Immune response to fungal infections

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Abstract

The immune mechanisms of defence against fungal infections are numerous, and range from protective mechanisms that were present early in evolution (innate immunity) to sophisticated adaptive mechanisms that are induced specifically during infection and disease (adaptive immunity). The first-line innate mechanism is the presence of physical barriers in the form of skin and mucous membranes, which is complemented by cell membranes, cellular receptors and humoral factors. There has been a debate about the relative contribution of humoral and cellular immunity to host defence against fungal infections. For a long time it was considered that cell-mediated immunity (CMI) was important, but humoral immunity had little or no role. However, it is accepted now that CMI is the main mechanism of defence, but that certain types of antibody response are protective. In general, Th1-type CMI is required for clearance of a fungal infection, while Th2 immunity usually results in susceptibility to infection.

Aspergillosis, which is a disease caused by the fungus *Aspergillus*, has been the subject of many studies, including details of the immune response. Attempts to relate aspergillosis to some form of immunosuppression in animals, as is the case with humans, have not been successful to date. The defence against *Aspergillus* is based on recognition of the pathogen, a rapidly deployed and highly effective innate effector phase, and a delayed but robust adaptive effector phase. *Candida albicans*, part of the normal microbial flora associated with mucous surfaces, can be present as congenital candidiasis or as acquired defects of cell-mediated immunity. Resistance to this yeast is associated with Th1 CMI, whereas Th2 immunity is associated with susceptibility to systemic infection. Dermatophytes produce skin alterations in humans and other animals, and the essential role of the CMI response is to destroy the fungi and produce an immunoprotective status against re-infection. The resolution of the disease is associated with a delayed hypersensitive response. There are many effective veterinary vaccines against dermatophytes. *Malassezia pachydermatis* is an opportunistic yeast that needs predisposing factors to cause disease, often related to an atopic status in the animal. Two species can be differentiated within the genus *Cryptococcus* with immunologic consequences: *C. neoformans* infects predominantly immunocompromised hosts, and *C. gattii* infects non-immunocompromised hosts. *Pneumocystis* is a fungus that infects only immunosuppressed individuals, inducing a host defence mechanism similar to that induced by other fungal pathogens, such as *Aspergillus*.

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1. Mycoses

Mycoses, conditions in which fungi pass the resistance barriers of animals and establish infections, are a group of diseases with very varied clinical manifestations. Mycoses are of growing importance for the following reasons (Garcia and Blanco, 2000):
They are produced by fungi that are widely distributed in the environment and, therefore, very difficult to eradicate.

The clinical manifestation of disease caused by fungal infection can be highly variable. For example, in the case of aspergillosis, with effects on very diverse organs, there is a variety of responses, such as local (aspergilloma), systemic (renal, lung, nervous central system, etc.) or even allergic (allergic bronchopulmonary aspergillosis in human).

Diagnosis of these diseases can be problematic because of the difficulty of interpreting the very different clinical pictures in individuals in the presence of colonization, infection and/or disease.

There is a few varieties of vaccines available against these diseases, which are therefore difficult to prevent. At this time, vaccines are limited to a few animal species, to only a few processes and with variable effectiveness.

Treatment is problematic: compared to the antibacterials, the number of antifungal drugs available at present is very small, with much greater difficulty in production, with many side-effects, and with the possibility of the appearance of resistance, as has happened with antibiotics in the treatment of bacterial infection.

In human medicine, the appearance of AIDS and the evolution of immunosuppressive treatments essential for the success of organ transplants, have highlighted the importance of fungal diseases, and efforts have been made to understand these diseases and to develop means for their prevention and control. This evolution has been much slower in veterinary medicine; on many occasions the fungal diseases have been relegated to post-mortem discoveries.

At present, the two main foci of attention in the study of fungal diseases are as follows:

1. The mechanisms of pathogenicity that cause usually saprophytic fungal species to transform into an aggressor toward an animal host causing disease or even death.
2. Mechanisms of the host resistance to infection and disease.

In this review, we discuss this second point in detail in an attempt to identify the main mechanisms used by the host immune system to counter fungal infection. A disease outcome is simply a result of the clash between the mechanisms of pathogenicity of the fungi and the mechanisms of resistance of the host, leading to the removal of the infection or its progression according to the imbalance of these mechanisms.

At present, much of what is known about fungal infections is limited to what happens in man. There are many preliminary results obtained with laboratory animals, mainly with murine models. Less is known in the case of domestic animals concerning fungal diseases, especially by species that produce disseminated processes. These are considered to be characteristic of immunocompromised individuals in humans, while in many cases this immunosuppression is not detected in animals. The question is raised of whether immunosuppression exists or have we not been able to detect it.

2. Immune response to fungi: innate and adaptive immunity

Host defence mechanisms against fungi are numerous, and range from protective mechanisms that were present early in the evolution of multicellular organisms (innate immunity) to sophisticated adaptive mechanisms that are induced specifically during infection and disease (adaptive immunity) (Romani, 2004).

Traditionally, innate immunity has been considered as simply a first line of defence. Nevertheless, innate immunity has recently received attention because, despite a certain lack of specificity, it effectively distinguishes self from non-self and activates adaptive immune mechanisms by the provision of specific signals (Medzhitov and Janeway, 1997; Romani, 2004). The innate immune system confers rapid recognition of microbial infection through a limited repertoire of germ line-encoded receptors that recognize a group of conserved molecular patterns common to broad groups of microbial species (Janeway and Medzhitov, 2002; Roeder et al., 2004).

The first of the defensive innate mechanisms is the physical barriers that separate the organism from the environment: i.e. skin and the mucous membranes of the respiratory, gastrointestinal and genito-urinary tracts. The skin and mucous membranes are physical barriers, and they have antimicrobial substances on their surface, some of them synthesized by the epithelial and endothelial cells. Also, they have a commensal microflora of saprophytic microorganisms that impede colonization by pathogenic microorganisms (Romani, 2004).

Once the fungi have passed the physical barriers, they are met with a series of innate mechanisms of defence, including cellular membranes, cellular recep-
tors and several humoral factors. In the tissues, phagocytes, consisting of neutrophils, mononuclear leukocytes (monocytes and macrophages) and dendritic cells, have an essential role, and natural killer cells, γδ-T-cells and non-hematopoietic cells, such as epithelial and endothelial cells are involved in the host defense. The host defense mechanism can adapt to different kinds of fungal infection; For example, macrophages are the primary cells involved in fungal killing during infection by Cryptococcus and Pneumocystis, whereas neutrophils are the primary effector cells in preventing infection by Candida albicans and Aspergillus fumigatus (Traynor and Huffnagle, 2001).

A critical point in the defence is the production of chemotactic factors at the site of fungal infection for effective recruitment of leukocytes to that site. These chemotactic factors are very varied, and include peptides derived from activation of the complement pathway, leukotrienes and products synthesized by the fungi; certain cytokines have secondary chemotactic activity. Another important group of chemotactic factors is the chemokines, a supergene family of small inducible peptides with potent chemotactic activity for leukocyte subpopulations. These chemokines are produced by a variety of cells, including leukocytes, epithelial cells, endothelial cells, fibroblasts and smooth muscle cells following stimulation by cytokines or microbial products. Chemokines regulate an array of biological activities in addition to chemotaxis, such as hematopoiesis, angiogenesis, cytokine induction, antigen presentation and Th cell differentiation. All of these activities are important in both acute and chronic fungal infections (O’Garra et al., 1998; Murphy et al., 2000; Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000; Traynor and Huffnagle, 2001).

The synthesis of these chemotactic factors is activated by invariant molecular structures shared by large groups of pathogens (also known as pathogen-associated molecular patterns, [PAMPs]) that are recognized by germ line-encoded proteins (pattern recognition receptors [PRRs]) present in different cells of the organism, mainly monocytes, macrophages, dendritic cells, B-cells, T-cells and endothelial cells. PRRs include the Toll-like receptors (TLRs), a protein family of cellular receptors that mediate recognition of microbial pathogens and subsequent inflammatory response in vertebrates (Janeway and Medzhitov, 2002; Roeder et al., 2004; Romani, 2004). TLRs and other PRRs confer PAMP recognition and their signalling triggers synthesis followed by release of proinflammatory cytokines, and induces expression of co-stimulatory molecules for promoting activation of adaptive immunity during antigen presentation. The simultaneous activation of multiple PRRs by one fungal pathogen endows the immune system with a broad range of possibilities for a specific and effective immune response (Roeder et al., 2004).

Bacterial and viral infections have been the major focus of research, and less is known about the function of TLRs against fungal pathogens and fungal PAMPs, though its participation in the defence against C. albicans, A. fumigatus, C. neoformans, Pneumocystis and Coccidioides has been reported (Wang et al., 2001; Netea et al., 2002, 2006; Meier et al., 2003). In contrast, several studies have demonstrated that pathogens are able to manipulate or escape innate immune recognition. Then, certain fungal pathogens use PRR-based strategies to evade host defence (down-modulate the microbial functions of leukocytes, or escape immune recognition); in certain circumstances, fungal pathogens induce a strong anti-inflammatory cytokine profile, which may be a mechanism to escape the host defence (Netea et al., 2006).

C. albicans induce immunosuppression through TLR2-mediated IL-10 release, and this leads to generation of CD4⁺CD25⁺ T-regulatory cells with immunosuppressive potential (Netea et al., 2004). A. fumigatus evades immune recognition by germination into hyphae with subsequent loss of TLR-4 recognition, whereas the TLR2-mediated IL-10 pathways remain intact, thus shifting the balance towards a permissive Th2-type profile (Netea et al., 2003). Emphasized is the fact that dectin-1 recognizes the β-glucans at the level of budding scars in the yeasts, but it cannot recognize the β-glucans in the hyphae, where they are shielded by a layer of mannans (Gantner et al., 2005).

Also are important the host defence peptides, also known as antimicrobial peptides. They are effector molecules of the innate immune system that show broad antimicrobial action against gram-positive and -negative bacteria, and also against fungi. Furthermore, they likely play a key role in activating and mediating the innate as well as adaptive immune response in infection and inflammation (Aerts et al., 2008; Steinstraesser et al., 2008). Then, they are antimicrobial and immunomodulating agents that present an important link between the innate and adaptive immune response (Zasloff, 2002). One major function of these antimicrobials peptides is to inactivate microbes, including fungi, through multiple direct effects on their membranes. They have the ability to attack specific external targets simultaneously (Ganz, 2003; Aerts et al., 2008; Steinstraesser et al., 2008). Another major function is their active role in the transition to the adaptive immune
response by being chemotactic for monocytes, neutrophils, and T-cells and by exhibiting adjuvant and polarizing effects in influencing dendritic cell development (Steinstraesser et al., 2008). They have a direct influence on the adaptive immune responses by activation of different immune factors such as tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1, and gamma-interferon (IFN-γ) (Ganz, 2002).

Although much progress has been made in recent years, the complete molecular basis of the mode of antimicrobial action for most of these antimicrobial peptides still needs to be unravelled (Aerts et al., 2008).

3. Immune response to fungi: humoral and cellular immunity

The relative contribution of humoral and cellular immunity to host defence against fungal infections has been controversial in the field of medical mycology. For a long time, only two main observations were considered, which had been made repeatedly for many fungal pathogens. First, cell-mediated immunity (CMI) had consistently been shown to mediate protection against many fungi, as demonstrated by adoptive lymphocyte transfer studies, enhanced susceptibility for hosts with CMI deficiencies and the finding that granulomatous inflammation is often essential for control of infection in tissue. Second, humoral immunity (HI) had been difficult to demonstrate by either transferring immune sera or correlating antibody titres with protection (Polonelli et al., 2000). Although a few studies suggested that antibody might have a role in protection, the role of HI was uncertain because of inconsistent results (Casadevall, 1995). The conclusion was that the CMI was important, whereas HI had little or no role.

Nevertheless, in the last decade it has been shown that HI can protect against fungal infection if certain types of protective antibodies are available in sufficient quantity. The main recognized functions of antibodies in fungal infections include prevention of adherence, toxin neutralization, antibody opsonization and antibody-dependent cellular cytotoxicity (Polonelli et al., 2000). These findings have been supported by experiment; for example, the identification of protective and non-protective antibodies for both C. neoformans and C. albicans, indicating that HI response to fungi could elicit antibodies of variable efficacy (Casadevall, 1995). Explanations for the negative historical results in passive protection experiments with transfer of polyclonal immune sera include inadequate amounts of protective antibody, the presence of antibody of a non-protective specificity, and/or the presence of antibody of a non-protective isotype (Polonelli et al., 2000). The relative composition and proportion of protective and non-protective antibodies against fungi might vary greatly. Also, the amount, specificity, isotype and idiotype of antibodies have marked effects on protective efficacy (Casadevall et al., 2002). Research is in progress to identify antibodies that are protective, the peptide mimetics that elicit them specifically and putative candidate vaccines that elicit protective immunity.

The long debate between the relative merits of humoral and cellular immunity in medical mycology has given way to a new consensus, that CMI remains the main mechanism for defence against fungal infections but that certain types of antibody responses can also protect (Polonelli et al., 2000). In conclusion, it is clear that the immune system works as a whole, and that very diverse components contribute to the defence of the host organism. Some parts contribute more than others under certain circumstances, but they are all important for the overall protection against infection.

The type of CMI induced is critical in determining resistance or susceptibility to fungal infection. In general, Th1-type CMI is required for clearance of a fungal infection, while Th2 immunity usually results in susceptibility to infection or allergic responses (Traynor and Huffnagle, 2001). Th1 cells produce predominantly cytokines such as IFN-γ, and promote cell-mediated immunity and phagocyte activation. In contrast, Th2 cells produce predominantly cytokines such as interleukins 3 and 4 (IL-3 and IL-4) and tend to promote antibody production (Traynor and Huffnagle, 2001; Crameri and Blaser, 2002; Belloccchio et al., 2005).

4. Practical applications of the immune response: diagnosis and vaccination

The development of an effective HI response against fungal infections has driven the research in two practical aspects of great interest: the development of immunologic methods of diagnosis and the application of vaccines.

4.1. Immune diagnosis

The development of an HI response as a consequence of fungal infection, and the fact that in domestic animals these diseases generally take place in immunocompetent individuals, at least as concerns the synthesis of antibodies, open the possibility of immune diagnoses as a screening method for the detection of fungal diseases.
The results obtained using this kind of test would be useful for early diagnosis, and have the advantage of requiring only simple and routine collection of blood samples (Garcia et al., 2001b).

Nevertheless, determination of the level of antifungal antibodies in serum highlights a series of problems that should be kept in mind (Blanco and Garcia, 2000):

(1) Some fungi, as Aspergillus, are ubiquitous microorganisms that are in continuous contact with man and animals, with colonization even in healthy individuals. This stimulates an immune response in the host, so that all the animals routinely have a certain level of antifungal antibodies, which makes precise high dilutions of serum necessary for an immunologic diagnosis to differentiate between infected and non-infected individuals.

(2) Different fungal genera have many common antigens, thus giving rise to cross-reactive immune reactions, and it is difficult to make an exact diagnosis.

Besides diagnostic procedures, we have used these methodologies to monitor the evolution of treatment against disease in such a way that antifungal treatment should not be discontinued before the titre of antifungal antibodies subsides to negative values that allow us to guarantee an effective cure (Garcia et al., 2001a,b, 2004a,b).

4.2. Fungal vaccines

A clearer understanding of the mechanisms involved in the immune response to fungal infection is needed to aid the development of effective vaccines. Besides the traditional concept of vaccines that protect against fungal diseases, it is important to consider the administration of immunomodulators and the use of the so-called therapeutic vaccines.

Research has involved both cellular and humoral immunity, but recent discoveries related to the activity of the HI have reactivated the search for vaccines, once it was recognised that protective antibodies to fungal antigens would be potentially useful for adjunctive therapy of fungal infections. Encouraging results have been obtained with certain antigen preparations and it is considered that a vaccine can be developed in the near future against diseases like aspergillosis or candidiasis (Polonelli et al., 2000).

Unlike the fungi for which virulence factors have been defined, such as C. neoformans, Histoplasma capsulatum, and Blastomyces dermatitidis, identification of similar properties for A. fumigatus has been difficult (Feldmesser, 2005). Such knowledge has allowed for the development of conjugate vaccines aimed at eliciting effective immunity to these targets, such as the glucuronoxylomannan conjugate for C. neoformans, or for the development of attenuated strains by targeted disruption of these molecules that can function as live attenuated vaccines, such as the B. dermatitidis BAD-1-deleted strain (Wuthrich et al., 2000; Casadevall et al., 2002; Feldmesser, 2005).

An additional approach toward vaccine development has stemmed from finding a broad-spectrum protective action of killer antidiotypic monoclonal antibodies. Antiidiotypes to a monoclonal antibody specifically reacting with killer toxins from yeasts and are lethal to pathogenic microorganisms expressing specific cell wall receptors. Because the target of this protective antibody is involved in β-glucan synthesis, the development of host response to this compound could be beneficial. β-Glucans are important constituents of the cell wall of many fungi, including Aspergillus spp., which, like many carbohydrates, are themselves poorly immunogenic (Cenci et al., 2002).

On the basis of the observations of antibody-mediated protection against fungi, the following criteria need to be taken into account when considering the possibility of protective antibodies against fungal diseases (Casadevall, 1995):

(1) Specificity: The experience with C. neoformans and C. albicans indicates that specificity is a critical variable in determining antibody efficacy. For example, C. neoformans protective antibodies against cryptococcosis are specific for glucuronoxylomannan; however, not all antibodies to glucuronoxylomannan are protective, suggesting that individual epitopes in this polysaccharide antigen differ in their ability to elicit protective and non-protective monoclonal antibodies.

(2) Ig isotype: The experience with C. neoformans suggests that antibody isotype is an important variable in determining the efficacy of antibodies against fungi. However, the relative efficacy of the various isotypes may differ, depending on the fungal pathogen. IgM and IgG1 are effective against cryptococcosis, but IgG3 is not. IgM and IgG3 are protective against candidiasis.

(3) Titre: Antibody-mediated protection against fungal pathogens is likely to depend on an optimal amount of serum antibody. For C. neoformans, the passive antibody is effective only at certain doses.
An ideal vaccine formulation would result in reproducible and predictable fulfilment of each of the three criteria described above.

At the moment, no standardized vaccine exists for the prevention of any of the fungal infections in humans, a situation that is attributable to both the complexity of the pathogens and their sophisticated strategies for survival in the host (Romani, 2004). In contrast, there are useful fungal vaccines in veterinary medicine, especially in the field of dermatophytosis, as will be discussed below, and for protection against other groups. For example, the fungus *Pythium insidiosum* causes a debilitating cutaneous, subcutaneous or systemic disease in horses and other mammals. Two vaccines have proved to be of use to both prevent the infection and to treat horses infected with *P. insidiosum*. The former is composed of sonicated hyphal antigens, and the latter is prepared from culture filtrate antigens (Deep, 1997).

5. Aspergillus

Of all the fungal diseases, aspergillosis has been the subject of the most intensive study, including the immune response to the disease. It has been described in many species of mammals and birds, where cellular characteristics have been described that might predispose to respiratory aspergillosis (Tell, 2005).

Human invasive aspergillosis is a disease affecting highly immunocompromised subjects. It is a rapidly progressive, often fatal, disease characterized by tissue destruction associated with abundant hyphae in necrotic tissue (Bozza et al., 2002). Host defects that result in predisposition to invasive aspergillosis include neutropenia, defective neutrophil function (such as that seen in patients with chronic granulomatous disease), receipt of corticosteroid therapy, immunosuppressive agents for solid organ transplant and late-stage human immunodeficiency virus (HIV) infection (Feldmesser, 2005).

In humans, the lungs are the most common location of *Aspergillus* infections. In domestic animals, the situation is different and varies between species. For example, in cows, the forestomachs, especially the omasum, are mostly affected, from which the infection may spread to other organs, including the placenta (Garcia et al., 2001a). A few cases of pulmonary involvement and later dissemination have been reported in dogs (Clercx et al., 1996).

Various authors have tried to relate these processes to some form of immunosuppression in animals, as is the case with humans, but until now it has not been possible to establish this relationship with neutrophils, T lymphocytes or macrophage function (Garcia et al., 2001a).

5.1. Pulmonary aspergillosis

In humans, aspergillosis is fundamentally a disease of pulmonary origin, acquired by inhalation of conidia, that affects immunocompromised individuals. There have been numerous studies carried out with humans and laboratory animals, mainly rats and mice, that reveal a lung disease in which the immune system behaves as it does against other fungal genera. It is likely that pulmonary aspergillosis in other animals follows the same pattern.

On the basis of these studies, host defence against *Aspergillus* comprises the following sequence of events (Phadke and Mehrad, 2005): (1) recognition of the pathogen; (2) a rapidly deployed and highly effective innate effector phase (non-specific or innate immunity); and (3) a delayed but robust adaptive effector phase characterized by immunologic memory (adaptive immunity).

There are three different mechanisms of defence: (a) physical barriers; (b) phagocytosis; and (c) humoral compounds.

5.1.1. Physical barriers

Include mucous membranes, mucociliary clearance and local secretion of inflammatory mediators by innate immunity cells (Crameri and Blaser, 2002).

Most of the *A. fumigatus* conidia, as well as most of the inspired particles, are eliminated by the action of the cilia of the pseudoestratified ciliated cylindrical epithelium of the upper part of the tracheobronchial tree. *A. fumigatus* synthesizes toxins, such as gliotoxin, fumagillin and helvolic acid, that are able to inhibit this ciliary movement (Amiotani et al., 1995). Also, the endothelial and epithelial cells are capable of internalizing conidia, which can facilitate the infection (Paris et al., 1997).

The airway mucus constitutes a physical, chemical and biological barrier of secretory products from the mucous membrane that facilitates the elimination of inspired particles, including fungal conidia. This fluid contains glycoproteins, proteoglycans, lipids, lysozyme and surfactants (McCormack and Whitsett, 2002).

Host proteins that participate in mediating the interaction between innate and adaptive immunity are very relevant for the host defence against *Aspergillus*. There are various PRRs such as lung surfactant proteins A and D (SP-A and SP-D), mannan-binding lectin (MBL) and TLRs, which strengthen the innate immune response. SP-A, SP-D and MBL belong to a group of
mammalian lectins called collectins, which contain collagenous regions (McCormack and Whitsett, 2002; Madan et al., 2005). It is known that they interact with carbohydrate structures on the surfaces of a wide range of pathogens, such as viruses, bacteria and fungi, and thus enhance phagocytosis and killing by neutrophils and alveolar macrophages. SP-A and SP-D are known to interact with phagocytic cells and enhance their chemotactic, phagoctytic and oxidative properties (Wright, 1997; Van de Wetering et al., 2004). Any defects in these innate immune molecules might contribute to increased susceptibility to *Aspergillus* infections. The collectins are not primitive, innate molecules but are highly specialized and able to modulate responses to foreign agents (Madan et al., 2005).

### 5.1.2. Phagocytes

The role of phagocytes in the defence against *Aspergillus* is essential to avoid the development of the disease.

- The first defensive cells that inhaled conidia meet are macrophages. The ability of the macrophages to prevent germination of the *Aspergillus* conidia depends on the anatomic location; human alveolar macrophages are competent in this activity, while those in the peritoneum are not (Latge, 1999). This is of importance in cases when the entry pathway of fungi is not pulmonary.

  Attachment of the conidia with macrophages is via non-specific receptors, such as mannosylfucosyl, and does not rely on the presence of opsonization factors such as complement or immunoglobulin. The conidia are internalized by the macrophages and prevented from growth for several hours until the macrophage begins to destroy them. At 24 h after internalization, ~90% of the conidia are killed. In spite of the enormous capacity of the macrophages to kill conidia, their effectiveness is not 100% (Latge, 1999). This first defensive mechanism greatly reduces pathogenicity by impeding germination and development of the fungus (Romani, 2004).

- Polymorphonuclear neutrophils are responsible for the destruction of the hyphae of *A. fumigatus*, and they are able to kill the conidia that escape destruction by the macrophages (Duong et al., 1998; Romani, 2004). The hyphae are too large to be engulfed and the neutrophils binding the surface without the need for complement or immunoglobulin, although they may help such action. The contact between neutrophils and hyphae triggers secretion of reactive oxidative intermediary agents that rapidly damage the hyphae; 50% of hyphae are destroyed in ~2 h (Latge, 1999).

  Resting conidia are relatively resistant to killing by either reactive oxygen intermediates or neutrophil cationic peptides and their ingestion triggers neutrophil degranulation and the respiratory burst only weakly. Once conidial swelling has occurred, the response of neutrophils is enhanced, such that phagocytosis, degranulation and reactive oxygen production are increased and conidia are killed more readily (Latge, 1999).

- Natural killer cells, an additional component of innate responses, function in host defence against invasive aspergillosis, with a very important role in neutropenic patients (Morrison et al., 2003). In this sense has been showed the important role of NK cells as an important effector cell in aspergillosis (Walsh et al., 2005).

The monocyte chemotactic protein-1 (MCP-1) is required for host defence against *Aspergillus* in neutropenic hosts; this effect is mediated by the early recruitment of NK cells to the lungs. It was not found induction of MCP-1 in the lungs of immunocompetent animals challenged with *Aspergillus* conidia. This observation suggests than in normal hosts, other components of innate immunity are sufficient to clear the pathogen. Then, NK cells have a very important role in neutropenic patients (Morrison et al., 2003).

- It has been described that human platelets can have a role in the protection against aspergillosis, since they are able to binding to the wall of invasive hyphae of *A. fumigatus* and to be activated (Christin et al., 1998). The thrombocytopenia that takes place in patients suffering long periods of neutropenia could increase the risk of aspergillosis. Platelets, like neutrophils, attach to cell walls of the invasive hyphal form of *A. fumigatus*, that induce its activation. Organisms were damaged as showed by loss of cell wall integrity and release of defined hyphal surface glycoproteins (Christin et al., 1998).

- Dendritic cells can phagocytose conidia and hyphae of *A. fumigatus* via two different phagocytic mechanisms. Similarly, the fate of each fungal form is different once it is engulfed by a dendritic cell. While hyphae were degraded progressively, conidia were still alive 3 h after phagocytosis, a finding that confirmed the more resistant nature of this morphological form of the organism (Bozza et al., 2002). Pulmonary dendritic cells ingest conidia and hyphae, migrate to draining lymph nodes and induce local disparate T helper-cell response (Romani, 2004).

Sometimes, in spite of these defensive mechanisms, the fungus is able to germinate, develop and form invasive hyphae. In this case, phagocytes in
different parts of the host are able to present the same defence. The hyphae are too large for the phagocytes to internalize for destruction; however, monocytes and macrophages have extracellular antifungal activity that is able to damage the fungi in an important way (Cox, 1989).

Activated lymphocytes did not prevent growth of *A. fumigatus* and did not damage the fungus, but did affect the adherence of hyphae to plastic surfaces. Since adherence is one of the first steps in microbial virulence, this effect might interfere with the pathogenicity of *A. fumigatus* (Clemons et al., 2000).

In later steps of the disease, T cells have an important role in defence in conjunction with macrophages. Immunohistochemistry studies of visceral lesions in dogs demonstrated a correlation between the number of T cells and the number of macrophages, which suggests a very important role of T cells in the local proliferation and activation of macrophages (Perez et al., 1996).

5.1.3. Humoral compounds

If the two defensive mechanisms described above are negotiated, the fungus spreads from the lung towards the bloodstream.

Joining together of *A. fumigatus* and components of the serum has been observed, but the role of this joining in the course of the disease is not known. For example, it is known that the levels of fibrinogen are increased during invasive aspergillosis, and that fibrinogen is able to join to *A. fumigatus*. C-reactive protein, which activates the complement cascade, can join to certain fractions of *Aspergillus*. The direct activation of the alternative complement pathway by *A. fumigatus* has been demonstrated, although the mechanism that begins the cascade of the complement resting conidia, germinated conidia and hyphae seems to be different (Feldmesser, 2005). Also, the capacity of the C3 component of complement to join to the fungus depends on changes in the biochemical composition of the surface of each of those three forms (Kozel, 1996).

Cytokines are important tools that have helped us to understand the control, immunoregulation and activation of pulmonary host defences. Understanding these properties provides a rational foundation for developing strategies for immune augmentation in the prevention and treatment of invasive pulmonary aspergillosis (Walsh et al., 2005).

Granulocyte colony-stimulating factor (G-CSF) enhances the antimicrobial activity of neutrophils against a wide variety of bacteria and fungi, including *A. fumigatus* (Roilides et al., 1993, 1995), and accelerates recovery from neutropenia and thus shortens the duration of the risk period for development of invasive pulmonary aspergillosis (Walsh et al., 2005).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) primes macrophages to release pro-inflammatory mediators, including IL-1 and TNF-α (Rodriguez-Adrian et al., 1998), and enhances the antifungal activities of intact neutrophils and/or mononuclear cells against *A. fumigatus* (Walsh et al., 2005).

Macrophage colony-stimulating factor (M-CSF) promotes the differentiation, proliferation and activation of mononuclear cells and macrophages (Gonzalez et al., 2001; Walsh et al., 2005).

TNF-α stimulates neutrophils to damage *Aspergillus* hyphae, enhances phagocytosis of conidia, and augments neutrophil oxidative respiratory burst and degranulation induced by opsonized fungi (Roilides et al., 1998; Phadke and Mehrad, 2005).

IL-6 is produced rapidly after intranasal infection with *A. fumigatus*, and appears to have a protective proinflammatory and immunomodulatory role in pulmonary host defence in response to organism-mediated pulmonary injury (Cenci et al., 1999).

IL-10 suppresses the antifungal activity of mononuclear cells against *Aspergillus* hyphae, while increasing their phagocytic activity (Walsh et al., 2005).

IFN-γ is a highly potent activator of phagocytes against opportunistic fungal pathogens in humans. IL-12 augments the oxidative antifungal activity of mononuclear cells against *A. fumigatus* via an IFN-γ independent route (Roilides et al., 1999).

The immune response against an *A. fumigatus* infection is usually a mixed response that is as much humoral as cellular, but it is effective only if it is associated with a cellular answer, with increase of CD4 lymphocytes, and elevation of the levels of IL-2, IFN-γ and IL-12. If the response is largely humoral, with an increase in the production of antibodies, IL-4, IL-5 and IL-10, it is usually associated with progression of the disease (Mehrad and Standiford, 1999; Roilides et al., 1998, 1999).

It can be assumed that this pattern of pulmonary aspergillosis, perfectly defined in experimental murine models and in man, happens in pulmonary aspergillosis in domestic animals.

5.2. Disseminated aspergillosis

The pulmonary entrance pathway in animals seems not to be predominant. What happens in these cases from the point of view of the immune system? The response of the immune system is related strongly to the
pathogenicity of the disease, and especially the pathway of entrance of the fungus.

The dog is the domestic animal species for which most is known about the working of the immune system in aspergillosis. In the dog, however, there is a symptomatology that is not usually accompanied by pulmonary affectionation, unlike the pulmonary clinical picture seen in humans. Therefore, they differ from the conventional disease pattern that appears in human medicine (Guerin et al., 1993; Clercx et al., 1996).

With the increase in knowledge about canine aspergillosis, it is clear that the clinical picture in the literature does not apply to all cases or species; for example, lesions can occur in very different sites and can be accompanied by very varied symptoms. Until the 1980s it seemed clear that the fungus involved in these processes was *Aspergillus terreus* (Day and Penhale, 1991; Berry and Leisewitz, 1996). However, an increasing number of studies were reported in which a different fungus was identified as the cause of the disease, including *Aspergillus flavus*, *Aspergillus flavipes*, and *Aspergillus flavipes*, as well as members of other genera, such as *Acremonium*, *Penicillium*, *Paecilomyces*, etc. (Jang et al., 1986; Watt et al., 1995; Gene et al., 2003; Zanatta et al., 2006). For this reason, years ago we proposed that these cases should not be called aspergillosis but should be described as systemic mycosis or disseminated mycosis, at least until the causal agent is identified. However, the actions of the immune system against all those fungal species are probably very similar.

The entrance pathway of fungi into the host organism is not still clear, although it has been suggested as possibly through old wounds, medium or inner chronic otitis, or a urinary pathway (Mullanery et al., 1983; Patterson et al., 1983; Littman and Goldschmidt, 1987; Starkey and McLoughlin, 1996; Garcia and Blanco, 2000). A digestive pathway of entrance has been proposed, since on some occasions lesions have been observed as mesenteric lymphadenitis with fungal hyphae (Pastor et al., 1993). Recently has been described the transuterine transmission (Elad et al., 2008). Once the fungus has gained entry in the dog, it can spread to different locations, mainly the rachis, where the disease can develop slowly, with dissemination to the brain, liver, spleen and abdominal lymph nodes. A clinical disease would be detected only after several years.

Theories about the primary spread of the fungal agent in the host suggest that it is intimately related to the ability of members of the genus *Aspergillus* to synthesize substances, such as elastase, that are able to invade non-necrotic tissues and penetrate into the bloodstream (Markaryan et al., 1994; Blanco et al., 2002). This capacity is associated with the production of certain types of lateral spores, named aleuriospores, which are usually observed when the fungus grows in vivo (Gelatt et al., 1991; Dallman et al., 1992; Wilson and Odeon, 1992; Kauffman et al., 1994; Butterworth et al., 1995; Perez et al., 1996). Once the fungus has penetrated into the bloodstream, spread to the organs. Development begins upon arrival at the capillary vessels, causing an inflammatory local reaction by the host, in which neutrophils and monocytes participate (Wilson and Odeon, 1992; Butterworth et al., 1995; Clercx et al., 1996; Starkey and McLoughlin, 1996). Therefore, because the entrance is not through mucous membranes, but directly into the bloodstream, the first line of defence that is so effective in immunocompetent individuals with lung infection is eluded, and it raises the possibility that the disease occurs in immunocompetent individuals.

In humans, local infection attributable to contamination of a wound should not develop into disseminated disease if the cell-mediated immunological system is intact (Garcia et al., 2000). However, an apparent predisposing immunosuppressive disease was not found in cases of canine systemic mycoses, at least with respect to the synthesis of antibodies, the function of neutrophils, T lymphocytes and macrophages, the levels of serum proteins, the levels of complement compounds, or skin test reactivity (Day et al., 1985; Day and Penhale, 1998).

At first, little importance was assigned to the HI response in the defence of the host in canine aspergillosis. It appears that the humoral factors by themselves are unable to prevent fungal development and that they are not important in the first stage of the infection, given the fact that disaggammaglobulinemia or hypogammaglobulinemia is not translated into risk of aspergillosis. In spite of this, it is obvious that antibodies have a role in the immune defence of the host, limiting the growth of fungal hyphae in conjunction with phagocytes. The level of antibodies in the blood of dogs with invasive aspergillosis shows a significant increase in the levels of IgG (Gelatt et al., 1991; Dallman et al., 1992; Wilson and Odeon, 1992; Guerin et al., 1993; Watt et al., 1995; Clercx et al., 1996). Some of these studies have shown low levels of IgA and IgM. Several authors have described the predisposition of German Shepherd dogs to suffering aspergillosis (Day et al., 1985; Berry and Leisewitz, 1996), probably due to reduced levels of IgA. However, in perhaps the most complete study so far, low levels of
IgA are reported for only 3 of 12 affected dogs. Nonetheless, the authors suggested a predisposition of the breed related to some genetically determined immunodeficiency, but none has been detected so far (Day and Penhale, 1998). The same thing was described for the Beagle dog, suggesting that the deficiency of IgA is due to defective synthesis or secretion, because the cells producing IgA are present in normal numbers (Guerin et al., 1993).

The results obtained in immunohistochemistry studies of the lesions found in different organs of animals with invasive aspergillosis revealed a high level of IgA in the lesions (Day and Penhale, 1991; Perez et al., 1996), with an intense immunoreactivity of IgA on the hyphae and in the cytoplasm of cells in the periphery of the mycotic granuloma. These discoveries demonstrate an unusual systemic distribution of IgA that suggests some defect in their production or distribution. In the same studies, a seemingly disproportionate local cellular response was described surrounding a small necrotic focus that included fungal hyphae. Very similar lesions were observed in laboratory animals with the experimental reproduction of the disease. Such lesions were compared with those present in equally infected animals, but they revealed an immunosuppression state caused by administration of cortisone, and they had a different appearance, with a large quantity of hyphae that sometimes invaded the adjacent vessels, accompanied by a limited inflammatory response. These results suggest that the lesions present in the immunocompetent animals are effective in stopping the advance and dispersion of the fungus. However, although the pattern of lesions in the dogs was similar to that in the immunocompetent mice, in any case they were indications that suggest resolution of these lesions (Day and Penhale, 1991).

In conclusion, the combination of the epidemiological studies with the results for the immune and inflammatory local responses allows speculation about the possibility of the existence of an immunologic deficit of genetic origin that would have an important role in the pathology of canine aspergillosis (Day et al., 1985).

In canine nasal aspergillosis, the immune response in the nasal cavity is mixed, involving antigen-presenting cells (macrophages and dendritic cells), T lymphocytes and plasma cells. Mast cells and eosinophils were not a significant component of the inflammatory response. IgG+ plasma cells were the predominant type within the lesions, whereas IgA+ plasma cells were predominant in the nasal mucosa of healthy dogs (Peeters et al., 2005). These data are in contrast with findings in dogs with disseminated aspergillosis, where IgA+ plasma cells predominated in the granuloma throughout the body, and IgA dysregulation was thought to be a predisposing factor to the disease (Day and Penhale, 1991; Perez et al., 1996). The predominance of major histocompatibility complex (MHC) class II+ antigen-presenting cells, and IgG+ plasma cells, together with the presence of numerous CD4+ and CD8+ T cells may indicate a Th1 immune response that is effective in preventing systemic dissemination of the fungus but ineffective in clearing the infection from the nasal cavity and frontal sinus (Peeters et al., 2005, 2007).

Clinical pictures of disseminated aspergillosis similar to those described in the dog have been reported in other animal species. In bovine mycotic abortion and in ovine Aspergillus mastitis an immune response similar to that described in the dog can be assumed: the rupture of the defensive barrier of skin and mucous membrane allows the fungus to enter directly into the bloodstream, where the immune system is unable to stop its development (Jensen et al., 1989; Las Heras et al., 2000; Garcia et al., 2004a).

Aspergillus spp. have been recognized since the early 1800s as an important cause of pulmonary disease in birds. In contrast to mammals, birds are highly susceptible to invasive aspergillosis (Feldmesser, 2005; Garcia et al., 2007). A study of avian aspergillosis in chickens found that the appearance of serum antibody reactive with hyphal fragments is correlated with the development of resistance to infection, and the appearance of precipitins coincides with clearance of hyphae from the air sac and lungs, suggesting a role for antibody in acquired resistance. The increased incidence accompanying captivity, some farming practices and/or mating has been noted repeatedly, leading to the hypothesis that susceptibility is stress-induced. A number of features have been proposed to have a role, such as anatomic differences from mammals and differences in innate immune cells, including a lack of surface macrophages in the lung and dependence upon heterophils, which, unlike mammalian neutrophils, do not have myeloperoxidase or any other oxidative mechanism for killing fungal hyphae (Tell, 2005).

Mould-infected feedstuff can have a dual role in the process; by dissemination of the fungus, and by containing small quantities of mycotoxins that would lead to immunodepression and, consequently, to favour the appearance of the disease.

5.3. Vaccine against Aspergillus

Studies of vaccines that use whole cells, either in the form of conidia or disrupted whole germlings or
mycelia, support the view that protective immunity against pulmonary or systemic infection can be induced. This suggests the possibility of the development of an effective vaccine against aspergillosis (Cenci et al., 2000).

A vaccine that is not fully protective but improves the chances of survival with antifungal therapy would still be extremely useful as a means of augmenting the host response to an established disease, providing additional time for immune reconstitution. Since 1939, a wide spectrum of vaccination strategies against aspergillosis, involving combination of a variety of proteins, glycoproteins, live or inactivated conidia used as immunogens or the adoptive transfer of immunity cells have been attempted (Belllocchio et al., 2005; Feldmesser, 2005). For example, an endotoxin isolated from A. fumigatus gave limited protection to hyperimmunized rabbits subsequently inoculated with conidia. Some protection was seen in ducks and mice using live conidia injected subcutaneously. It has been shown clearly in a murine model that vaccination with A. fumigatus extracts can confer protection against subsequent challenges with conidia (Belllocchio et al., 2005).

One attempt at using a live attenuated conidial vaccine has been reported. Vaccination of mice with an avirulent p-aminobenzoic acid auxotrophic mutant produced by chemical mutagenesis protected against systemic infection with the virulent parental strain (Sandhu et al., 1976). Recent experimental evidence suggests that vaccination against Aspergillus through the use of fungus-pulsed dendritic cells is feasible (Bozza et al., 2003; Belllocchio et al., 2005).

Recent studies have proceeded in three important directions: (1) toward a focus on manipulation of the immune response with different adjuvants; (2) identification of specific antigens capable of inducing protective responses; and (3) the development of a vaccine with efficacy against a broad range of pathogens, including A. fumigatus.

6. C. albicans

C. albicans is part of the normal microbial flora in human beings and domestic animals, and is associated with the mucous surfaces of the oral cavity, gastrointestinal tract and vagina. Immune dysfunction can allow C. albicans to switch from a commensal to a pathogenic organism capable of infecting a variety of tissues and causing a possibly fatal systemic disease (Stevens et al., 1998; Romani, 1999; Traynor and Huffnagle, 2001). Mucosal infection is the most usual form of the disease but cutaneous lesions are seen on occasion (Lehmann, 1985).

In cattle, as a consequence of the abundant use, and occasional abuse, of antibiotics in the treatment of mastitis, there is a selection of flora, mainly members of the genus Candida, that are new etiological agents of these processes, which are initially difficult to diagnose because their presence is not expected. Candidiasis in birds is related to malnutrition and stress, generally produced by the same strains that are found naturally on the food plants of these animals. Arthritis caused by yeasts in horses is relatively frequent as a consequence of contamination of wounds or after surgical treatment. In pigs, candidiasis usually takes the form of digestive alterations in young animals, and is usually related to problems that predispose to the disease, like treatment with antibiotics (Garcia and Blanco, 2000). C. albicans is a common causative agent of stomatitis in the dog (Jadhav and Pal, 2006).

Although fungi need predisponent factors to produce the disease, it is known that saprophytic colonization of the mucous membrane by C. albicans does not need the host to be immunocompromised, since it is detected in immunocompetent individuals (Hostetter, 1994; Garcia and Blanco, 2000).

The clinical spectrum of C. albicans infections ranges from mucocutaneous to systemic life-threatening infections. The main risk factors that predispose to severe Candida infections are congenital or acquired defects of CMI, including quantitative and qualitative defects in neutrophils and dysregulated Th-cell reactivity (Romani, 2004). Candida infections in dogs have been described in cases with no clinical evidence of an underlying disease or immunosuppression (Brown et al., 2005; Kuwamura et al., 2006).

Resistance to infection by C. albicans is associated with Th1 CMI, whereas Th2 immunity is associated with susceptibility to symptomatic infection. Neutrophils are considered to be the primary effector cells for C. albicans killing in vivo, although macrophages are involved in CMI to control infection. In addition to their role as effector cells, neutrophils may have an immunoregulatory role in development of the Th response. The fact that neutrophils are (i) abundant at the sites of C. albicans infection and (ii) capable of selectively producing the directive cytokines IL-12 and IL-10, suggests that these cells may be important in determining the type of anti-Candida Th cell response (Lehmann, 1985; Stevens et al., 1998; Traynor and Huffnagle, 2001).

Keratinocytes, neutrophils, macrophages, eosinophils and basophils are the first line of host defence against mucosal C. albicans infections (Oliveira et al.,...
Neutrophils, monocytes, and monocyte-derived macrophages, as well as committed tissue macrophages (Kupffer, splenic macrophages, and pulmonary alveolar macrophages) are the key effector cells in host defence against deeply invasive candidiasis (Roilides and Walsh, 2004).

Cytokines such as TNF-α, IL-6, G-CSF and GM-CSF have been determined to be important for neutrophil recruitment during candidiasis (Kullberg et al., 1999). GM-CSF accelerates hematopoiesis at early and late steps of differentiation of myeloid cells, resulting in increased production of neutrophils, metamyelocytes and eosinophils. This cytokine also augments the antimicrobial activities of terminally differentiated neutrophils and monocytes (Roilides and Walsh, 2004). GM-CSF increases the fungicidal activity of human neutrophils against Candida pseudohyphae and of human monocytes against blastoconidia of C. albicans (Smith et al., 1990; Roilides and Walsh, 2004). M-CSF accelerates proliferation and differentiation of mononuclear/macrophage progenitors, recruits monocytes to sites of infection and activates mature macrophages. M-CSF is a potent immunomodulator of antifungal activity of monocytes and tissue macrophages against a variety of fungi, including Candida spp. (Roilides et al., 2000; Roilides and Walsh, 2004). The efficacy of HI mechanisms against C. albicans has been a controversial subject for decades. In the last decade, evidence has accumulated that antibodies specific for certain cell surface epitopes may be beneficial for the host (Han and Cutler, 1995; Cassone et al., 1997; Polonelli et al., 2000).

The finding of protective and non-protective antibodies, and the observation that these have different epitope specificity suggest an explanation of why some investigators have reported evidence for protective antibodies and others have not. Specifically, when presented with an enormously complex antigenic stimulus, such as a yeast cell, the immune system will respond in a variable manner to a finite number of the displayed determinants. Predicting the resulting antibody specificities and titres of each antibody may be impossible, and would likely vary from animal to animal. Thus, even though the final total antibody titres may be somewhat similar quantitatively amongst individual experimental animals immunized against whole fungal cells, their sera will be expected to differ qualitatively. This same line of reasoning might be invoked to explain why the presence of fungal-specific antibodies in severely ill candidiasis patients is not necessarily evidence against protective antibodies (Polonelli et al., 2000).

In animal fungal diseases, it has been shown that the levels of IgM, IgG and IgA are increased. This fact has been considered of great importance with diagnostic and prognostic value, although not necessarily involve a protective status against the disease (Bromuro et al., 1994).

It is of interest that many authors have reported that saprophytic colonization of certain mucocutaneous locations triggers the production of antibodies and sometimes it is possible to detect systemic fungal antigens. This can occur in the absence of clinical disease and even without systemic colonizations (which always entails spread of the agent by the lymphohematogenous pathway) (Hernando et al., 1993). There exists a CMI response characterized by the appearance of reactive T cells that have a vigilance role in avoiding active infection (Bromuro et al., 1994).

C. albicans can cause invasive disease in immunocompromised animals. In contrast to the resistance seen at the skin or mucosal surface, T cell-mediated immunity seems to be unimportant in resistance to this form of the disease, in which neutrophils have the central role (Lehmann, 1985).

In the invasive process, C. albicans reaches the bloodstream. These blood-borne organisms then adhere to the endothelial cell lining of the vasculature (Klotz, 1992; Clemons et al., 2000). Next, they invade endothelial cells by inducing their own endocytosis (Filler et al., 1995; Clemons et al., 2000). Once inside the endothelial cells, C. albicans injures and eventually kills these cells (Fratti et al., 1998; Clemons et al., 2000). Injury of the endothelial cells leads to loss of integrity of the vascular lining, enabling the pathogen to invade the deep tissues. Moreover, the loss of endothelial cells results in the exposure of the sub-endothelial cell basement membrane, which can be bound by additional organisms (Clemons et al., 2000).

Endothelial cells are not passive in this process, they actively respond to Candida infection by synthesizing a variety of pro-inflammatory cytokines and expressing leukocyte adhesion molecules (Orozco et al., 2000; Clemons et al., 2000). These pro-inflammatory mediators recruit activated leukocytes to the site of vascular invasion to aid the host defence. If a sufficient number of functional leukocytes are present, the invading organisms can be killed and the infection can be aborted (Clemons et al., 2000).

It has been shown in dogs that CMI is an important determinant of the systemic spread of Candida spp., where circulating neutrophils appear to be the major source of defence. Disseminated candidiasis has been associated with parvoviral infection, and with treatment...
with antimicrobials and corticosteroids, administration of cyclophosphamide and with a defect in the immunological system (Brown et al., 2005).

The disease can be controlled in poultry by immunization via intradermal or intramuscular injection of ethanol-killed C. albicans (Lehmann, 1985).

7. Dermatophytes

Dermatophytosis (ringworm) is an infection by fungal species belonging to the genera Microsporum, Trichophyton and Epidermophyton of the keratinized superficial tissues of man and animals; the corneum stratum, hair and skin (Sparkes et al., 1993).

Dermatophytosis is important in the dog, not so much for the severity of the process, which is never life-threatening, but fundamentally for their zoonotic character. It is much more frequent in domestic cats, which are the main source of infection for man. It is important in cattle, since there are a great number of these animals affected; in fact, in North and Central Europe it is considered one of the most important zoonoses, and vaccines that in principle are fully effective are used in many countries (Garcia and Blanco, 2000).

Microsporum canis followed by Trichophyton mentagrophytes, are the dermatophytes most frequently isolated from dog and cat (Cabanes et al., 1997), whereas Trichophyton verrucosum and T. mentagrophytes are the most important species isolated from cattle and horses (Garcia and Blanco, 2000).

Dermatophytes are not part of the normal flora of the skin. Due to their keratinophilic and keratinolytic nature, they are able to use cutaneous keratin as a nutrient producing the infection in this way (Garcia and Blanco, 2000). Although the infection is confined to the superficial keratinised tissues, it induces a humoral and a cellular immune response (DeBoer et al., 1991; DeBoer and Moriello, 1993; Mignon et al., 1999a; Sparkes et al., 1993, 1995).

Skin in itself represents a very effective physical barrier against fungal invasion, in which the action of neutrophils, epidermal cellular proliferation and keratinisation have a very important role in the initial response of the host, restricting the microorganism to the superficial stratum of the skin, and promoting rapid elimination of the fungus (Osawa et al., 1998).

After the organism is exposed to a dermatophyte, the following sequence of events occurs in the skin (Gudding and Lund, 1995; Hunsaker and Perino, 2001):

1) Presentation of the antigen: Antigens are caught by the antigen-presenting cells of the immune system of the skin (Langerhans cells), which move to the regional lymphatic nodes, where the antigen is presented to the T lymphocytes through the MHC II (Teunissen, 1992). This movement is probably favoured by cytokines; GM-CSF has a stimulatory effect on the Langerhans cells, promoting its capacity to present the antigen (Kaplan et al., 1992). Since Langerhans cells have little phagocytic capacity, macrophages have an important role in the capture and degradation of the antigen into smaller fragments. In the same way, keratinocyte have the capacity to degrade antigens by the phagocytosis, and to transfer them to Langerhans cells to be presented to T lymphocytes (Suter, 1993).

2) Recruitment of cells: In cattle experimentally infected with T. verrucosum it has been demonstrated that there is an increment of macrophages, CD4+ and CD8+ lymphocytes, and T γ/α cells in the dermis, and the most abundant are CD4+ T helper cells (Pier et al., 1993). A dense neutrophil population has been observed in the skin.

3) Resolution of the process: Resolution of the disease is generally accompanied by the development of a delayed hypersensitive response, while the persistence of the infection seems to be associated with the absence of this response and with a poor in vitro lymphoproliferation (DeBoer and Moriello, 1995; Mignon et al., 1999b). This implies a protective status against re-infection (Sparkes et al., 1993; DeBoer and Moriello, 1995).

In response to contact with the fungus, epidermopoiesis in the skin is increased, which produces an increase in the rate of regeneration of epidermal cells and a consequent removal of the fungus from the skin surface. The CMI response is essential to the establishment of an immunoprotective status against dermatophyte infection (Sohnle, 1993; Mignon et al., 1999b). The sequence of events in the HI response to dermatophytosis is unclear, although the action of specific antibodies could have a direct fungistatic effect by means of opsonization and complement activation (Pier et al., 1993; Sparkes et al., 1994).

7.1. Vaccine against dermatophytosis

The cellular wall of dermatophytes is composed mainly of chitin, glucans and glycopeptides, which are the main antigens of these fungi (Wagner and Sohnle, 1995). The most important antigens are the proteic portion of glycopeptides that stimulate the HI response, and keratinases, which produce a delayed hypersensi-
tivity response when they are inoculated intradermally (Dahl, 1993).

The development of effective immunoprophylactics offers an interesting alternative in the control of this disease, once the protective status of antigenic extracts is proven. A great variety of veterinary vaccines effective against fungal disease have been marketed in different countries, in some cases for many years.

The inactivated vaccines stimulate the CMI, as demonstrated by skin tests and leukocyte migration inhibition tests. Vaccines containing *T. verrucosum* conidia inactivated with formalin have been described for use in cattle (Wawrzkievicz and Wawrzkievicz, 1992).

An inactivated vaccine plus adjuvant containing conidia and mycelium of two *T. equinum* strains has been used in the immunization of horses (Pier and Zancanella, 1993). The vaccine does not prevent the disease, but the lesions are less severe in vaccinated animals in compared to non-vaccinated animals.

The most widely used inactivated vaccine is Insol Dermatophyton 
°, developed in Switzerland by Boehringer Ingelheim. The manufacturers indicate that it is effective in horse, dog and cat, and that it can be used as treatment of the disease, improving the clinical outcome. It contains strains of *T. verrucosum, T. mentagrophytes, T. sarkisovii, T. equinum, M. canis, M. canis var. distortum, M. canis var. obesum,* and *M. gypseum*.

The commercial vaccine Feo-O-Vax MC-K 
° was developed by Fort Dodge in USA. It is an inactivated vaccine containing the mycelium of *M. canis* and an adjuvant. This vaccine produces anti-dermatophyte antibody titres similar to those developed in the course of the natural infection, with a low CMI. All vaccinated cats developed the disease after a topical application of *M. canis* conidia; however, the lesions were smaller than those in the control animals. The fact that all of the animals vaccinated had lesions suggests that high titres of antibody against *M. canis* may not be enough for protection against the infection (DeBoer and Moriello, 1995).

The inactivated vaccine Dermatovac-IV has been developed in guinea pigs. It contains an adjuvant and an optically standardized inactivated suspension of conidia and mycelium of the fungi *M. canis, T. equinum, M. gypseum* and *T. mentagrophytes* (Pier et al., 1995).

Without doubt, the most effective and widely used have been the live vaccines. The Ringvac bovis LTF-130 
° vaccine, marketed by Alpharma, and elaborated with the LTF-130 strain of *T. verrucosum*, which has a characteristic high level of immunogenicity, low virulence and great stability, fundamental requirements of a strain used in a vaccine. It has been used effectively in Russia and Norway, where its effectiveness was demonstrated with experimental animals as well as in field studies. It is administered intramuscularly, and it contains a residual virulence able to stimulate the appropriate immune response, producing a delayed hypersensitivity reaction, which is considered essential for the removal of ringworm lesions (Rybnikar et al., 1998).

The live vaccine Permavax-Tricho 
°, marketed in the Czech Republic by Bioveta Ivanovice, contains an attenuated strain of *T. verrucosum*. This vaccine triggers a protective immunity status 28 days after the second inoculation, preventing the appearance of the clinical disease for 1 year after vaccination (Rybnikar et al., 1998, Mignon et al., 1999b).

8. *Malassezia pachydermatis*

The lipophilic yeast *M. pachydermatis* is part of the normal cutaneous microflora of most warm-blooded vertebrates. The opportunistic nature of this yeast has been demonstrated, confirmed by the excellent response to specific antifungal therapy. The normally commensal yeast may become a pathogen whenever alternation of the skin surface microclimate or host defence occurs (Akerstedt and Vollset, 1996; Guillot and Bond, 1999, 2006, Ashbee, 2006, 2007).

*M. pachydermatis* is the only species in the genus that does not require an exogenous source of lipid for growth (Blanco et al., 2000; Ashbee, 2006). In veterinary medicine, it is the most important species in the genus, although another species has been implicated in skin disorders. *M. pachydermatis* is the species most frequently isolated from the skin, mucosa or ear canal of healthy dogs and cats.

In dogs, this yeast acts as an opportunistic secondary pathogen within the ear canal. Otitis externa associated with *M. pachydermatis* is often characterized by a waxy, moist, brown or yellow exudate with variable erythema and pruritus (Griffin, 1993; Guillot and Bond, 1999). The factors that favour proliferation of *M. pachydermatis* and its transition from a commensal organism to an apparent pathogen on canine skin are poorly understood, but presumably reflect disturbances of the normal physical, chemical or immunological mechanisms that restrict microbial colonization of skin. Breed predilections have been identified, and geographical variations are apparent (Guillot and Bond, 1999). Concurrent diseases have been recognized in many, but not all, cases of *Malassezia* dermatitis in dogs, and correction of the concurrent disease may
prevent, or reduce the frequency of relapse in some dogs (Garcia and Blanco, 2000). Some atopic dogs carry large numbers of *M. pachydermatis* yeasts in both lesional skin and in unaffected areas (Rossychuck, 1994; Ashbee, 2007). Immediate intradermal test reactivity has been observed in atopic dogs following the injection of *M. pachydermatis* extracts at concentrations that cause no reaction in healthy dogs, suggesting that hypersensitivity responses to yeast allergens may be involved in the pathogenesis in some cases of atopic disease (Morris and Rosser, 1995). These observations are analogous to the suggestion that IgE-mediated hypersensitivity to *Malassezia*-derived allergens may be important in the pathogenesis of the “head-neck” form of atopic dermatitis in humans (Kieffer et al., 1990; Guillot and Bond, 1999).

In cats, dermatitis associated with *Malassezia* spp. is frequently reported in those with endocrine and metabolic diseases, neoplasia, and infection with feline leukaemia virus (FLV) and feline immunodeficiency virus (FIV). Some reports suggest that *Malassezia* spp. overgrowth may be found with feline allergic skin diseases (Ahman et al., 2007; Ordeix et al., 2007).

*M. pachydermatis* has the ability to stimulate the immune system via the classical and alternative complement pathways, acting as an adjuvant and elicits both humoral and cellular immune responses in healthy individuals and individuals with conditions associated with *Malassezia* spp. (Sohnle and Collins-Lech, 1983; Takahashi et al., 1986; Ashbee et al., 1994a,b; Suzuki et al., 1998). The activation of the complement pathways is responsible for the inflammation associated with seborrhoeic dermatitis. In contrast, it is able to resist phagocytic killing by neutrophils and down-regulate cytokine responses when co-cultured with peripheral blood mononuclear cells (Richardson and Shankland, 1991; Kesavan et al., 1998). These apparently contradictory behaviours may be related to the lipid-rich capsular-like layer that surrounds the yeast cells, and is responsible for the lack of inflammation associated with *Malassezia* in its commensal state (Mittag, 1995; Ashbee and Evans, 2002; Ashbee, 2007).

Understanding the apparently contradictory ability of *Malassezia* to upregulate or suppress the immune response directed against it may well be the key to understanding how *Malassezia* spp. occur both as commensals and as pathogens (Ashbee, 2006).

With the interaction of *Malassezia* with keratinocytes, the yeast induces the production of different cytokines, more in *M. pachydermatis* than in other species of *Malassezia*, especially IL-6. Yeast-cell contact is required for this stimulation, and cytokine production is not caused by a soluble factor. This higher level of cytokine induction by *M. pachydermatis* may explain the greater severity of disease associated with this specie. In normal skin or mucous membranes, *Malassezia* reduces the inflammatory response, enabling it to live as a commensal (Watanabe et al., 2001; Ashbee, 2006).

The ability of *Malassezia* spp. to stimulate the immune system is well documented (Ashbee and Evans, 2002), but the antigenicity is lower in comparison to other organisms. Then, 20–100-fold more protein from *Malassezia* than from *C. albicans* was required to stimulate the cellular immune response (Sohnle and Collins-Lech, 1980).

The interaction of *Malassezia* with phagocytic cells may serve to amplify the inflammatory response and encourage further recruitment of phagocytic cells. The ability of neutrophils to kill *Malassezia* seems limited, in contrast to its function against other yeasts, such as *C. albicans* (Richardson and Shankland, 1991; Ashbee and Evans, 2002). One possible reason for this limited killing ability of phagocytes may be the production of azelaic acid by *Malassezia*. Also, the lipids associated with the cell wall of *Malassezia* may be antiphagocytic and involved in protection against killing by neutrophils (Akamatsu et al., 1991; Ashbee and Evans, 2002).

The majority of individuals have some antibodies to *Malassezia*, even from a relatively young age, with IgG and IgM. Levels of IgA are generally low, suggesting that mucosal sensitisation by *Malassezia* is not important (Ashbee and Evans, 2002).

9. Cryptococcus

Recently, a differentiation has been made between two species, *Cryptococcus gattii* and *Cryptococcus neoformans*. This differentiation was made on the basis of genetic variation and a lack of evidence for genetic recombination between both genotypes (Duncan et al., 2006a,b). These genetic differences are consistent with differences in habitat, geographical distribution and, most importantly, in pathogenicity and the effectiveness of the response of the host immune system. *C. neoformans* infects predominantly immunocompromised hosts, while *C. gattii* has not been associated with a suppressed immune system (Duncan et al., 2006a,b). This can explain many historical contradictions about the pathogenicity of this fungus, and especially the need for a state of immunosuppression in the host for the success of the disease.

*C. neoformans* is a widespread fungus found in environmental niches such as soil and avian excreta, whereas *C. gattii* is found on eucalyptus trees and in the
soil (Traynor and Huffnagle, 2001; Duncan et al., 2006a).

The primary route of entry for Cryptococcus is via the lungs, where the fungus may establish a primary infection. If the initial pulmonary infection is not controlled, the fungus can disseminate to other organs and the central nervous system, resulting in fatal cryptococcal meningoencephalitis (Traynor and Huffnagle, 2001; Aguirre et al., 2004; Chen et al., 2007).

Human disseminated cryptococcosis has two unusual features that distinguish it from other disseminated fungal infections. First, patients have cryptococcal polysaccharide antigen in their body fluids, and detection of this is useful in diagnosis of the disease. Second, there is a limited inflammatory response in tissues harbouring C. neoformans. One could interpret these findings to mean that individuals with the highest levels of cryptococcal polysaccharide in their body fluids simply had higher number of organisms in their tissues and therefore were the most likely not to respond to antifungal therapy. Another interpretation could be that the cryptococcal polysaccharide had an adverse effect on host defence mechanisms, thus allowing a progressive disease, concluding that cryptococcal polysaccharide in the bloodstream exacerbates disease (Diamond and Bennett, 1974; Murphy, 1989; Clemons et al., 2000).

Clinical cryptococcosis has been reported worldwide in many animal species. It is the most common systemic fungal infection in cats and is often described in dogs (Duncan et al., 2006b). In cats, the disease takes the form of rhinitis when the process is primary, and is systemic with alterations mainly of the central nervous system and with important affection of lymph nodes when it is secondary to the infection by FIV.

The role of FIV in feline cryptococcosis is under debate: some studies show an equivalent prevalence of FIV in cats with and without cryptoccosis in hospitalized animals, whereas other studies show that concurrent infection with FIV or FELV in cats with cryptococcosis was much higher than the prevalence of those viral diseases in a population of hospitalized cats. In the majority of these studies, the causative agent was assumed to be C. neoformans and not C. gattii. This is important, because C. neoformans is most commonly isolated from immunosuppressed individuals, whereas C. gattii should be considered a primary pathogen because it infects immunocompetent hosts, even in areas where the organism is endemic (Duncan et al., 2006a). Therefore, it may be necessary to reconsider the exact etiologic entity in those cases.

In other animal species, cryptococcosis can appear to be related to immunodepression, although not always. Then, have been described outbreaks of ovine and caprine cryptococcosis with respiratory signs, and without immunodepression situation detected in those animals (Garcia and Blanco, 2000). In these cases, respiratory symptoms associated with cachexia were the predominant clinical picture; liver and brain involvement was also documented (Baro et al., 1998).

Cryptococcosis may occur in horses as a disseminated cryptococcal infection with osteomyelitis of both the axial and appendicular skeleton produced by C. gattii. The administration of corticoids led to clinical deterioration due to immunomodulating effects. Administration of systemic corticoids occurred after lesions had appeared; however, the horse deteriorated significantly after corticosteroid treatment (Lenard et al., 2007).

9.1. Immune response

Clearance of Cryptococcus infection requires the development of a Th1-type CMI and the subsequent pulmonary recruitment and activation of leukocytes. The leukocytic infiltrate in response to cryptococcal infection includes a mix of myeloid and lymphoid cells, all of which are capable of inhibiting the growth of, or killing, the organism in vitro (Traynor and Huffnagle, 2001; Romani, 2004). Neutrophils and macrophages are the two phagocytic cells in the natural host defence that are most likely to be responsible for clearing the cryptococcal cells from the tissues. There was very little inflammatory response, i.e. influx of neutrophils, lymphocytes and macrophages, into infected host tissues (Clemons et al., 2000).

In this Th1-CMI, IFN-γ, TNF-α, IL-2, IL-12, IL-15, IL-18, monocyte chemotactic protein-1, macrophage inflammatory protein-1α and nitric oxide have been shown to have important roles in murine models. GM-CSF plays a complex role in the development of anticytotoxic immunity in the lungs: it is required for early recruitment of leukocytes into the lung, movement of recruited leukocytes into the alveolar space and the formation of inflammatory foci in the lungs (Traynor and Huffnagle, 2001; Chen et al., 2007).

The limited leukocyte infiltration into infected tissues in cryptococcosis patients is due to the circulating glucuronoxylomannan (GXM, predominant polysaccharide of high molecular weight) stimulating L-selectin to shed from the surface of neutrophils, thus preventing the first step in extravasation. This is the mechanism responsible for the reduced number of leukocytes that are seen in Cryptococcus-infected tissues (Clemons et al., 2000).
The Th2 cytokines IL-4 and IL-5 were not secreted at significantly higher levels in Cryptococcus-infected brains of immune mice compared to control mice (Uicker et al., 2005).

CD4+ T-cells are critical for the control of Cryptococcus. An antigen-specific CD4+ T cell response occurs when the T cell receptor recognizes processed antigen fragments presented by MHC II. Then CD4+ T cells secrete cytokines and proliferate (Kwon-Chung et al., 2000).

Nevertheless, in the lung, both CD4+ and CD8+ T cells are required to clear the cryptococcal infection. If yeasts escape and colonize the brain, rapid proliferation leads to serious central nervous system disease. Work with severe T cell and B cell-deficient combined immunodeficient mice and wild-type controls has demonstrated a CD4+ T cell-mediated resistance to cryptococcal organisms that colonize the brain. Depletion of CD8+ T cells had no detectable effect on T cell-mediated resistance in brain in vivo. In contrast, CD8+ T cells play a well-defined and important role in containment of pulmonary infection (Aguirre et al., 2004).

10. Pneumocystis

Pneumocystis is a genus that has had an interesting tenure on the scientific stage. The first documentation of the existence of the organism known as Pneumocystis was as a part of the trypanosome life-cycle. Several years later, Pneumocystis was identified as an organism separate from trypanosomes. This includes a life-cycle similar to that of protozoans, based on the identification of a small trophic form, the larger cyst form may include five to eight progeny within the cyst. However, the disease caused by Pneumocystis was not thoroughly reported until World War II, where it was observed to be associated with pneumonia in malnourished children. Thereafter, Pneumocystis infections became increasingly evident in the immunocompromised patient population but it was the AIDS epidemic that brought Pneumocystis to the forefront of lethal, opportunistic fungal infections. During this time, another interesting observation roused the Pneumocystis field with the report that Pneumocystis was more closely related to fungi than to protozoan. Actually, Pneumocystis is clearly within the fungal kingdom, falling between ascomycetes and basidiomycetes (Edman et al., 1988; Steele et al., 2005). Although P. carinii had been isolated from many animal species, including man, traditionally a great specificity of the isolates has been demonstrated for the species that could infect. Then, P. carinii was considered as a group of heterogeneous populations, genetically isolated from each other, that have undergone a prolonged process of genetic and functional adaptation to each mammalian species (Dei-Cas, 2000). Further genetic analysis has shown that Pneumocystis isolated from different species has significant differences in gene sequence and chromosomes. This has prompted nomenclature changes and P. carinii is now reserved for the rat pathogen, P. carinii f.sp. muris for the murine pathogen, and P. jiroveci for the human pathogen (Stringer et al., 2002; Steele et al., 2005). It may be that this situation will be repeated in different animal species, like the description of the genetically different P. canis in dog (English et al., 2001).

Pneumocystis pneumonia is a well-recognized major opportunistic infection in HIV-positive individuals, and is growing in importance in HIV-negative patients undergoing immunosuppressive treatment for malignancy, connective tissue disease or organ transplantation. In the veterinary field, Pneumocystis has been described as producing pneumonia in dogs, causing serious pulmonary alterations. Pneumocystis is important in horses, where treatment with immunosuppressive drugs, like corticoids, is a relatively frequent practice in animals dedicated to sport, originating a pneumatic process with bronchoneumonic diffuse image. In pigs, this fungus gives rise to pneumatic processes, affecting animals 7–11 weeks old, with lung damage that includes a decrease of the size of the pulmonary septa with infiltration of mononuclear cells and appearance of exudates in the alveoli. This focal pneumonia evolves toward a diffuse pneumonia, very similar to what happens with this disease in children (Garcia and Blanco, 2000; Kondo et al., 2000; Cavallini et al., 2007).

Pneumocystis infects hosts by a respiratory route, and animal-to-animal airborne transmission has been clearly established (Dumoulin et al., 2000).

In humans, the persistence of Pneumocystis in the lung is a limited-time phenomenon inversely related to immunological improvement. A normal immune response completely eradicates Pneumocystis from the host. However, immunocompetent hosts can be parasitized transiently by Pneumocystis: increased titres of Pneumocystis antibodies were detected in hospital staff in close contact with Pneumocystis pneumonia patients, and immunocompetent experimental hosts were parasitized transiently by Pneumocystis after close contact with hosts developing pneumonia by this fungus (Dei-Cas, 2000).

10.1. Immune response

Host defence against Pneumocystis is indistinguishable from that observed for other medically important
fungal pathogens of the lung (Steele et al., 2005). As with fungal pathogens such as A. fumigatus, C. neoformans and H. capsulatum, alveolar macrophages are an essential component of the immune response against Pneumocystis and are ultimately responsible for clearing this fungus from the lungs (Brummer and Stevens, 1996; Ibrahim-Granet et al., 2003; He et al., 2003; Steele et al., 2005).

Natural killer cells are a subset of innate immune cells that may have a role in host defence against Pneumocystis, as has been demonstrated for A. fumigatus and C. neoformans.

Similar to other opportunistic fungal infections, Pneumocystis pneumonia is most often observed when the CD4+ T helper cell count falls below 200 cells/mm³. CD4+ T cells are absolutely critical for resolution of Pneumocystis, having an essential role in the recruitment and activation of effector cells against the organism. CD8+ T cells by themselves do not aid clearance of Pneumocystis from the lungs (Beck and Harmsen, 1998; Dei-Cas, 2000; Traynor and Huffnagle, 2001).

IFN-γ is not absolutely required for clearing Pneumocystis from the lungs but is required for modulating the inflammation (Dei-Cas, 2000).

B cells and/or Pneumocystis-specific antibody are important for clearance of Pneumocystis from the lungs. Antibodies against Pneumocystis are reported to be readily detectable in the early years of life, at ages similar to that reported for other pathogenic fungi such as C. albicans and C. neoformans (Goldman et al., 2001; Steele et al., 2005), and most data suggest that the organism is widely encountered in nature and that antibody production is part of the natural host response, predominantly of the IgG class, but also IgM (Furuta et al., 1985; Steele et al., 2005). In this sense, many ELISA-based assays have been developed and employ a wide variety of Pneumocystis preparations and antigens to detect the presence of Pneumocystis-specific antibodies (Steele et al., 2005).

In pigs, the infection is due to the naïve system of young pigs; immunohistochemistry revealed Pneumocystis infection in suckling pigs and a serological study observed an increase in the titre of antibodies to the organism in pigs after weaning, concluding that HI response contributes to the defence against Pneumocystis. A relation has been described with porcine reproductive and respiratory syndrome virus infection, which produces a decrease in the number of CD4+ T cells (Kondo et al., 2000).

In dogs, the disease is usually recognized in young animals, and is thought to be associated with an underlying immunodeficiency. In a study with Cavalier King Charles Spaniels dogs it was concluded that there is a defect in immunity, with a lower concentration of IgG and a higher concentration of IgM compared to the levels in control individuals, and an impaired cell-mediated immunity is possible. The concentration of serum IgA was not abnormal (Watson et al., 2006).

In horses, cases have been described with laboratory evidence of immunosuppression based on low levels of IgG, and IgM deficiency, which could have been secondary to the observed decreased expression of MHC II molecules, leading to reduced B cell stimulation. Congenital diseases such as common variable immune deficiency should also be considered (MacNeill et al., 2003).

It may be that infection by Pneumocystis in domestic animals always needs an immunocompromised situation in the affected organism, as happens in man.

11. Conclusion

We have discussed the response of animal immune systems against fungal infection, citing some examples. Although a considerable body of knowledge has accumulated, there is still much to learn. It is important to avoid generalizations, which can lead to mistakes and confusion; we know that differences exist among the immune systems of different animal species, which can lead to success or failure in the resistance to fungal infection. Generalizations should be avoided also for the fungi-like pathogens; there are many pathogenicity mechanisms that can develop at any given moment, and the mechanisms can be very different between species. The example of canine aspergillosis discussed above illustrates both aspects. Although traditionally they are not considered alterations of immunodepression in affected animals, the deficiencies that are observed in certain breeds of dog for the synthesis of IgA can be important in the susceptibility against disease. Immune depression differs, depending on species, and even among breeds of the same species. There are very few reports of A. fumigatus producing disseminated aspergillosis in dogs, as seen in other species within the genus Aspergillus and in other genera. It is important to understand why A. fumigatus does not usually produce an invasive process in dogs, while it develops disseminated mycoses in many other animal species, and it is the main invasive pathogenic fungus in humans. We need to know if this is related to mechanisms of pathogenicity of the fungus, or to the defensive mechanisms of the dog, or to a mixture of both.

There is an obvious temptation to assume that all of the discoveries made in murine models and confirmed
in humans apply to all animal species. However, as discussed here, differences in the immune system of different animal species could be essential in the fight against specific fungal diseases, and the recently described TLRs serve as a clear example.

In conclusion, further research is needed, and that will require collaboration between clinicians, veterinarians and immunology investigative laboratories. Clinical cases of animal mycosis should be studied in depth taking all immunologic variables into consideration in order to reach an understanding of the pathogen–host interaction for individual animal species with the different fungal species.

References


Steele, C., Shellito, J.D., Kolls, J.K., 2005. Immunity against the

Starkey, R.J., McLoughlin, M.A., 1996. Treatment of renal aspergil-


Wagner, D.K., Sohnle, P.G., 1995. Cutaneous defenses against der-


