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Neutrophil FcγRI as Target for Immunotherapy of Invasive Candidiasis

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Invasive candidiasis represents a life-threatening disease for immunocompromised patients. This study focused on new immunotherapeutic approaches for systemic Candida albicans infections in a human FcγRII-transgenic mouse model. FcγRI (CD64) is a potent immunoactivating receptor on phagocytic and dendritic cells. In vivo targeting of C. albicans toward neutrophil-FcγRI by bispecific Abs and G-CSF effectively protected FcγRII-transgenic mice from lethal candidiasis. Nontransgenic mice were not protected, and treatment with bispecific Ab or G-CSF alone did not reduce mortality. Furthermore, infected FcγRII-transgenic mice developed high titers of anti-C. albicans IgG, and survival was extended on secondary infection without further treatment. These findings document the capacity of FcγRI to initiate potent anti-C. albicans immunity and support the development of FcγRI-directed immunotherapy of invasive fungal disease. The Journal of Immunology, 2001, 166: 7019–7022.

Candida albicans is responsible for the majority of severe systemic fungal diseases occurring in immunocompromised patients (1, 2). High mortality (~60%) is observed among these patients, despite treatment with antifungal drugs (3, 4). Neutropenia represents a crucial risk factor, because neutrophils (polymorphonuclear leukocytes (PMNL2)) are indispensable for antifungal immunity (5). G-CSF increases circulating PMNL numbers, stimulates differentiation, and modulates PMNL activity (6, 7). G-CSF has documented therapeutic efficacy in fungal infection models and is well established for the treatment of neutropenic and nonneutropenic patients (5, 8). Covalent attachment of polyethylene glycol (peg) to G-CSF increases its circulating half-life and may further optimize G-CSF treatment (9). Recently, peg-G-CSF has been documented to prolong survival of mice with disseminated C. albicans infection (10).

PMNL express different receptors capable of phagocytosis of microorganisms, including FcR, complement receptors, and a number of carbohydrate-binding molecules. Opsonins (i.e., Ab or complement components) engaging these receptors are essential for elimination of C. albicans by PMNL (11). PMNL express three classes of FcγR, FcγRIIa (CD32), FcγRIIb (CD16), and FcγRI (CD64), on activation with IFN-γ or G-CSF (12). Both FcγRIIa and FcγRI are, in contrast to FcγRIIb, potent immunoactivating receptors capable of mediating phagocytosis, Ab-dependent cellular cytotoxicity, and initiation of inflammatory cytokine release. FcγRI is exclusively expressed on phagocytic and dendritic cells, in contrast to the widely distributed FcγRII (13, 14). Moreover, FcγRI represents the only FcγR with a well-documented capacity to facilitate immunological memory in vivo (15–17).

Targeting C. albicans toward PMNL FcγRI results in potent PMNL fungicidal activity in vitro (18). Because FcγRI is a high affinity IgG receptor, which is saturated with serum IgG in vivo, conventional Ab are ineffective in targeting FcγRI. Bispecific Abs (BsAb), which contain dual specificity for both target (C. albicans) and effector (PMNL FcγRI), will by binding outside the Ab-binding site of FcγRI overcome this problem (18). BsAb may furthermore improve the selectivity and efficacy of Ab-based therapeutics (19). The increasing need for novel therapeutic approaches for fungal disease prompted us to investigate the therapeutic efficacy of FcγRI-targeting during invasive candidiasis in a transgenic (Tg) mouse model. This study demonstrates FcγRI-directed BsAb and G-CSF to effectively protect mice against lethal candidiasis, supporting FcγRI as candidate target for immunotherapy.

Materials and Methods

Experiments were approved by the ethical committee of University Medical Center Utrecht.

Antibodies

BsAb (FcγRI × αCan) was produced as described previously (18). FITC-conjugated mAb 22 (Medarex, Annandale, NJ) was used to detect FcγRI expression. FITC-conjugated goat F(ab′)2 anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) detected mouse anti-C. albicans IgG in mouse sera.

G-CSF

Pegylated G-CSF (peg-G-CSF), kindly provided by Dr. J. Andresen (Amgen, Thousand Oaks, CA), was synthesized by coupling recombinant human G-CSF N-terminally to 20-kDa peg as described (9). Previous work indicated a single s.c. injection of 15 μg to elevate circulating neutrophil numbers in mice for 3–4 days (10).

C. albicans

C. albicans strain UC820 is described as a clinical isolate (11). After overnight culture at 37°C in Sabouraud maltose broth (Difco, Detroit, MI),
yeasts were centrifuged, washed three times in sterile 0.9% NaCl (saline), and counted. For phagocytosis assays, yeast particles were FITC labeled as described previously (18).

Mice

Because Ab to mouse FcγRI are not available, human FcγRI Tg mice have been developed that exhibit similar FcγRI cell distribution and expression patterns as in humans (15). Female Tg FVB/N × C57BL/6 F1 mice 9–12 wk old were used. Nontransgenic (NTg) FVB/N × C57BL/6 littermates served as controls. Mice were screened for FcγRI expression by incubating blood (25 μl) with FITC-labeled mAb 22, followed by analysis on a FACScan (Becton Dickinson, San Jose, CA). Human FcγRI is constitutively expressed by monocytes and macrophages, whereas expression on PMNL of FcγRI Tg mice is induced by IFN-γ or G-CSF, similar to the situation in humans (15).

Isolation of mouse PMNL

Mice were injected s.c. with 15 μg peg-G-CSF (in 150 μl saline), or with saline alone (control). On day 3, mice were injected i.p. with 1 ml thioglycollate (Difco). The mice were killed after 4 h, and 5 ml ice-cold RPMI 1640 (Life Technologies, Gaithersburg, MD) were injected into the abdominal cavity to harvest peritoneal cells. The cells were washed in RPMI 1640 containing 10 μg/ml BsAb (FcγRI × αCan) for 30 min at 37°C. C. albicans phagocytosis was quantified by measuring FITC fluorescence intensities of PMNL by flow cytometry. PE-conjugated GR-1 (PharMingen, San Diego, CA) was used to identify mouse PMNL. The percentage of PMNL that phagocytozed one or more C. albicans yeast particles was determined. In addition, phagocytosis was analyzed in cytospin preparations by light microscopy.

Phagocytosis assays

Isolated mouse PMNL (1 × 10^6) were incubated with FITC-labeled C. albicans particles (4 × 10^6) in RPMI 1640 alone (control) or in medium containing 10 μg/ml BsAb (FcγRI × αCan) for 30 min at 37°C. C. albicans phagocytosis was quantified by measuring FITC fluorescence intensities of PMNL by flow cytometry. PE-conjugated GR-1 (PharMingen, San Diego, CA) was used to identify mouse PMNL. The percentage of PMNL that phagocytozed one or more C. albicans yeast particles was determined. In addition, phagocytosis was analyzed in cytospin preparations by light microscopy.

Infection protocol

On day 0, mice (Tg and NTg) were injected i.v. with 5 × 10^5 viable C. albicans (in 100 μl saline). Groups of at least five mice received either no treatment (controls) or peg-G-CSF (two s.c. injections on days −3 and 0) combined with BsAb (FcγRI × αCan) treatment (three i.v. injections on days 0, 1, and 2), as outlined in the underlying treatment scheme. Other groups were given peg-G-CSF or BsAb (FcγRI × αCan) alone. Survival of mice was assessed twice daily.

Results

Peg-G-CSF enhances FcγRI-mediated PMNL phagocytosis

The effect of peg-G-CSF treatment of Tg and NTg mice on PMNL function was first analyzed in vitro. Three days after a single injection of peg-G-CSF, membrane FcγRI expression was strongly up-regulated on Tg, but not on NTg PMNL (Fig. 1A). Furthermore, circulating PMNL numbers increased from 12% (±2%, n = 6) before treatment to 50% (±4%, n = 4) of total leukocytes at day 3 in both Tg and NTg mice. Next, the effect of peg-G-CSF on C. albicans phagocytosis by Tg and NTg PMNL was analyzed by different detection methods. NTg PMNL did not mediate phagocytosis in the presence of FcγRI-directed Ab, irrespective of peg-G-CSF treatment (Fig. 1B, top). Tg PMNL, on the contrary, potently internalized C. albicans via FcγRI after peg-G-CSF treatment (Fig. 1B, bottom). No phagocytosis was observed by PMNL in the absence of Ab, and preabsorption of Ab with C. albicans removed the capacity of Ab to induce phagocytosis (data not shown).
FcγRI targeting of C. albicans protects mice from invasive candidiasis

Next, we investigated the therapeutic efficacy of in vivo FcγRI-targeting in a murine candidiasis model. FcγRI Tg and NTg mice received an invasive C. albicans infection, which was lethal within ~1 mo. Mice were untreated, treated with FcγRI-BsAb or peg-G-CSF alone, or treated with both (see Materials and Methods for details). Untreated mice (Tg/NTg) showed 100% mortality within 34 days, and treatment with BsAb alone did not prolong survival (Fig. 2). Mice (Tg/NTg) that received peg-G-CSF alone and NTg mice receiving both peg-G-CSF and BsAb all showed 100% mortality within 50 days (data shown for NTg mice treated with peg-G-CSF and BsAb). However, combined treatment with peg-G-CSF and BsAb was highly therapeutic in Tg mice, decreasing mortality to ~20%. Mice were monitored for >150 days and remained healthy.

Prolonged survival of FcγRI Tg mice on secondary infection (rechallenge)

Because combined therapy of peg-G-CSF and FcγRI-directed Ab resulted in ~80% survival of Tg mice, we investigated anti-C. albicans immunity of surviving mice. Mouse sera were tested for the presence of specific anti-C. albicans IgG at day 60 after infection. All surviving mice contained anti-C. albicans IgG with titers ranging from 1/20 to 1/2000 (Fig. 3A), in contrast to uninfected Tg mice. Next, groups of surviving Tg mice were rechallenged with a lethal C. albicans dose without any further treatment. Significant prolonged survival and decreased mortality was observed at this secondary infection, compared with survival of Tg mice dealing with primary disease (Fig. 3B).

Discussion

There is an increasing need for new therapeutic strategies for patients with systemic fungal disease. In this study, we describe a novel immunotherapeutic approach for experimental invasive candidiasis. In vivo targeting of C. albicans toward FcγRI, combined with peg-G-CSF treatment, effectively protects mice from lethal candidiasis. Moreover, FcγRI targeting results in prolonged survival on C. albicans rechallenge, consistent with induction of immunological memory.

To develop effective antifungal immunity in vivo, there is a requirement for 1) sufficient fungicidal effector cells, i.e., PMNL, and 2) opsonins engaging phagocytic receptors. The significance of elevated PMNL numbers and PMNL activation is emphasized by the well-established effects of G-CSF during experimental candidiasis. With respect to the importance of phagocyte receptor engagement, we selected human FcγRI as the target for immunotherapy of invasive fungal disease based on the following grounds. Targeting C. albicans toward FcγRI results in potent fungicidal activity by both human and FcγRI Tg mouse PMNL in vitro (18). Moreover, FcγRI represents the only activating FcR, which is exclusively expressed by phagocytes and APC. Recent studies support evidence for induction of "vaccine effects" on in vivo Ag targeting toward FcγRI (15–17). FcγRI, being a high affinity IgG receptor, however, is likely to be saturated with serum IgG in vivo. We therefore focused on targeting an epitope located outside the ligand-binding region of FcγRI by using BsAb. Such BsAb are not hindered by serum IgG and furthermore interact more efficiently with FcγR than conventional Ab (19).

First, we showed peg-G-CSF to be crucial for effective C. albicans phagocytosis by PMNL FcγRI, which was linked to increased FcγRI expression levels. Consistent with this, protection against lethal candidiasis in Tg mice was achieved only when FcγRI-BsAb therapy was combined with peg-G-CSF treatment. This indicates that mononuclear cells, which constitutively express FcγRI (13), are not effective in clearing C. albicans in our in vivo model. The requirement for G-CSF corresponds well with recently observed antitumor activity induced by FcγRI-BsAb and G-CSF (16). It is currently unknown whether G-CSF is needed for induction of increased levels of FcγRI only, or whether the growth factor is also important for PMNL activation. The observation that mortality of treated NTg mice was not decreased (Fig. 2) proved protection to be mediated by human FcγRI. Moreover, we observed in vivo phagocytosis of C. albicans by blood PMNL of infected Tg mice treated with peg-G-CSF and BsAb, and a reduced fungal outgrowth in kidneys, in contrast to controls (data not shown). This indicates PMNL phagocytosis via FcγRI to represent the underlying mechanism of BsAb-mediated protection to invasive candidiasis.

Excitingly, survival of Tg mice was significantly prolonged on C. albicans rechallenge without further treatment. This suggests that FcγRI targeting, combined with peg-G-CSF treatment, induces anti-C. albicans immunity. Indeed, treated Tg mice developed specific anti-C. albicans IgG in serum, which may have contributed to the prolonged survival. Because the cell wall of C. albicans is composed largely of carbohydrates, anti-Candida Ab
are typically of the IgM isotype (20–22). Most anti-Candida Ab available are directed against intracellular Ags, further emphasizing the difficulty of developing protective IG responses.

Elevated Ab responses on in vivo Ag targeting toward FcyRI have been documented before using a number of Ags (15, 17). Moreover, induction of antitumor activity by FcyRI-directed Ab triggers resistance to tumor rechallenge (16). APC effectively internalize Ags via FcyRI and are proposed to initiate immunological memory by enhancing Ag presentation (23, 24). A unique intracellular trafficking motif in the cytoplasmic tail of FcyRI proved crucial for its capacity to facilitate Ag presentation (25). Not only is MHC class II-restricted Ag presentation enhanced on FcyRI targeting but also MHC class I presentation (Refs. 25 and 26; L. Bevaart, H. H. van Ojik, P. M. Guyre, J. G. J. van de Winkel, and M. J. van Vugt, unpublished observations). This suggests that Ag targeting toward FcyRI primes both CD4+ and CD8+ T cell responses.

Although survival of Tg mice was prolonged after infection rechallenge, mortality was still ~80%. This implies that endogenous anti-C. albicans IgG, by itself, is not sufficient to cure mice from invasive candidiasis. The in vivo role of Ab in immunity to C. albicans is controversial (27, 28), mainly because candidiasis patients often contain anti-C. albicans Ab. Ab protection is dependent on many variables, including Ab quantity, isotype, affinity, and Ag specificity. Saturation of FcyRI with IgG or engagement of inhibitory FcyR may also contribute to lack of protection. This again underlines the value of using BsAb for immunotherapy of fungal disease.

In summary, this study demonstrates FcyRI-directed Ab combined with peg-G-CSF to protect mice from lethal candidiasis and to prolong survival upon fungal rechallenge. These data support FcyRI to prolong survival upon fungal rechallenge. These data support combined with peg-G-CSF to protect mice from lethal candidiasis and of immunotherapies for invasive fungal disease.

Acknowledgments

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References