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Antibody Immunity and Natural Resistance to Cryptococcosis

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Abstract

Purpose of Review To review recent data on the role that B cells and/or antibody-based immunity play in host defense against *Cryptococcus neoformans* (Cn).

Recent Findings Cn, an encapsulated fungus, causes cryptococcal meningitis (CM). There are ~180,000 deaths per year worldwide attributed to CM, which is the most common cause of meningitis in adults with HIV in sub-Saharan Africa. HIV infection with advanced immunodeficiency is the most important predisposing risk factor for CM, highlighting the critical role that T cell-mediated immunity plays in disease prevention. However, numerous studies in the past decade demonstrate that antibody immunity also plays a role in resistance to CM. In mice, B cells reduce early dissemination from the lungs to the brain, and naïve mouse IgM can enhance fungal containment in the lungs. In concert with these findings, human studies show that patients with CM have lower IgM memory B cell levels and/or different serum profiles of Cn-binding and natural antibodies than controls.

Summary There is sufficient evidence to support a possible role for B cells and certain antibodies in natural resistance to CM. This underscores the need for a deeper understanding of mechanisms by which natural and Cn-binding antibodies may reduce Cn virulence and protect against Cn dissemination and human CM.

Keywords *Cryptococcus neoformans* · B cells · Antibodies · IgM · Host immunity · Adaptive response

Introduction

Cryptococcus neoformans (Cn) is an encapsulated basidiomycetes yeast widely distributed in the environment. It is the most common cause of meningitis in HIV-infected adults in sub-Saharan Africa, Asia, and South America [1, 2]. Cn is acquired by inhalation, makes the first stop in the lungs, colonizes this organ and in most people enters a state of latency [3, 4]. In some people, mainly those with advanced immune suppression, Cn can disseminate to the central nervous system and cause meningoencephalitis, or cryptococcal meningitis (CM) [2]. In 2014, it was estimated that there were 215,000 cases and 180,000 deaths due to Cn worldwide [5], primarily in HIV-infected persons. It is noteworthy that despite antiretroviral therapy (ART) roll out, the incidence of CM has not changed substantially in Africa or Asia [5–7].

B Cells and Resistance to CM: Historical Studies

As the HIV/AIDS pandemic unfolded and an unprecedented number of cases of CM occurred beginning in the 1980s, the link between CM and AIDS-associated CD4 T cell loss in patients established a role for T cells in resistance to CM. Studies in mice largely confirmed clinical observations in patients. On the other hand, a role for B cells was more difficult to establish. In part, this reflected an insufficient understanding that HIV infection also causes profound B cell defects. In addition, tools to study B cell effects in mice were limited. For example, one study did not reveal a difference in the susceptibility of B cell sufficient and B cell-depleted mice to Cn [8]. In this study, newborn mice were rendered B cell deficient by administration of rabbit anti-mouse-μ antiserum prior to

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intravenous infection with Cn. There was no difference in mortality, colony forming unit (CFU) in different organs, or antigen level in the sera of control and B cell-deficient animals. However, a subsequent study in a B cell knockout (uMT) mouse model showed these mice were more susceptible to Cn than wild type mice [9]. Notably, B cells were the predominant cell type in the lungs of A/JCr mice infected with Cn [10], demonstrating they contribute to the immune response to Cn. These early studies suggested B cells can enhance natural immunity and resistance to Cn.

Many studies dating to over 40 years ago show that administration of immune sera containing glucuronoxylomannan (GXM) capsular polysaccharide specific antibodies elicited by vaccination can protect naïve mice against Cn [11, 12]. Extensive work with monoclonal GXM antibodies showed that they can be protective, non-protective, or detrimental depending on isotype, specificity, and host factors [12]. These elegant studies revealed that the effect of specific IgG (or IgM) elicited by an acquired antibody response on the outcome of Cn infection is highly complex. One important study showed B cells were an important component of acquired resistance to Cn [13]. In this model, although Cn-infected μMt knock out (B cell deficient) mice developed an immune response that protected them against CM, SCID mice reconstituted with lymphocytes from these mice had high fungal burdens, failed to contain Cn, and had reduced survival, whereas mice reconstituted with lymphocytes from B cell-sufficient mice had lower lung and brain CFU. Thus, B cells contributed to control of Cn when T cell-mediated immunity was impaired [13]. This scenario resembles the immune status of patients with HIV/AIDS, whereby profound T cell loss occurs in the setting of marked B cell defects [14, 15]. Of note, a vaccine-elicited GXM IgG1 produced excessive lung inflammation and did not protect uMt mice from lethal Cn, suggesting that normal B cells and/or natural antibodies have a role in regulating inflammation stemming from antibody-mediated immunity to Cn [9]. Overall, these studies showed that administration of defined antibodies formed during an acquired immune response to Cn mediates protection against CM. However, they did not address the role that B cells or antibody may play in natural resistance to Cn.

B Cells and Their Role in Natural Resistance to Cn

Mice, like humans, are highly resistant to Cn. In humans, infection is common, disease is rare [3, 4]. In the past 10 years, numerous studies investigated the role that B cells play in natural resistance to CM in mice [16–19]. One study showed that B-1 and B-2 cells each contribute to the early immune response to Cn [17]. In this model, capsular and acapsular Cn bound to B-1 cells. B-1a cells were required for early Cn clearance from the lungs; they enhanced Cn phagocytosis and reduced dissemination to the brain [17]. This revealed a new paradigm to understand how B cells may augment host defense against Cn. In contrast to prior work that sought to establish a role for B cells and/or antibody in acquired immunity to Cn (see above), this study showed that B-1a cells are a key contributor to the early innate mouse immune response to Cn. In another study, the role of B-1 cells in resistance to intranasal infection with Cn was examined in X-linked immunodeficient (XID) mice [19]. XID mice, which have a mutation in the Bruton's tyrosine kinase (Btk) gene in B cells [20], lack B-1 cells and have reduced levels of natural IgM. These mice had higher lung and brain CFU than controls 3 weeks after infection [19]. Lung Cn phagocytosis was impaired and histopathology revealed a diffuse, disorganized inflammatory pattern with significantly more enlarged extracellular Cn. In contrast, control mice had numerous small, intracellular yeast cells [19]. This finding was of interest, because Cn can undergo morphological changes that result in the formation of Titan cells, which have enhanced virulence [21–24]. A Cn infection model in $Rag1^{-/-}$ mice showed that these mice, which lack B and T cells [16], exhibited earlier and more Cn dissemination to the brain than wild-type mice. Adoptive transfer of wild-type B cells to these mice led to reduced brain CFU and reversal of an abnormal inflammatory lung histopathology pattern to one that resembled the wild-type response.

How Might B Cells Augment Natural Resistance to Experimental CM?

Several hypotheses may explain how B cells enhance resistance to CM. One is that they help curtail Cn dissemination. This may be mediated by the antibodies they produce. B-1 cells mainly produce IgM. B-1 and B-2 cells each produce IgM in the early innate immune response to Cn that binds GXM and Laminarin (Lam, a mainly 1,3-β-glucan) [17]. Multiple studies now show IgM enhances early resistance to Cn dissemination in mice [16, 18]. IgM can also augment macrophage recruitment and phagocytosis of Cn [18, 25]. Therefore, naïve IgM may enhance early antifungal immunity in the lungs [16]. This was examined in $sIgM^{-/-}$ mice, which lack secreted IgM. In this model, lung CFU were similar in $sIgM^{-/-}$ and control mice after intranasal infection with Cn, but mortality was higher in $sIgM^{-/-}$ mice and they had higher brain CFU and marked brain inflammation [18]. Adoptive transfer of IgM restored control levels of Cn alveolar macrophage phagocytosis.

As noted above, adoptive transfer of B cells reduced Cn dissemination from lungs to brain in $Rag1^{-/-}$ mice [16]. Transfer of naïve IgM from wild-type mice in the same model enhanced alveolar macrophage phagocytosis of Cn. Taken together, these studies establish that B cells and/or their secreted product, IgM, enhance early innate immunity to Cn in the lungs of mice. Given that naïve IgM enhanced Cn

phagocytosis in multiple models, e.g. (wild-type, sIgM$^{-/-}$, and Rag1$^{-/-}$ mice), it is logical to posit it contains antibodies that bind Cn determinants that augment phagocytosis and/or other host defense mechanisms in the lungs. Consistent with this idea, naïve IgM enhanced host defense against pneumocystis in mice via antibodies that reacted with beta glucans [25]. Thus, B cells and/or naïve IgM reduce early Cn dissemination, making them a component of the early innate immune response to Cn in the lungs.

Another mechanism by which IgM could enhance resistance to Cn is by restricting fungal size in the lungs and promoting fungal containment. Though this remains a hypothesis, it is reasonable to posit that absence of IgM in the lungs may have contributed to Cn enlargement in XID mice [19], possibly inhibiting Titan cell formation. In support of this idea, a mouse1,3-β-glucan-binding IgG2b (2G8) isolated from a Lam-vaccinated mouse bound the Cn cell wall, mediated non-opsonic Cn killing in vitro, protected mice against lethal Cn challenge [26]. Cn isolated from lungs and brain of 2G8-treated mice were smaller than those in control mice, suggesting the antibody may have inhibited Titan cell formation.

Translating Knowledge Gained from Mice to Humans

Numerous serological studies dating from the mid-1990s have demonstrated differences, often featuring lower levels of GXM-IgM, between the GXM antibody profiles of HIV-infected persons, HIV-infected persons with a history of CM, and HIV-uninfected controls [27–31]. These studies linked perturbations in GXM antibody repertoires with HIV infection and CM. However, differences were not limited to those with HIV infection, a study of HIV-uninfected solid organ transplant (SOT) recipients showed pre-transplant levels of GXM-IgM were lower in those who developed CM post-transplant than those who did not [32]. Since IgM memory (CD10+CD27+IgM+) B cells, (see [31]) which resemble B-1 cells in mice [33], are the source of ~ 50% circulating IgM and are depleted in HIV infection [14, 31], perturbations in IgM may be due to a B cell repertoire defect.

Links Between IgM Memory B Cell Levels and Risk for CM

Consistent with studies in mice linking B-1 cells to resistance to Cn dissemination, an association between lower levels of peripheral IgM memory B cells and HIV-associated CM was identified in two cohorts [31]: (1) HIV-infected persons with a past history of CM and HIV-infected persons with no history of CM (retrospective cohort); and (2) HIV-infected males in the multicenter AIDS cohort study (MACS) who subsequently developed CM and those who did not matched for CD4 T cell count (> 300 cells/ul, prospective cohort). Persons in both cohorts who had/or developed CM had lower levels of memory (CD19+CD27+) and IgM memory B cells than those with no history of or who did not develop CM. A similar study with a cohort of HIV-uninfected persons with and without CM had similar findings; those with a history of CM had lower levels of memory and IgM memory B cells than those who did not [34]. CD4 T cell levels were statistically comparable and CD8 T cell levels were numerically higher, but not statistically significantly so in those who developed CM. X-linked hyper IgM (XHIM). The latter, which is marked by elevated serum IgM, low IgG levels and reduced levels of IgM memory B cells, has also been associated with cryptococcosis in children [35]. A mutation in the Bruton tyrosine kinase is the cause of X-linked agammaglobulinemia [36], and as above, XID mice are more susceptible to Cn dissemination. Notably, cases of CM are increasingly reported in adult patients treated with the Bruton tyrosine kinase inhibitor, ibrutinib [37–41].

Links Between IgM and Natural Antibodies and Risk for CM

IgM memory B cells produce ~ 50% of the IgM in human sera [33]. Human serum IgM and IgG bind carbohydrate and polysaccharide Ags, including β-glucans, conserved fungal determinants found on Cn and many other fungi [42]. For example, human sera from HIV-infected patients with CM bound to glycosylated determinants on the Cn cell wall and inhibited its growth [43].

Two recent studies examined levels of Cn and Lam (β-glucan) binding antibodies in persons at risk for and with CM [44, 45]. Normal human serum antibodies bind Lam, a branched (mainly) 1, 3-β-glucan [42]. One study compared HIV-infected persons with positive or negative serum assays for cryptococcal antigen (CrAg) [45]. CrAg positive persons are at high risk for CM [46]. The results showed that Lam-binding-IgM and IgG were lower in CrAg positive persons and negatively correlated with CrAg positive status. Using an iterative statistical model, this study found that in combination, plasma IgG2, IgM, GXM-IgG, Lam-IgM, and Lam-IgG had an 80% ability to predict CrAg positive status. This suggests statistical modeling may hold promise for identifying serological biomarkers of risk for CM. In another study, plasma levels of IgM, GXM-IgM, and Lam-binding-IgM were lower in HIV-infected patients who developed cryptococcal-immune reconstitution inflammatory syndrome (C-IRIS) after ART initiation [44]. These findings suggest β-glucan-binding antibodies may have a role in preventing CM and/or the inflammatory manifestations of C-IRIS, and may also hold promise as biomarkers of risk for C-IRIS. Since β-D-glucan (BDG) levels may be elevated in patients with CM [47], associations between lower levels of Lam antibodies, CM, and

C-IRIS suggest these antibodies may play a role in controlling BDG-mediated inflammation. Finally, we note that multiple studies show that serum levels of GXM-IgG are higher in HIV-infected persons, those with a history of CM, and those with positive CrAg assays than controls [27, 28, 34]. These data suggest the hypothesis that GXM-IgG levels may reflect fungal burden, an idea that requires further study.

Conclusion

While recognition of the role that cell-mediated immunity plays in resistance to CM is longstanding, particularly in HIV-infected persons, ample data now show B cells also contribute to resistance to CM in mice and may play a similar role in humans. In mice, B cells and naïve IgM enhance immunity to Cn in the lungs. In humans, lower levels of IgM memory B cells associate with CM in HIV-infected and HIV-uninfected persons, and lower levels of GXM-IgM, Lam(β-glucan)-binding IgM and IgG can associate with HIV-associated CM, risk for CM, and/or C-IRIS. These findings require validation in larger, racially diverse cohorts, and statistical modeling may help identify robust serological markers. Nonetheless, available data point to a possible role for B cells and certain antibodies in natural resistance to CM and underscore the need for a deeper understanding of mechanisms by which natural and Cn-binding antibodies may reduce Cn virulence and protect against Cn dissemination and human CM.

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Compliance with Ethical Standards

Conflict of Interest Nuria Trevijano-Contador and Liise-anne Pirofski declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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