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Abstract

Increased incidence of fungal infections in the immunocompromised individuals and fungi-mediated allergy and inflammatory conditions in immunocompetent individuals is a cause of concern. Consequently, there is a need for efficient therapeutic alternatives to treat fungal infections and inflammation. Several studies have demonstrated that antibodies or immunoglobulins have a role in restricting the fungal burden and their clearance. However, based on the data from monoclonal antibodies it is now evident that the efficacy of antibodies in fungal infections is dependent on epitope specificity, abundance of protective antibodies and their isotype. Antibodies confer protection against fungal infections by multiple mechanisms that include direct neutralization of fungi and their antigens, inhibition of growth of fungi, modification of gene expression, signaling and lipid metabolism, causing iron starvation, inhibition of polysaccharide release and biofilm formation. Antibodies promote opsonization of fungi and their phagocytosis, complement activation and antibody-dependent cell toxicity. Passive administration of specific protective monoclonal antibodies could prove to be beneficial in drug-resistance cases, to reduce the dosage and associated toxic symptoms of anti-fungal drugs. The longer half-life of the antibodies and flexibilities to modify their structure/forms are additional advantages. The clinical data obtained with two monoclonal antibodies should incite interests in translating pre-clinical success into the clinics. The anti-inflammatory and immunomodulatory role of antibodies in fungal inflammation could be exploited by intravenous immunoglobulin or IVIg.

Keywords: Fungi . Immunoglobulin . Inflammation . Aspergillus . Candida . Intravenous immunoglobulin . Therapy –Natural antibodies

Background

Fungi are among the most common microbes encountered by mammalian hosts. Approximately, 1-10 fungal spores are ingested in each breath, making it a natural route of infection for most filamentous fungal pathogens. Medically important fungi include *Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Malassezia*, *Paracoccidioides* and *Pneumocystis* [1-3]. Fungi are experts in sensing their surrounding environment and respond suitably to the different fluctuating environmental factors. Due to their acclimatization capabilities, fungi can interact with plants, animals and humans and establish symbiotic, commensal, latent or pathogenic relationships. For example, *Candida albicans* are commensal organisms in humans until the host becomes immune deficient, which can lead to life-threatening disease [4]. Omics-based approaches have revealed a link between fungal metabolism, morphogenesis and response to stress during adaptation to the host environment. These processes not only enhance fungal virulence but also provide opportunities for identifying potential therapeutic targets [5].

Many fungal pathogens as well as commensal fungi have co-evolved with their mammalian hosts over millions of years. This shows that fungi have developed effective and complex strategies to antagonize immune responses in the host. One recent report shows that air borne fungal spores of *Aspergillus fumigatus* evade the innate immune recognition and immune responses by expressing surface "rodelt layer" [6, 7]. This layer is composed of hydrophobic RodA protein covalently bound to the conidial cell wall through glycosylphosphotidylionositol remnants. RodA extracted from conidia of *A. fumigatus* was immunologically inert and did not induce

dendritic cell (DC) or alveolar macrophage maturation and activation. The disruption of this 'rodlet layer' chemically (using hydrofluoric acid), genetically (Δ*rodA* mutant) or biologically (germination) resulted in a conidial morphotype that induce immune activation. These observations show that the fungal pathogens have immune evasive mechanisms.

Innate immune responses are the first line of defense against fungal infections that lay foundation for the long lasting, more specific and effective adaptive immune responses. The fungal pathogen-associated molecular patterns (PAMPs) such as heat-shock protein 60 (Hsp60), β-glucans, phospholipomannan, O-linked mannans, zymosan and fungal DNA are recognized by various pattern recognition receptors that include toll-like receptors (TLRs) (such as TLR 2, 4 and TLR9) and C-type lectin receptors (such as Dectin-1 and DC-SIGN) [8-10]. These detection mechanisms are also complemented by other defense mechanisms such as microbial antagonism, defensins, collectins and complement system.

The detection of fungal pathogens by phagocytes especially macrophages and DCs initiate downstream intracellular events that activate immune responses resulting in efficient clearance of fungi through phagocytosis and direct pathogen killing. Neutrophils play a key role in clearing hyphae, the tissue-invasive form of molds. DCs migrate to secondary lymphoid tissues and polarize diverse $CD4^+$ T-cell (T helper, Th) responses including Th1, Th2, Th17, and regulatory T (Treg) cell responses. This has been shown in case of *Histoplasma capsulatum*, *Cryptococcus neoformans*, *C. albicans* and *A. fumigatus*. The Th cells in turn direct B cells to produce antigen-specific antibodies that mediate humoral immunity.

Role of humoral immune response in the protection against fungal infections: data from experimental models

Antibodies or immunoglobulins (Igs) are glycoproteins and one of the vital components of the immune system. Five classes or isotypes of antibodies have been identified that include IgG, IgM, IgA, IgE and IgD and their prevalence in the blood in the order of IgG>IgA>IgM>IgD>IgE. Further IgG is divided into four subclasses such as IgG1, IgG2, IgG3 and IgG4 in human and IgG1, IgG2a, IgG2b and IgG3 in mice. IgA is most abundant antibody at mucosa and is divided into IgA1 and IgA2. Studies to prove the beneficial effects of antibodies in the protection against fungal infections have mostly came from *in vivo* studies in experimental models. These data suggest that antibodies provide protection against fungal infections via several and possibly interdependent mechanisms. In fact, antibodies are well known effector molecules of the adaptive immune system and neutralize the pathogens and their derived molecules in part through activation of the complement. In addition, they also exert regulatory role in the activation of innate immune cells by signaling via diverse Fc receptors. However, initial studies to understand the role of antibodies in anti-fungal immunity were largely inconclusive. These inconclusive reports could be due to occurrence of insufficient proportion of protective antibodies in the serum that are capable of clearing fungal infection. On the other hand, there could be inhibitory antibodies that neutralize the effect of protective antibodies [11, 12].

Several reports demonstrated that natural antibodies have an important role in the defense against fungal infection. In fact, administration of normal mice serum to μMT mice was shown to restrict the fungal growth in various models [13-16]. Natural

antibodies are polyreactive, generally germ-line encoded and are characterized by low to medium affinity. Natural antibodies belong to IgM, IgA and IgG classes and are produced mainly by B1 cells [17-23]. A substantial fraction of serum antibodies from naive mice recognize fungal antigens including *C. albicans* [24, 25]. Further, passive administration of a monoclonal natural IgM antibody 3B4 recognizing self-antigen keratin and germ tubes of *C. albicans*, protected mice from *C. albicans*-induced death. The anti-fungal mechanisms of this natural antibody include direct suppression of germ tube formation and enhancing the macrophage-mediated phagocytosis of candida by opsonization [24, 26] (Fig. 1). In line with these observations, murine studies have shown that administration of opsonizing antibodies results in protection against invasive candidiasis [27, 28] although beneficial effects could not be observed in vaginal candidiasis [29].

Natural IgM are important for the resistance to *C. neoformans and Pneumocystis murina* in mice by diverse mechanisms. It was proposed that natural IgM enhance the recruitment of macrophages to the site of infection and phagocytosis of fungi; guide the recognition of fungal antigens by DCs and their migration to draining lymph nodes; and support B cell class-switch by helping differentiation of Th2 cells [30, 31]. In line with these observations, mice with X-linked immunodeficiency that have significantly lower levels of IgM displayed higher susceptibility to *C. neoformans* infection [32].

B-cell depleted mice show higher susceptibility to systemic candidiasis [33]. Systemic challenge of *C. albicans* in athymic mice [34], severe combined immunodeficiency (SCID) mice [35] and antibody deficient CBA/N mice lacking

Lyb-5 B cells [36] showed that humoral immune responses play an important role in conferring protection against systemic candidiasis. Further, studies in B-cell knockout (J_HD μ KO) mice have shown that these mice are susceptible to experimental systemic candidiasis but resistant to mucosal and systemic candidiasis of endogenous origin [37]. These J_HD mice lacked circulating B cells or secretory antibodies due to disruption of immunoglobulin gene J_H that arrests B cell development in the bone marrow. After oral immunization, these mice developed protective immunity to intravenous challenge. However, the mice showed colonization of *C. albicans* in the gut, indicating that the mode of infection does influence the outcome of immune responses by the host.

A report by Romani and colleagues reveals that antibodies have a critical role in the generation of memory anti-fungal immunity [16]. By evaluating the susceptibility of wild-type and B-deficient (μMT) mice to *C. albicans* or *A. fumigatus* infections by intravenous or intra-tracheal route respectively, they found that μMT mice could efficiently limit the fungal growth both upon primary and the secondary infections. Their results thus point out that Th1 cells are important to mediate protective immunity to these two fungal pathogens. However, μMT mice were incapable of surviving the re-infection with *C. albicans*. These results thus indicated that although the resistance to *Aspergillus* is independent of B cells and antibodies, protection against *Candida* appears to be mediated both by antibody-dependent and independent mechanisms [16]. Administration of normal mice serum to μMT mice further restricted the fungal growth, thus confirming that antibodies do have a role in restricting the fungal burden and in the clearance of pathogens, but as discussed later,

their efficacy might be dependent on epitope specificity, abundance of protective antibodies and their isotype.

The findings of Romani and colleagues also suggest that the functions of antibodies in the protection against fungal infections might go beyond neutralization of pathogens, opsonization, antibody-dependent cytotoxicity or preventing adherence [16, 38] (Fig. 1). Thus, they identified a novel mechanism through which antibodies might participate in the protective immunity to *Candida* infections. It is known that circulating antibodies and B cells have remarkable ability to modulate the immune responses by regulating the functions of antigen presenting cells such as DCs [39-46]. Romani and colleagues reported that the inability of μMT mice to survive re-infection with *C. albicans* was associated with failure to generate IL-10-producing $CD4^{+}CD25^{+}$ Tregs. Interestingly, antifungal opsonizing antibodies could restore IL-10 production in DCs indicating that antibodies could limit the exaggerated inflammatory responses to fungal infections and might educate the DCs for the development of long-lasting anti-fungal immunity [16] (Fig. 1).

In experimental paracoccidioidomycosis, a chronic granulomatous disease caused by thermally dimorphic fungi *Paracoccidioides brasiliensis*, circulating normal antibodies were shown to control *P. brasiliensis* growth and organization of the granulomatous lesions by regulating the infiltration of inflammatory cells [47].

Several reports also demonstrate that protective anti-fungal antibody responses could be induced in mice by vaccination with appropriate fungal antigens. Thus, vaccination with a liposomal-mannan admixture mediated antibody-dependent

protection against *C. albicans* [14]. Importantly, synthetic glycopeptide vaccines that combine β-mannan and peptide epitopes (corresponding to those proteins expressed during human candidiasis and their cell wall association) also induced high titred antibodies to β-mannan and test antigens that include fructose-bisphosphate aldolase-Fba, methyltetrahydropteroyltriglutamate-Met6 and hyphal wall protein-1. In addition, these antibodies rendered protection against experimental disseminated candidiasis following DC vaccination approach [48]. Further, passive transfer of immune sera either from peptide (Fba)-vaccinated mice or glycol-peptide ([β-$(Man)_3$]-Fba)-vaccinated mice, conferred protection in naïve mice [49, 50]. Similarly, vaccination with other antigens was also shown to elicit protective antibody responses. A glycol-conjugate vaccine consisting of laminarin, a β-glucan from *Laminaria digitata*, and diphtheria toxoid CRM197 (Lam-CRM conjugate) protected mice against *A. fumigatus* and *C. albicans* by eliciting anti-β-1,3-glucan antibodies [51, 52]. Intravaginal immunization with secreted aspartic proteases family (Sap2t) of *C. albicans* elicited protective mucosal IgG and IgA antibodies to Sap2t. Passive transfer of these antibodies or anti-Sap2t IgM and IgG monoclonal antibodies protected mice against vaginal candidiasis [53]. A virosomal vaccine containing Sap2t also induced similar immune responses and protection against vaginal candidiasis [54]. In line with these reports, immunization with purified or recombinant major surface glycoprotein of *Pneumocystis carinii* elicited protective humoral and cellular responses in rats [55]. These data thus suggested that abundance of protective antibodies is the key factor that determines the role of antibodies in the protection against fungal infections.

DNA vaccination strategy was also explored for eliciting protective antibody response to fungal pathogens. *Pneumocystis pneumonia* infection is the most prevalent respiratory pathogen of AIDS patients and the options for immunotherapy have been limited given the poor $CD4^+$ T cell immune responses. DNA vaccination with a *Pneumocystis* antigen, kexin linked to CD40 ligand, induced strong antibody response in mice, and that B cells or IgG from vaccinated mice were highly protective upon adoptive transfer [56, 57]. This approach is highly desirable for patients who have $CD4^+$ deficiency or dysfunction as this method could induce protective humoral responses independent of $CD4^+$ T cells.

Demonstration of protective role of antibodies in fungal infections by using monoclonal antibodies

As relative abundance of protective specific antibodies was postulated as one of the factors that determine the protection afforded by circulating antibodies, various groups have evaluated this hypothesis by using monoclonal antibodies (MAbs). Most of the protective antibodies described to date recognize surface molecules that include, but not limited to glucans, mannans, and glucuronoxylomannans. In addition, proteins and glycolipids could also induce protective antibodies upon immunization.

By using *C. neoformans* capsular glucuronoxylomannan-specific murine MAbs, Casadevall and colleagues compared the protective capacities of various isoforms of antibodies upon passive transfer to lethally infected mice. They found that on a weight basis, IgA isotype antibody was most effective as compared to IgG1> IgM> IgG3. However, IgA has a shorter half-life than IgG1 in the circulation and hence more IgA antibodies would be required for the protective effects. Therefore,

authors further performed the experiments by using antibody concentrations that closely mimics *in vivo* situation and found that IgG1 was more effective than IgA in conferring the protection against challenge [15]. In addition, other reports also confirmed the therapeutic potential of murine IgG1 MAb to capsular polysaccharide (CNPS), IgM MAb that binds to melanin and murine IgG2b MAb to glucosylceramide and β-glucan (laminarin) of *C. neoformans* [58-61].

The subclass of IgG also plays an important role in the protection against *C. neoformans*. The relative efficacy of IgG subclass antibodies was in the order of IgG1, IgG2a, and IgG2b ≫ IgG3. Switching from IgG3 to IgG1 converted a nonprotective glucuronoxylomannan-reactive MAb into a protective antibody [62, 63]. Thus these data indicated that by simple isotype/subclass switching, a non-protective antibody could be converted into a protective antibody and hence suggesting that those non-protective antibodies reported for fungal infections should be re-examined for the isotype. These *C. neoformans* protective glucuronoxylomannan-specific IgG MAbs seem to work in cooperation with nitric oxide, and both Th1 and Th2 cytokines [64, 65]. In addition, binding of protective glucuronoxylomannan-reactive 18B78 IgG MAb and IgM MAbs (12A1 and 13F1) to *C. neoformans* also modifies the gene expression of the fungi, phosphorylation of proteins and lipid metabolism [66]. (Fig. 1) The complement component C3 was found to be dispensable for the protection by these IgGs [67]. In addition, mouse background also shown to influence the protection given by IgG subclass antibodies [68] thus underscoring the complex relationship between the cellular and humoral components of the immune system. Further studies from the same group revealed that epitope specificity of the MAbs is the critical factor that determines the serotype-

specific protection rendered by the anti-*C. neoformans* MAbs and to confer protection against distinct serotypes of *C. neoformans* [69].

Han et al., showed that transfer of β-1, 2-mannotriose [β-$(Man)_3$]-specific IgM MAbs enhance the resistance to disseminated candidiasis in normal, SCID and neutropenic mice, and to vaginal infection [14, 70, 71] and was dependent on the specificity of the antigens but independent of isotype (IgM or IgG3) of antibodies [72]. Structural analysis revealed that internal antigenic determinants dominate recognition of β-$(Man)_3$ by IgG3 MAb [73]. β-mannan-specific IgM MAb could also reduce the dose of amphotericin B when used in combination in experimental candidiasis [74]. In contrast to *C. neoformans*-specific MAbs, complement was found to be essential for the protection by *C. albicans* β-mannan-specific IgM and IgG3 MAbs and was associated with enhanced phagocytosis and killing of the yeast cells by phagocytic cells [75, 76] (Fig. 1). These results thus suggest that the mechanisms of anti-fungal antibodies might also depend on the fungal species and epitope specificity of the antibodies and that a generalized mechanism cannot be drawn.

An IgM MAb C7 that reacts with Als3p and enolase of *C. albicans* cell wall exerted three anti-candida actions such as candidacidal activity and inhibition of both adhesion and filamentation [77]. Subsequently it was found that the candidacidal activity of this MAb was linked to interference with iron acquisition by *C. albicans* [78] (Fig. 1). Of note, MAb C7 also showed reactivity against several species of Candida as well as in *C. neoformans*, *Scedosporium prolificans* and *A. fumigatus* thus pointing towards broad-spectrum activities of this antibody [78].

Compared to *Candida* and *Cryptococcus*, studies on the development of therapeutic MAbs to *A. fumigatus* are limited. An *A. fumigatus*-specific IgG1 MAb directed against cell wall glycoprotein of *A. fumigatus* exhibited protection against experimental aspergillosis in mice and significantly reduced the fungal load in the kidneys. The protection by this MAb might be due to its effects on germination of *A. fumigatus* [79]. The same group also developed an IgM MAb against immunodominant catalase B of *A. fumigatus* that exerted anti-*A. fumigatus* activities *in vitro* [80]. In a murine pulmonary aspergillosis model, *A. fumigatus*-specific IgM MAb-alliinase conjugate enhanced survival of immunosuppressed mice by causing specific killing of *A. fumigatus* without damaging the lung tissue [81].

IgG2a and IgG2b MAbs to gp43 of *P. brasiliensis* were shown to reduce fungal burden and was associated with the enhanced phagocytosis of *P. brasiliensis* by macrophages leading to increased nitric oxide production [82]. Prophylaxis passive intranasal administration of anti-glycoprotein A IgM or IgG1 switch variant MAb protected against murine *P. carinii* [83]. Further studies revealed that complement was required for the protection conferred by anti- *P. carinii* IgG1 antibodies [84].

Passive transfer of cell surface histone-like protein-specific IgM MAbs protected the mice against *Histoplasma capsulatum* by altering the intracellular fate of the fungus in the macrophages in a complement receptor 3-dependent process [85, 86]. This protection was associated with the enhanced IL-4, IL-6 and IFN-γ in the lungs either on day 2 or day 7 post-infection. Similar to this report, passive transfer of *H. capsulatum* Hsp60-specific protective IgG1 and IgG2a MAbs significantly sustained the survival of mice infected with *H. capsulatum*. Administration of these

MAbs could alter the pathogenesis *H. capsulatum* by modulating its intracellular fate and by significantly boosting Th1 cytokine responses such as IL-2, IL-12, IFN-γ and TNF-α but not IL-4 in various organs either at day 7 or day 14 post infection [87]. Thus, enhancement of Th1 cytokine responses and modulation of intracellular fate of the fungus seems to be common factors associated with protection rendered by *H. capsulatum* MAbs. However, the regulation of cytokine responses might be dependent either on isotype of MAb or time-point of analysis.

Role of antibodies in the protection against fungal infections: Data from human studies

Normal human serum or repertoire contains natural antibodies to various pathogens. Candidal mannan-specific human IgG antibodies from normal human serum were shown to mediate classical complement pathway initiation [88]. Affinity purified natural mannan-specific human IgG displayed prozone-like effect and hence therapeutic use of monoclonal version of these natural IgGs requires careful dose titration studies [89]. A full-length human recombinant anti-mannan IgG1 (M1g1) was generated from anti-mannan Fab that was isolated from a phage Fab display combinatorial library containing Fab genes of bone marrow lymphocytes [90]. M1g1 activated complement pathway, enhanced phagocytosis and phagocytic killing of *C. albicans* by murine macrophages and rendered resistance to disseminated candidiasis in mice [90]. The complement activation and deposition of C3 on *C. albicans* by M1g1 could be independent of Fc-region as Fab fragments could activate alternative pathway to initiate C3 deposition [91]. Natural antibodies that react with candida antigens are also part of mucosal immunoglobulin repertoire wherein IgA from saliva

were shown to recognize *Candida* antigens such as phosphoglycerate kinase and fructose bisphosphate aldolase [92].

Confirming the experimental studies on the role of immunoglobulins and B cells in the protection against fungal infection, a primary hepatic invasive aspergillosis with progression has been reported in a patient following rituximab therapy for a post transplantation lymphoproliferative disorder [93]. This report was further substantiated by another report wherein rituximab therapy was significantly associated with increased risk for invasive aspergillosis in patients with lymphoproliferative diseases after autologous hematopoietic stem cell transplantation [94].

Though, many break-through studies have dissected the role of antibodies in anti-fungal immunity, the translation of these pre-clinical studies to patients is still under progress. The presence of specific antibodies in patients with progressive fungal infections has provided evidence against a protective role of antibodies in fungal infections. Also, it has been shown that naturally acquired antibodies develop during infancy to *C. albicans* and in early childhood to *C. neoformans* [95]. However, the individuals still could not fight against fungal infections indicating that the presence of antibodies does not necessarily prevent fungal infections. Based on the reports from experimental studies, it is now clear that these patients' data might not reveal fundamental incapacity of antibodies to protect against fungal infections rather point towards inadequate amounts of protective antibody and/or the concurrent presence of both protective and non-protective antibodies. In fact, higher levels of IgG protective antibodies including those against Met6p, Pgk1p and Hsp90 are

associated with good-prognosis in invasive candidiasis patients [96]. Another report indicated that patients who survived candidiasis display amplified antibody reactivity towards C-terminal epitope of mp58 mannoprotein [97]. Nevertheless an absence of relationship between hypogammaglobulinemia and susceptibility to fungal infections in general (with the exception of case studies) suggests that cellular responses have a major role in the protection against fungal infections and that antibodies might play a supportive role by reducing the fungal burden and by shaping the immune responses. Therefore, further research is warranted to understand the natural antibody responses to fungal pathogens in humans.

As passive administration of specific protective antibodies shown promising results in experimental models, two antibodies have entered clinical trials in recent years.

Patients with cryptococcosis elicit specific antibodies to glucosylceramide and affinity purified these antibodies exhibit inhibitory activity on cell budding and fungal growth of *C. neoformans* [98]. Human IgM MAb specific to glucuronoxylomannan prolonged survival of *C. neoformans*-infected mice [99]. Based on these experimental data, a murine-derived anticryptococcal IgG1κ MAb 18B7 reacting to glucuronoxylomannan entered phase I, multi-institution, open-label, nonrandomized, dose-escalation study in HIV-infected subjects who had been successfully treated for cryptococcal meningitis [100]. Preclinical study has demonstrated that MAb 18B7 recognizes all four serotypes of *C. neoformans*, opsonizes *C. neoformans* serotypes A and D, increase the antifungal actions of human and mouse effector cells, and activate the complement pathway leading to deposition of complement C3 on the capsule of

Cryptococcus [101]. Also, MAb 18B7 therapy in mice led to quick clearance of serum cryptococcal antigen. Phase I study revealed acceptable safety for this antibody and suggested further investigation at a maximum single dose of 1.0 mg/kg. Cryptococcal antigen titers in the serum of these patients dropped by a median of two-fold at first week and a median of three-fold at two weeks post-therapy. The half-life of the MAb 18B7 in the serum was found to be nearly 53 h. Further randomized clinical trials are awaited for this antibody.

A strong and sustained antibody response to hsp90 was associated with recovery of patients from invasive candidiasis following treatment with amphotericin B [102-104]. An immunodominant epitope on the Hsp90 of *C. albicans* is present both in filamentaous fungi and in yeasts including *C. parapsilosis, Torulopsis glabrata, Candida tropica*lis, *Candida krusei* and *A. fumigatus* [105]. Therefore, a single-chain variable fragment of a human monoclonal antibody Efungumab (Mycograb®) recognizing immunodominat epitope on the Hsp90 of *C. albicans* has entered clinical trials in patients with invasive candidiasis. A double-blind, randomized study demonstrated that Efungumab combined with lipid-associated amphotericin B produce significant clinical and culture-confirmed improvement in outcome for patients with invasive candidiasis [106]. A pre-clinical data also supported synergy between Efungumab and caspofungin [107]. However, a recent study suggested that Efungumab potentiation of amphotericin B could be non-specific [108]. Although a status of an orphan drug has been given in USA for this antibody, its use in Europe is not permitted by European Medicines Agency due to potential side effects and concerns about aggregation of the antibody. Also, Novartis discontinued the clinical development of this anti-fungal antibody in 2010.

Treatment of fungal-mediated inflammatory conditions: quest for unusual savior, intravenous immunoglobulin

Intravenous immunoglobulin (IVIg) is a therapeutic preparation of normal IgG obtained from pooled plasma of several thousand healthy donors. Depending on the exposure of donors to infectious diseases and vaccines and also on the endemic nature of the infectious diseases, IVIg contains antibodies to various pathogens of bacterial, viral, fungal and parasitic origin [40, 109]. In addition, natural antibodies represent major composition of IVIg [110].

Initially used for the replacement therapy of primary and secondary immunodeficiencies, high-dose (1-2 g/kg body weight) IVIg is currently used in the therapy of diverse autoimmune and inflammatory diseases such as Kawasaki disease, Guillain-Barré Syndrome, inflammatory myopathies, immune thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, vasculitis, graft versus host disease, and others as an immunomodulatory agent with no reports of serious side effects [110-115]. In addition to invasive disease, fungi species are also associated with several inflammatory conditions such as both IgE and eosinophilia-driven hypersensitivity diseases including severe asthma, allergic bronchopulmonary mycoses, chronic sinusitis, hypersensitivity pneumonitis, atopic eczema/dermatitis syndrome and gut inflammation. Of note, IVIg has been used as an off label drug in allergy and asthma [116-121] and shown protective effects in experimental models of allergic airway inflammation [122-126].

IVIg could act as immunomodulatory agent in inflammatory conditions via several mutually nonexclusive mechanisms (Fig. 1). Thus, IVIg could inhibit the

activation of innate cells such as DCs, macrophages, neutrophils, iNKT cells and the secretion of inflammatory cytokines while enhancing the anti-inflammatory mediators such as IL-1RA and IL-10. IVIg inhibits the differentiation and expansion of Th17 cells and reciprocally expands Tregs, regulates the functions of B cells, activation of endothelial cells and their secretion of cytokines and chemokines. In addition, IVIg could neutralize the pathogens including fungi and their antigens [123, 124, 126-143]. This broad range of activities of IVIg reflects the importance of circulating immunoglobulins in the maintenance of immune homeostasis.

In an open-label study with eight severe steroid-dependent asthma children aged between 6 to 17 years, treatment with IVIg (six monthly infusion at 2g/kg) resulted in significant reductions in steroid requirements. In addition, IVIg therapy led to decrease in serum IgE levels and a progressive diminution in skin test reactivity to allergens [116]. Other anecdotal studies also supported the use of IVIg in severe asthma with a steroid-sparing effect [117, 144-146]. The immunological analysis revealed that IVIg treatment decreased number of activated $CD3^+$ T cells, $CD4^+$ T cells in endobronchial biopsies with a reduction in peripheral blood T cell activation, decreased total serum IgE and IL-8 [144]. Also, IVIg could synergistically act with dexamethasone to inhibit lymphocyte activation and improve glucocorticoid receptor binding affinity [117, 147]. A multicenter, randomized, double-blind, placebo-controlled trial of high-dose IVIg although failed to show benefits in corticosteroid-dependent asthma [118], this study period was only two months and patients included were over 40 years. Therefore, based on this report, it might be concluded that younger patients probably benefit from IVIg therapy. Also modifications in the immune system due to previous drugs/therapies in adult patients might influence the

immunomodulatory functions of IVIg. Although further randomized clinical trials are required to support the use of IVIg in asthma and allergy, IVIg is not currently used as a first line therapy due to the availability of several new generation drugs. But these studies and experimental models provided a clue that IVIg could benefits fungal inflammatory conditions.

Conclusion

Treatment of disseminated fungal infections are still challenging due to costs associated with the treatments, growing reports of antifungal drug resistance, toxicity of anti-fungal drugs and non-availability of protective vaccines. Although, humoral immunity might not have a major role in conferring protection against fungal infections in human, passive administration of specific protective antibodies could prove to be beneficial in drug-resistance cases, to reduce the dosage and associated toxic symptoms of anti-fungal drugs. The longer half-life of the antibodies and flexibilities to modify their structure/forms are additional advantages with anti-fungal antibodies. The clinical data obtained with two antibodies should incite interests in translating pre-clinical success into the clinics. In addition, clinically proven benefits of IVIg in various inflammatory diseases substantiate the necessity of testing this therapeutic preparation in fungal-mediated allergy and inflammatory conditions.

Most of the protective antibodies described to date recognize surface molecules of the fungi. Though “one antibody for all pathogenic fungi” is still elusive, there are experimental evidences that suggest that common cell wall component-specific protective antibodies like β-glucan exert protection across the several species of fungi [51, 61]. Another option could be broad-spectrum recombinant single chain

fragment (ScFv) anti-idiotypic antibodies bearing the internal image of a yeast killer toxin (KT). These killer antibodies are lethal to yeasts and filamentous fungi including *C. albicans, A. fumigatus* and *P. carinii* that express specific β-1,3 D-glucan cell-wall receptor (KTR). These KT-scFv were reported to have fungicidal properties against *C. albicans* both *in vitro* and *in vivo* model of experimental vaginal candidiasis [148]. A decapeptide resulting from the variable region sequence and containing part of the CDR1 segment of the KT-scFv light chain also exerted therapeutic activity against experimental mucosal and systemic candidiasis [149]. Killer anti-idiotypic MAb bearing the internal image of a yeast killer toxin showed protection against early invasive aspergillosis in a murine model of allogenic T-cell-depleted bone marrow transplantation [150]. In addition, natural yeast KT-like antibodies with candidacidal properties were also identified in the vaginal fluid of candida infected human vaginitis patients [151].

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Figure Legend:

Fig. 1. Multi-faceted functions of antibodies in the protection against fungal infections and fungi-mediated inflammatory conditions. Antibodies confer protection against fungal infections by multiple mechanisms that include direct neutralization of fungi and their antigens, inhibition of growth of fungi, modification of gene expression, signaling and lipid metabolism, causing iron starvation, inhibition of polysaccharide release and biofilm formation. Antibodies promote opsonization of fungi and their phagocytosis, complement activation and antibody-dependent cell toxicity. Growing evidences also indicate that antibodies have a key role in immunomodulation and in preventing inflammation-mediated tissue damage.