Fungal Genetics and Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Molecules at the interface of *Cryptococcus* and the host that determine disease susceptibility

Karen L. Wozniak [a,b], Michal A. Olszewski [c,d], Floyd L. Wormley Jr. [a,b,*]

[a] Department of Biology, The University of Texas at San Antonio, San Antonio, TX, United States
[b] South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, TX, United States
[c] Veterans Affairs Ann Arbor Health System, Ann Arbor, MI, United States
[d] University of Michigan Medical School, Ann Arbor, MI, United States

ARTICLE INFO

Article history:
Received 25 July 2014
Revised 22 October 2014
Accepted 25 October 2014
Available online xxxx

Keywords:
Cryptococcosis
Cryptococcus neoformans
Cryptococcus gattii
Fungal pathogenesis
Host–fungal interactions
Medical mycology

ABSTRACT

Cryptococcus neoformans and *Cryptococcus gattii*, the predominant etiological agents of cryptococcosis, are fungal pathogens that cause disease ranging from a mild pneumonia to life-threatening infections of the central nervous system (CNS). Resolution or exacerbation of *Cryptococcus* infection is determined following complex interactions of several host and pathogen derived factors. Alternatively, interactions between the host and pathogen may end in an impasse resulting in the establishment of a sub-clinical *Cryptococcus* infection. The current review addresses the delicate interaction between the host and *Cryptococcus*-derived molecules that determine resistance or susceptibility to infection. An emphasis will be placed on data highlighted at the recent 9th International Conference on *Cryptococcus* and Cryptococcosis (ICCC).

1. Introduction

Cryptococcus neoformans and *Cryptococcus gattii*, the predominant etiological agents of cryptococcosis, are encapsulated fungal pathogens that cause disease ranging from asymptomatic infection to mild bronchopneumonia to life-threatening infections of the central nervous system (CNS) (Mitchell and Perfect, 1995). Cryptococcal meningoencephalitis is the most common disseminated fungal infection in AIDS patients (Vibhagool et al., 2003). Global estimates show that one million cases of cryptococcal meningitis occur in AIDS patients each year resulting in approximately 625,000 deaths (Park et al., 2009). Protection or susceptibility to *Cryptococcus* disease in both humans and experimental animal models is dependent on the outcome of many host and pathogen derived factors. The virulence of the organism relies on specific molecular factors that target host defenses to allow establishment of infection. Conversely, host protection is facilitated by an array of factors produced by the host and other effector functions of the immune cells. Altogether, the complex interplay between host and pathogen derived factors determine whether cryptococcal infection is resolved, allowed to progress, or remains dormant within the host's tissues.

The current review will consider the interactions between the host and *Cryptococcus* derived molecules that determine resistance and susceptibility to infection. An emphasis will be placed on recent data highlighted at the 9th International Conference on *Cryptococcus* and Cryptococcosis (ICCC).

1.1. Host molecules interacting with Cryptococcus

1.1.1. Cytokines, chemokines, and antimicrobial peptides

Cytokines and chemokines are small proteins that signal through a variety of cellular receptors in order to execute effector functions aimed toward elimination of the fungal pathogen while minimizing host damage associated with the infection. The role of cytokines and chemokines in promoting resistance or susceptibility to cryptococcal disease has been examined for many years; however, we are still far from full understanding of the complex interactions between the numerous members of cytokine networks and their temporal and inter-cellular relationships. Specific cytokines and chemokines produced by host cells have been associated with promoting protective immune responses or, conversely, susceptibility and progressive disease. Cytokines commonly associated with protective anti-cryptococcal immune responses include the pro-inflammatory cytokines TNF-α, IL-1α,

* Corresponding author at: Department Biology, The University of Texas at San Antonio, One UTSA Circle, M.B.T. Room 1.242, San Antonio, TX 78249-0062, United States. Fax: +1 (210) 458 7021.
E-mail address: floyd.wormley@utsa.edu (F.L. Wormley Jr.).

http://dx.doi.org/10.1016/j.fgb.2014.10.013
1087-1845/

IL-1β and IL-6, the Th1-type cytokines IFN-γ and IL-12 as well as Th17-type cytokines IL-17A and IL-22 (reviewed in Williams et al. (2011) and Wozniak and Levitz (2011)) (Wormley et al., 2007; Wozniak et al., 2011, 2014, 2009). Th1-type immune responses appear to be the most critical for protective immunity against cryptococcosis. Clinical studies presented by Jarvis et al. at the 9th ICCC showed the efficacy of using IFN-γ as an adjunctive therapy with antifungal drugs to facilitate significantly faster clearance of *Cryptococcus* from the cerebral spinal fluid compared to anti-fungal therapy alone (Jarvis et al., 2012; Pappas et al., 2004) (Jarvis et al. seminar presentation 10.1, 9th ICCC). Nevertheless, Wormley et al. showed that IL-17A is important for optimal protective immune responses against cryptococcosis; particularly in aiding prevention of *C. neoformans* trafficking to the central nervous system (CNS) (Wormley et al. seminar presentation 8.2, 9th ICCC) (Wozniak et al., 2011). In addition, acapsular cryptococcal biofilms can activate the NLRP3 inflammasome resulting in production of IL-1β. NLRP3 KO mice have less cellular infiltration, increased fungal burden, and a decrease in survival compared to WT mice during cryptococcal infection, suggesting that activation of the inflammasome may be important for protective anti-cryptococcal responses (Meng et al., seminar presentation S7.1, 9th ICCC). Studies presented by Kawakami et al. showed a potential role for IFN-α in the protective response against *C. neoformans* infection. Cryptococcal infection of IFNAR1 KO mice showed that the immune response involved Th1-type, Th2-type, and Th17-type cytokines, and the KO mice had increased bronchial mucin production and expression of MUC5AC compared to WT mice, which was inhibited by treatment with anti-IL-4 antibody (Kawakami et al., seminar presentation S7.3, 9th ICCC). In addition to cytokines, antimicrobial peptides are small molecules that mediate protective responses against microbial pathogens. They are induced by the cytokines IL-17A and IL-22, which have been detected during protective anti-cryptococcal responses (Wormley et al., 2007; Wozniak et al., 2011, 2014, 2009). Antimicrobial peptide studies showed that S100A8, S100A9, lipocalin-2, and SAA3 were significantly increased during protective anti-cryptococcal immune responses (Wozniak et al., 2014).

In contrast, cytokines associated with susceptibility and/or exacerbated cryptococcal disease include IL-4, IL-5, IL-13, IL-10, and IL-33 (reviewed in Wozniak and Levitz (2011) and Williams et al. (2011)) (Flaczyk et al.; Piehler et al., 2011; Stenzel et al., 2009). Studies presented by Piehler et al. demonstrated that expression of the IL-33 receptor T1/ST2 correlates to Th2-type responses during cryptococcal infection, and antigen-specific T1/ST2$^+$ T cells are the primary source of IL-5 and IL-13. These Th2-type responses then lead to defective pulmonary control of *Cryptococcus*. Furthermore, the innate lymphoid cells type 2 (ILC2) also express T1/ST2, and increase in the lung upon cryptococcal infection and may be involved in the initiation of non-protective Th2-type responses (Piehler et al., seminar presentation S7.5, 9th ICCC). The studies by Nielsen et al. showed, in turn, that increased recognition of chitin on *Cryptococcus* Titan cells led to the induction of a non-protective Th2-type immune response (Nielsen et al., seminar presentation S3.4, 9th ICCC). Furthermore, specialized cryptococcal-specific Treg cells can suppress detrimental Th2-type responses during pulmonary cryptococcosis (Wiesner et al., seminar presentation S7.6, 9th ICCC).

The importance of DC and macrophage activation in protection against cryptococcosis depends on the cytokines present in the microenviroment during infection. Interplay between the pro-inflammatory cytokine TNF-α and regulatory cytokine IL-10 in the generation of protective immune responses has been attributed to their opposing effects on dendritic cell (DC) activation in studies presented by Olszewski et al. TNF-α was shown to stabilize the DC1 (a term describing proinflammatory DCs) phenotype in pulmonary DCs, which promotes the generation and maintenance of protective Th1 and Th17 responses required for clearance of *C. neoformans* (Eastman et al. poster presentation 133, 9th ICCC). A reciprocal role of IL-10 was defined in another study, in which blockade of IL-10 by anti-IL10 antibody therapy increased DC activation, improved Th1 polarization, and augmented infiltration of exudate macrophages into the lungs (Olszewski et al. poster presentation 132, 9th ICCC). In addition to these crucial cytokines, a novel role of Notch ligand on DCs and Notch receptors located on T cells have been demonstrated to be crucial for execution of effector T cell cytokine responses in *C. neoformans* infected lungs (Hadd et al. seminar presentation S8.5, 9th ICCC). In turn, studies presented by Hole et al. suggested that Th1-type cytokine and chemokine (particularly CXCL10 and CXCL11) production in the lungs of protectively immunized mice promotes the infiltration of plasmacytoid DCs which were shown to have fungistatic anti-cryptococcal activity (Hole et al. poster 127, 9th ICCC).

Additional studies demonstrated the crucial role of cytokines in macrophage activation/polarization which largely accounts for clearance versus persistence of cryptococcal infection. Piehler et al. at the 9th ICCC showed that IL-4 producing Th2 cells are directly responsible for the induction of alternative macrophage activation and the pathology associated with progressive pulmonary cryptococcosis in mice (Piehler et al. poster presentations 123 and 124, 9th ICCC). These observations were complemented by studies which demonstrated that changes in cytokine environment can plastically alter macrophage polarization and, thus, their fungicidal properties (Arora et al., 2011; Davis et al., 2013; He et al., 2012) even in macrophages that were previously polarized (Davis et al., 2013). Collectively these studies demonstrated that (1) macrophages are a distal effector cell, ultimately responsible for clearance or persistence of *C. neoformans*, (2) cytokine microenvironment is a major factor driving macrophage polarization and the resultant macrophage fungicidal activity, and (3) manipulation of cytokine environment and/or interference with cytokine receptors on macrophages creates unique therapeutic opportunities that may be highly effective, even in advanced stages of cryptococcosis (Olszewski et al. seminar presentation S. 81 and Piehler et al. poster presentations 123 and 124, 9th ICCC).

In addition, cytokine and anti-cryptococcal responses are influenced by the recognition of cryptococcal patterns (PAMPS) via specific pattern recognition receptors (PRRs) on the surface of macrophages, DCs and other cells. Data presented at the 9th ICCC by Geijtenbeek et al. showed that signaling via C-type lectin receptors, specifically Dectin-1, leads to activation of caspase-8 and induces protective Th1-type and Th17-type anti-cryptococcal responses (Geijtenbeek et al., seminar presentation S7.2, 9th ICCC). Previous studies have reviewed the importance of other C-type lectin receptors as well as toll-like receptors (TLRs) in protection against cryptococcal infection (Dan et al., 2008; Mansour et al., 2006; Yauch et al., 2004), reviewed in Geijtenbeek et al. (2004), Levitz (2002), Romani et al. (2002). In addition, genetic polymorphisms of PRRs – specifically polymorphisms in FCGR 3A and FCGR 2B – lead to susceptibility to *C. neoformans* infection (Zhu et al., seminar presentation S3.1, 9th ICCC).

1.1.2. Antibodies

The role of antibody mediated immunity (AMI) in provoking protective anti-cryptococcal immune responses has been extensively studied; yet answers regarding the precise impact of AMI on inducing protection against *Cryptococcus* remain elusive. Experimentally, B cell KO mice inoculated with an engineered *C. neoformans* strain shown to induce protective anti-cryptococcal immunity remained protected against a secondary challenge with a pathogenic *C. neoformans* strain, suggesting that B cells and antibodies are not required in this murine model of protection (Wozniak et al., 2009). Also, earlier clinical studies showed that

Please cite this article in press as: Wozniak, K.L., et al. Molecules at the interface of *Cryptococcus* and the host that determine disease susceptibility. Fungal Genet. Biol. (2014), http://dx.doi.org/10.1016/j.fgb.2014.10.013