (90). GalXM is composed of a backbone of \( \alpha \) (1\( \rightarrow \)6) linked galactose substituted with small side chains consisting of mannose, xylose and galactose (91). For individual strains there are differences in sugar composition in the GalXM, indicating that this polysaccharide is structurally heterogeneous (92). MP and GalXM often fractionate together in polysaccharide preparations. When GalXM-containing material is further fractionated on a concanavalin A affinity chromatography column, three peaks containing GalXM, MP1/MP2 and MP4 are observed. MP is proposed to be consisting of a protein backbone heavily substituted by short oligosaccharides mainly containing mannose although significant amounts of galactose and xylose are present (91). MP4 consists of 20% protein (predominant amino acids are serine, threonine, and alanine) and sugar moieties (xylose-mannose-galactose in a 1 to 7.7 to 0.8 ratio) (91).

### 3.2 Interference with neutrophil migration

The circulatory and migratory properties of leukocytes have evolved to allow efficient surveillance of tissues for infectious pathogens and rapid accumulation at sites of injury and infection. It is currently believed that leukocytes leave the circulation by first adhering to activated endothelial cells, followed by migration through the interendothelial junctions (93). Localized inflammation results in dilatation of vessels and diminished blood flow, allowing the transient adherence of PMN to endothelial cells by selectin-mediated tethering and rolling along the vessel wall (94;95). CD62L-mediated rolling precedes a further functional up-regulation of PMN following exposure to pro-inflammatory cytokines and chemoattractants (96), resulting in firmer, integrin-mediated adherence. Up-regulation of integrins is accompanied by shedding of CD62L from the cell surface (94;96). Further stimulation of PMN phenotypically high in CD11b/CD18 (CR3, Mac-1) initiates transendothelial migration via an interaction of Mac-1 with its counterreceptor ICAM-1 (97). Finally, chemotactic gradients guide migrating PMN to the site of infection. Patients who are genetically deficient in the leukocyte integrins have been described. Neutrophils in these leukocyte adhesion deficiency I (LAD-I) patients fail to cross the endothelium and accumulate at inflammatory sites and in vitro are deficient in binding to and migrating across resting or activated endothelial monolayers (98).
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The potency of *C. neoformans* culture supernatant to inhibit leukocyte migration was recognized almost half a century ago (99). Disseminated cryptococcosis is characterized by the presence of high levels of capsular antigens GXM and GalXM in the CSF and serum of affected patients (100;101). GXM titers in both serum and CSF from AIDS patients can reach levels up to 20 mg/ml (102). Despite the elevated IL-8 CSF/serum ratio and the elevated serum levels of TNF-α and IL-1β, the CSF of patients with cryptococcal meningitis typically contains few mononuclear cells and virtually no PMN (78). GXM is capable of interfering with leukocyte migration towards potent chemoattractants, such as fMLP and C5a (80;103;104). Furthermore, GXM can induce the production of IL-8 by human microglia but inhibits neutrophil migration towards IL-8 (79). GXM sheds L-selectin from neutrophils (105), and can bind to CD18 adhesion molecules thereby blocking interaction of activated neutrophils with ligands on the endothelium (106). To investigate if GXM actually interferes with leukocyte migration in clinical cryptococcosis, we compared retrospectively the GXM titer in serum and CSF with the CSF leukocyte cell counts of 35 Dutch HIV-infected patients with culture-proven diagnosis of cryptococcal meningitis (chapter 7). Furthermore, GXM has been described to delay translocation of PMN across the blood-brain barrier in a rabbit bacterial meningitis model (107).

Therefore, the initial aim to further study was to investigate the molecular mechanism by which GXM prevents PMN migration toward chemoattractants. Surprisingly, we found that cryptococcal culture filtrate (CneF) from GXM-producing and GXM-nonproducing (ΔCneF) strains, both prevented PMN migration towards IL-8 and fMLP. These results seriously question the opinion that GXM is the sole cryptococcal component preventing extravasation. The finding that MP-4 was primarily responsible for the inhibition of PMN migration is described in chapter 8.

4. OUTLINE OF THIS THESIS

In chapter 2 a survey of cryptococcosis cases in the Netherlands is described during a 14-year retrospective analysis of cases (1986-2000). In chapter 3, 4, 5 and 6 we follow *C. neoformans* on its route through the human body.

Cryptococcosis is likely to occur via inhalation of small, acapsular *C. neoformans*. The inhaled particles are small enough to reach alveolar spaces. One of the initial steps in host defense, is the interaction between acapsular cryptoccci and alveolar macrophages. In a laboratory environment, this interaction (binding and phagocytosis) is usually measured in tubes where both target (*C. neoformans*) and effector cells (for example alveolar macrophages) are in suspension. *In vitro*, however it is more likely that alveolar macrophages act as adherent cells. Therefore, in chapter 3 a method is described to measure phagocytosis of *C. neoformans* by adherent effector cells. Serum-free phagocytosis of *C. neoformans* by alveolar macrophages may be an
important part of the innate immune response because in the lung high concentration of serum opsonins, like complement factors, might be missing. **Chapter 4** describes the influence of non-serum opsonins, surfactant protein A and D on phagocytosis of *C. neoformans* by a number of effector cells, including human alveolar macrophages. A next step in immune response against *C. neoformans* includes influx of leukocytes to the side of infection, including PMN and mononuclear cells. Furthermore, in disseminated infection, cryptococci are able to escape to the vascular compartment. **Chapter 5** describes the cytokine profile of PBMC stimulated with *C. neoformans* and analysis the components responsible. Finally, yeast cells will be able to reach the brain compartment. In AIDS related cryptococcal meningoencephalitis brain macrophages and microglia were identified as principal inflammatory cells. In **chapter 6** a study is described which analysis which chemokines are produced by HIV-infected macrophages after stimulation with *C. neoformans* or GXM. CSF of patients with AIDS related cryptococcal meningoencephalitis contains few neutrophils despite high levels of neutrophil chemoattractant IL-8. As mentioned before cryptococcal polysaccharides are thought to play a role in the interference with neutrophil migration. In **chapter 7** patient data were analyzed for their titers of GXM in serum and CSF, and the results were correlated to matching CSF-counts. In **chapter 8**, the role of another cryptococcal polysaccharide, MP4, in interfering with leukocyte migration, is investigated.

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