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ELDERLY: ARE YOUR DEFENSES READY FOR FUNGAL INFECTIONS?

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The beginning of wisdom is to desire it.

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Elderly: are your defenses ready for fungal infections?

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Abstract

The accrual of life expectancy in developed countries brings new challenges to physicians. Among these is the increased incidence of fungal infections in the elderly, associated with higher mortality and morbidity rates. Fungi are known to cause disease mainly in susceptible hosts, as opportunistic pathogens. This arises the question about which are the age-related changes that makes elderly more vulnerable against fungi. Among other changes due to ageing, immunosenescence of both innate and adaptive immunity seems to create a suitable environment, through decreased cell-mediated immunity and chronic inflammation, for both endemic and opportunistic fungal infections. Age-related immunological impairments may lead to the imbalance between antifungal immunity and fungi tolerance. This may determine that a passive colonization by a fungus proceeds to a virulent phenotype in the elderly. As a paradigm of this, the *Candida* species play a major role in the elderly infections. An accurate clinical diagnosis, difficult due to misleading manifestations, is needed to successfully treat them. Physicians increased awareness about this emerging health problem and outreaching research programs in this field would bring new evidences that will lead to a better understanding of the fine tune between tolerance and resistance to fungi and ultimately will enhance healthy ageing.

Keywords: Ageing, elderly, immunosenescence, cell-mediated immunity, inflammation, fungal diseases, anti-fungal immunity.

Resumo

O aumento da esperança média de vida nos países desenvolvidos traz novos desafios para os profissionais de saúde. Um deles é o aumento da incidência das infeções fúngicas nos idosos, associadas a maiores taxas de mortalidade e de morbilidade. Os fungos são conhecidos por causarem doença, sobretudo, em indivíduos suscetíveis, sendo considerados agentes de infeção oportunista. Daqui surge a questão sobre quais são as alterações relacionadas com o envelhecimento que tornam os idosos mais vulneráveis aos fungos. Entre outras alterações relacionadas com a idade, a imunosenescência tanto da imunidade inata como da imunidade adaptativa parece criar, através da diminuição da imunidade mediada por células e da inflamação crónica, um ambiente favorável para o desenvolvimento de infeções fúngicas endémicas e oportunísticas. O comprometimento imunológico devido ao envelhecimento pode levar ao desiquilíbrio entre a imunidade contra fungos e a tolerância aos mesmos. Nos idosos, este ambiente pode tornar um fungo comensal num fungo virulento e levar ao desenvolvimento de uma infeção, com maior destaque para as infeções por espécies do género *Candida*. Por sua vez, um diagnóstico clínico preciso, por vezes difícil devido às características inespecíficas nos idosos, é necessário para se proceder ao tratamento eficaz destas infeções. A crescente consciencialização dos clínicos e o desenvolvimento de programas de investigação centrados neste problema de saúde emergente poderão trazer novas evidências para melhor perceber a estreita relação de tolerância e resistência aos fungos e, em último caso, a promoção do envelhecimento saudável.

Palavras-chave: envelhecimento, idosos, immunosenescência, imunidade mediada por células, inflamação, infeções fúngicas, imunidade contra fungos.

Abbreviations

AGE	Advanced glycation end products
CD	Cluster of differentiation
CHC	Chronic hyperplasic candidiasis
CLR	C-type lectin receptor
CMI	Cell-mediated immunity
CR	Complement receptor
DCs	Dendritic cells
DHEA	Dehydroepiandrosterone
DM	Diabetes Mellitus
DTH	Delayed-type hypersensitivity
FcγR	Fc-gamma receptor
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IRP	Immune risk profile
mDCs	Myeloid dendritic cells
MHC	Major histocompatibility complex
MR	Mannose receptor
NCR	Natural cytotoxicity receptors
NET	Neutrophil extracellular traps
NK	Natural Killer
NKT	Natural Killer T-cells
PAMP	Pathogen-associated molecular pattern
pDCs	Plasmacytoid dendritic cells
PMN	Polymorphonuclear leukocytes
PRR	Pattern recognition receptor
RAGE	Receptor for advanced glycation end products
ROI	Reactive oxygen intermediates
TCR	T-cell receptor
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Tregs	Regulatory T-cells

Introduction

Fungi are ubiquitous in nature and they can interact with humans by establishing symbiotic, commensal, latent or pathogenic relationships.[1] Although most fungi being considered harmless, any disruption of stable, but-also-dynamic, pathogen-host interaction may lead to fungal disease.[2] These diseases can range from simple cutaneous lesions and acute self-limiting pulmonary manifestations in immunocompetent individuals to severe inflammatory diseases and life-threatening invasive infections in immunocompromised patients.[2] In addition to the implications of immune status in manifestations of fungal infections, its risk factors have been described through the years and can be summarized as: prolonged antibiotic treatment, Diabetes Mellitus (DM), pregnancy, AIDS, solid organ transplant, hematopoietic stem cell transplantation, congenital immunodeficiency and age.[3]

In fact, in the last three decades there are several epidemiological studies reporting the increasing incidence of fungal infections,[4–8] mainly because of increased immunosuppressed individuals[9] and older adults.[3,10,11] Indeed, age is being consecutively linked with fungal infections and with higher morbidity and mortality rates,[12] with most infections being diagnosed in patients over than 65 years.[3] Also, the natural declining of cell-mediated immunity (CMI) that occurs as a result of ageing, known as immunosenescence, can increase the risk of cancer[13] and infections caused by intracellular bacteria, mycobacteria, fungi and virus.[14] The two current hallmarks of immunosenescence are immune risk profile (IRP) and inflamm-ageing found in healthy aged adults.[15] Firstly, IRP is characterized by inverted $CD4^+/CD8^+$ ratio, increased dysfunction of memory and effectors T-cells, and by depletion of naïve cells, which are able to recognize and combat new antigens.[16] Secondly, inflamm-ageing results in a pro-inflammatory status with high levels of IL-6, IL-1β, IL-18, IL-8, IL-12 and TNFα, contributing to deregulation of cell-mediated immunity and to decreased ability to prevent damage associated with inflammation.[17]

This reveals the inappropriate immunological response to pathogens associated with advanced age, in particularly against fungi, since they affect predominantly patients with deregulated immunity. Even the age-related comorbidities, as DM,[14] renal failure[3] and malnutrition[18] may have some role in modulating immune system during the ageing process, being associated with increasing opportunistic fungal infections, with *Candida* species getting the main role.[19] For example, risk factors for fatal candidemia caused by *Candida albicans* are old age itself, procedures related with intensive care and an acute sepsis.[20] Immunological impairment may also put healthy older adults at risk of endemic mycoses, since now they are more likely to travel and to participate in outdoor activities, being exposed to pathogens without prior exposure, and consecutively at risk of a primary infection.[19] *Coccidioides immitis* infection can be regarded as the paradigm of this, since chronic medical conditions and exposures were independently associated among elderly.[21]

The fungal infections in elderly have increased interest not only because they are more frequent and more severe, but also because the manifestations and symptoms are often different from those of younger patients, turning the diagnosis more difficult.[12] This becomes even more important when it's noticed that elderly population has been growing in developed countries and in the next forty years the number of people older than sixty five years in the world will exceed the number of young people for the first time in history.[13] This has huge implications in health care systems and in the way physicians and other health professionals handle this new, but expected, reality.

The aim of this review is to examine the impact of immunosenescence in host's response against fungal pathogens and its clinical consequences in the elderly. The focus will be the major alterations in immune system due to ageing process and its correlation with brand new breakthroughs about immunity against fungi, and then the major clinical consequences of these infections in the elderly.

Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published from January, 1995, to November, 2012, by use of the terms ”fungal infections”, “elderly”, “immunosenescence”, inflamm-ageing”, “fungal immunity”, “phagocytosis”, “neutrophils”, ”macrophages”, “T-cells”, “B-cells”, “Th17” and “Tregs”. Were included articles published in English, Spanish and Portuguese. Articles resulting from these search and relevant references cited in those articles were reviewed.

Is Immunosenescence the elderly's fate?

Although ageing cannot be considered a disease *per se*, it is associated with decreasing of total physiological reserves and with less likelihood to be sufficient to maintain and repair the ageing body. This leads to imbalanced reactive oxygen intermediates (ROI) metabolism that also affects immune system and critically alter immune mechanisms, summarized in table I.[15] However, the exact causes of immunosenescence are not clear and its multifactorial nature is being accepted. Age-related processes like thymic involution, chronic antigenic stimulation, signal transduction changes, endocrinosenescence and protein-energy malnutrition can be related to immunosenescence causes.[15,22] The deterioration of immune system involves innate, adaptive, and humoral immunity, all necessary to successfully overcome infection.

Table I: Age-related oxidative stress and immunosenescence

Biochemical mechanisms[23]	**Dysfunctions in immune system**
Decreased cellular functions due to oxidative damage	Phagocytosis, due to change and damage of membrane composition[24]
Apoptosis following oxidized molecular aggregates accumulation	Effectiveness of oxidative burst in ageing T-cells [25]
Decreased levels of enzymes with antioxidant activity	Pathogen processing ability of antigen presenting cells[26]
Impairment of proteasome degradative functions	Cellular ability to process and present MHC class II restricted antigens[27]
	TCR and B-cell receptor deregulation.[28,29]

MHC: major histocompatibility complex; TCR: T-cell receptor.

Innate immune system

Innate immune system is the first line of organism's defense against pathogens, manly bacteria and fungi, and comprises the earliest response to pathogens. These are less specific and lack immunological memory. Innate immunity is composed by cells [polymorphonuclear leukocytes (PMN), macrophages/monocytes, dendritic cells (DCs), natural killer (NK) and natural killer T (NKT) cells] and by soluble factors, as pro-inflammatory cytokines and

interferons (IFN).[30] Innate immunosenescence is more related to functional changes than to changes in cellular numbers, but there is still debate about its alterations. The main changes due to innate immunosenescence are summarized in figure 1.

In what regards cellular numbers, there are some studies that support (i) the preserved number of neutrophils through ageing in healthy elderly,[31] (ii) conserved overall number of macrophages/monocytes with increased $CD16^+$ monocytes subpopulation, considered pro-inflammatory,[32] (iii) decreased myeloid DC (mDCs) and $CD34^+$ precursors, most important antigen presenting cells to T-cells, and preserved plasmacytoid DC (pDCs),[33] and (iv) increased number of NK cells, mainly because of $CD56dim57^+$ NK cells accumulation, with poor responsiveness to cytokine stimulation.[34]

Concerning to functional alterations, cellular components of innate immune system display several and complex changes. With ageing, neutrophils show reduced chemotaxis, phagocytosis and intracellular killing (due to impaired oxidative burst[35] and deficient reactive nitrogen intermediates), and decreased production of neutrophil extracellular traps (NETs).[36] There are also some data that support that pattern recognition receptors (PRRs) may be functionally altered, like Toll-like receptors (TLR) implicated in macroautophagy (TLR4 and TLR7), needed to clear the pathogens.[37] These alterations seem related to impairment of intracellular signaling pathways and to membrane composition, and not to changes in number of receptors.[38]

Monocytes/macrophages seem equally to have reduced phagocytosis and free radical production in healthy older adults, as much as decreased capacity of antigen presentation due higher production of prostaglandin E2, which inhibits MHC class II expression and IL-12 production.[36] Also, expression and function of some TLRs seem altered with age,[39] such as increased expression of TLR1 and TLR2, decreased expression of TLR4 and TLR8, and TLR signaling pathways impairment, leading to activation of pro-inflammatory cytokines, as IL-6,

IL-8, IL-1β and TNFα.[15] Furthermore, macrophages' apoptotic cell death is induced by advanced glycation end products (AGE) found in plasma and tissue of diabetic and uremic subjects.[40]

Functional alterations in DCs are more prominent in frail elderly. mDCs have decreased endocytosis, chemotaxis, TLR-mediated signaling, production of IL-12 and ability to activate naïve $CD4^+$ T-cells via antigen presentation.[36] However, this cells subtype also contributes to inflamm-ageing by its constant activation status with increased IL-6 and TNFα production without stimulation.[41] pDCs have decreased capacity to secrete IFN-I via TLR stimulation (e.g. TLR7 and TLR9) and IFN-III, and also a reduced capacity to present antigens to $CD4^+$ and $CD8^+$ T-cells.[42] However, pDCs maintain their capacity to secrete pro-inflammatory cytokines, to activate $CD8^+$ T-cells and to stimulate IL-17 production.[43]

In NK cells, the decreased expression of natural cytotoxicity receptors (NCRs) can be responsible for decreased per cell cytotoxicity, since they are involved in NK cell recognition and killing target cells. The production of chemokines by NK cells is also decreased in response to IL-2 or IL-12.[36]

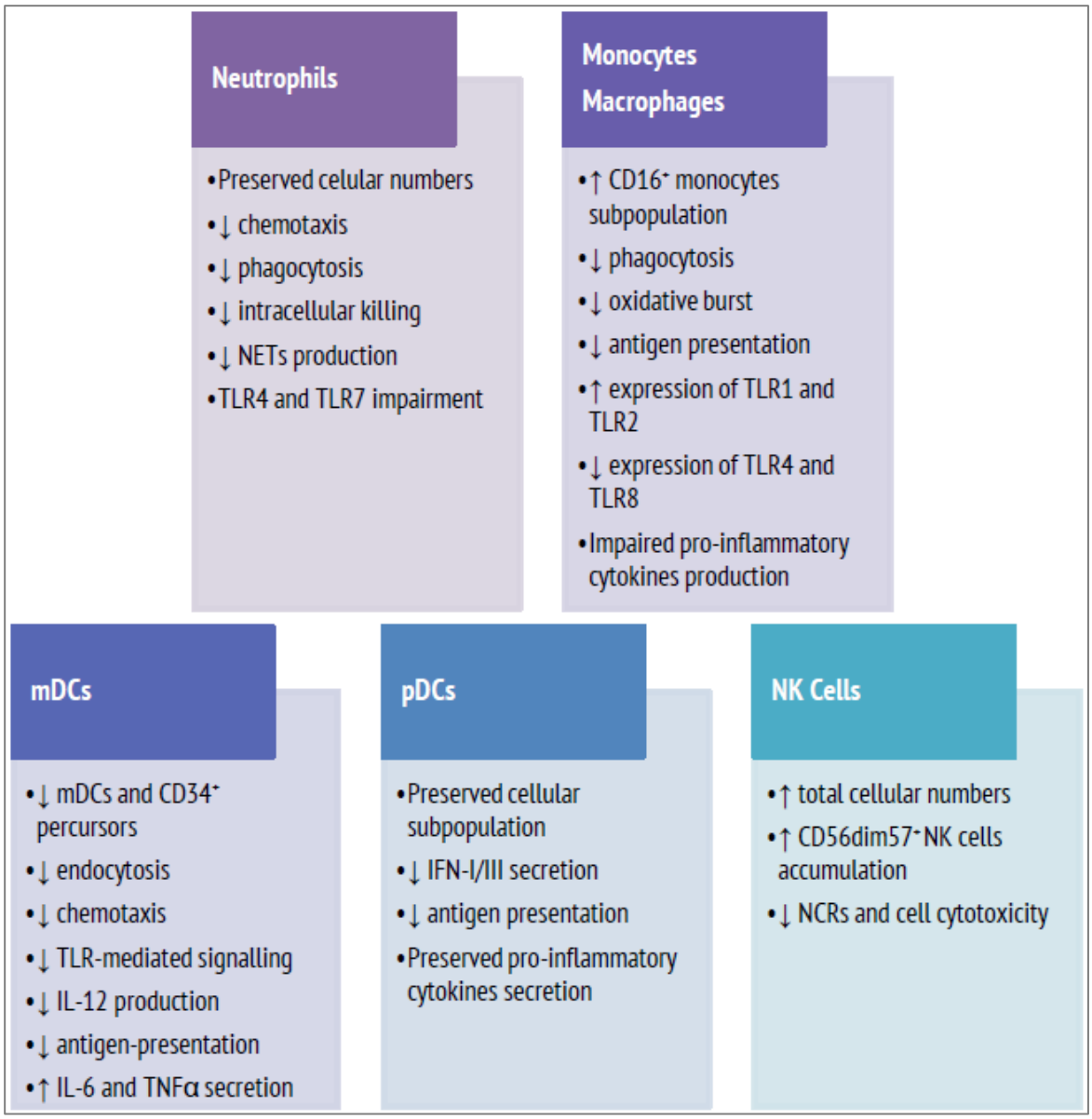

Figure 1: **Innate immunosenescence**
Main changes in cellular numbers and cellular function of innate immunity due to ageing process.

Adaptive immune system

Adaptive immune system is activated by innate immunity and is characterized by later, but specific, response against pathogens, maximizing their elimination. This system produces immunological memory through memory cells, providing fast and effective responses in further infections. Adaptive immunity is composed by lymphocytes, namely T and B-cells and antibodies production. Major alterations in this system are mainly related to decrease of

numbers and diversity of both naïve T and B-cells, with clonal expansion of memory and effector specific cells.[44] This can result in decreased responsiveness to new antigens and attenuated delayed-type hypersensitivity (DTH),[45] leading the organism more susceptible against new pathogens.

Number of naïve T-cells existing in the thymus is dramatically reduced with aged (Figure 2), mainly because of thymic involution, with major decline of their diversity after the age of 70.[46] Functionally, naïve T-cells also exhibit age-related changes such as shorter telomeres, restricted T-cell receptor repertoire, reduced IL-2 production and impaired expansion and differentiation into effector T-cells.[47] Related to cytotoxic T-cells ($CD8^+$), it has been shown the accumulation of $CD8^+CD28^-$ T-cells, mostly CMV-specific, with defects in antigen-presenting DC by CD80/CD86 costimulatory ligands for CD28.[48] T helper cells ($CD4^+$) also have age-related modifications through the emergence of $CD4^+CD28^-$ T-cells, with incompetence towards nominal exogenous antigens and the acquisition of autoreactivity, through restricted TCR repertoire.[49] Moreover, the proliferation of B-cells and humoral responsiveness is impaired, since the interaction between senescent $CD4^+$ T-cells and B-cells is disturbed due to reduced expression of CD154 (CD40Ligand), as well as defects in antigen presentation induced by CD80/CD86, as already shown.[50] However, the lack of CD28 in $CD8^+$ T-cells and in $CD4^+$ T-cells is being associated with the capacity to produce pro-inflammatory cytokines.[15,49]

Furthermore, other very important age-related changes in adaptive immunity are the alterations in T helper (T_h) cells response. Although, there is an intense debate about the effects of ageing in T_h1, T_h2 and T_h17 cellular numbers and function, and data is not consensual.[51] Increased T_h2/T_h1 ratio has been described in older adults, with reduced Th1 responses, through IL-2 and IFNγ, and predominant T_h2 response, characterized by increased IL-4, IL-6 and IL-10 cytokines' profile, leading to impairment of cell-mediated immunity and

to phagocytosis.[17,52] This ratio can also be caused by ageing of endocrine system, with increased cortisol and decreased dehydroepiandrosterone (DHEA).[22] This latter was also mainly linked with elderly women, due to decreased sexual hormones.[53] Concerning to the T_h17 cells, these display an important role in infection's management and seem to suffer with ageing too. Elderly seems to present decreased frequency of memory T_h17 cells and an increased differentiation of effector T_h17 cells from naïve $CD4^+$ T-cells, when compared with youngsters.[54] This may be due to the increased T_h17 promoting factors, such as IL-6.[51] However, circulating IL-17 is reduced with normal ageing.[51] Even the regulatory T-cells (T_{regs}) seem to be altered, by accumulating in older individuals through increased peripheral survival.[55] The accrual of T_{regs} in elderly are believed to inhibit DCs and effector T-cells, these latter through suppression of anti-inflammatory IL-10, probably leading to higher mortality and morbidity.[56]

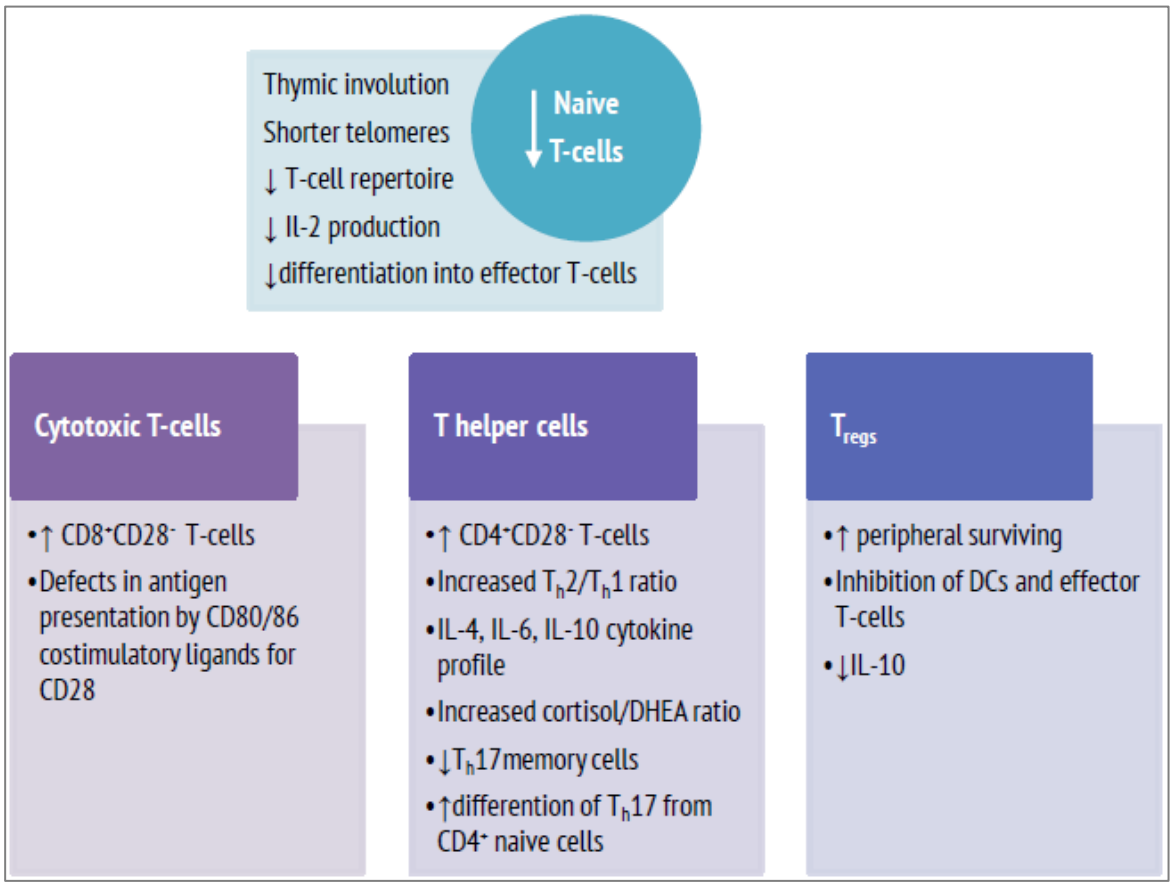

Figure 2: **T-cells immunosenescence**
Main changes in naïve T-cells and in T-cells subsets and functions due to ageing process.

Ageing also affects humoral immunity and consequently B-cells (Figure 3), the producers of antibodies, with a strong correlation to humoral abnormalities such as hypergammaglobulinema, autoantibody production, autoimmune and lymphoproliferative diseases.[57] With ageing, naïve B-cells are decreased due to reduced lymphopoiesis, and B-cell population becomes increasingly dominated by antigen-experienced cells, specific to environmental and autoantigens.[58] Furthermore, both the overall number of B-cells (even including some memory subsets) and peripheral B-cells repertoire appear to be diminished, with the latter being related to reduced health status.[44] Ageing-associated B-cells are being reported too, responding to innate but not to adaptive stimuli, producing cytokines, potentiating Th17 polarization and secreting autoantibodies.[59] Functionally, primary immune responses in elderly people develop more slowly, and are less protective than in younger people. During recall responses, antibodies production is diminished and has poorer functional quality.[44] Related to serum immunoglobulins, only IgG4 can be found decreased. Other IgG subclasses and IgA levels show a significant increase during the ageing process, probably conferring greater protection against viral and bacterial infections.[57]

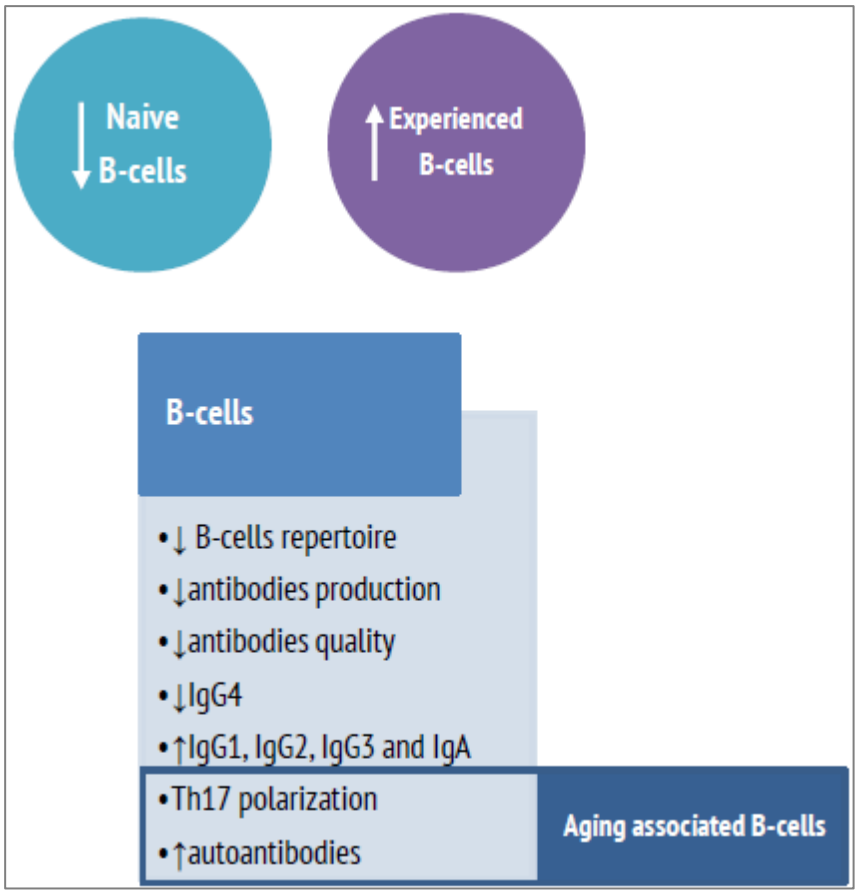

Figure 3: **B-cells and humoral immunosenescence**
Main changes in cellular B-cells population and in immunoglobulins production due to ageing process.

Immunosenescence: suitable environment to fungal infections?

The relation between fungi and humans is very intriguing since their interaction is mainly beneficial to both, and diseases are rarely developed. In fact, many fungi have co-evolved with humans and other mammals through millions of years and L. Romani recently suggested that this led to complex mechanisms of host's immune surveillance and to sophisticated fungal strategies to antagonize and evade immunity.[1] Immune system can also recognize commensal fungi and due to a tight balance between pro and anti-inflammatory responses, it maintains a stable homeostasis between host and fungi. This relation is preserved through the two-component anti-fungal response: resistance (the ability to limit fungal burden) and tolerance (the ability to limit the host damage caused by immune response).[60] This is important because, by controlling each other to avoid potential harmful inflammatory responses, fungi and host mutual relationship can yield benefits to both.[61] Any disruption to this homeostasis can lead to a parasitic relationship, resulting in disease. Besides, the impairment of host defenses influences the manifestation and the severity of fungal infections (Figure 4).[62]

Within the following section some important issues related to immunity against fungi will be discussed together with its correlation with immunosenescence, with the purpose to elucidate how age-related immune dysfunction is connected with elderly's increased susceptibility to fungal diseases.

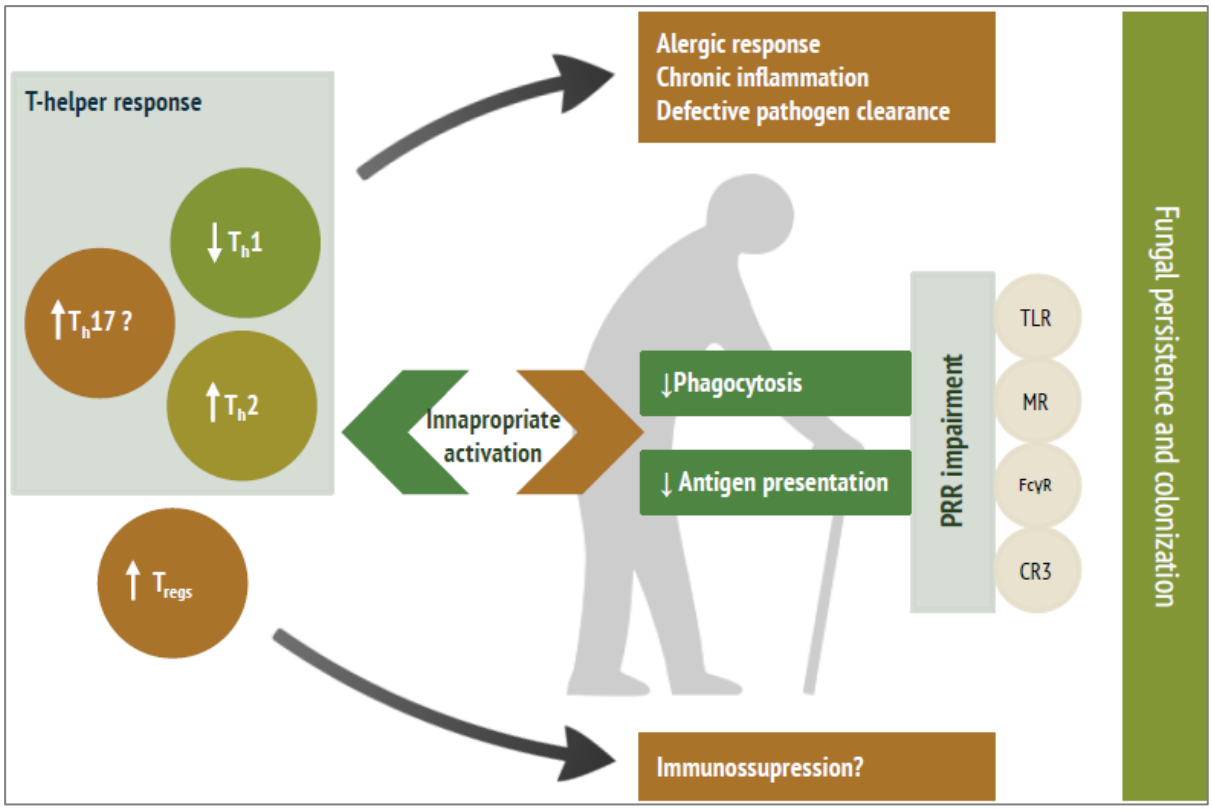

Figure 4: **Immunosenescence implications in antifungal immunity**
The age-related changes in receptors of innate immunity (PRRs) and the decreased function of innate cells, with decreased phagocytosis and antigen presentation, leads to inappropriate activation of adaptive immunity, needed to successfully resolve the infection. In its turn, adaptive immunity is also altered with age, leading to inappropriate activation of innate immunity to clear the pathogen. This global impairment of immune system, due to the ageing process, result in a suitable environment for fungal persistence and colonization and consequently, fungal diseases.

Phagocytosis

Phagocytosis, performed mainly by innate immune cells, neutrophils and macrophages, has a central role during fungal infections by engulfing the pathogen, destructing it through oxidative burst and initiating adaptive immune response by cytokines production. Innate immunity mechanisms are most present at sites of continuous exposure to fungi, such as skin and mucosal epithelial cell surfaces of respiratory, gastrointestinal and genitourinary tracts.[1] Like discussed earlier, the phagocytic efficiency decreases through ageing, probably predisposing the host to fungal diseases. It is described that reduced ability of neutrophils from aged-adults to kill *C. albicans* is mainly due to ineffective intracellular killing.[45] Also,

the age-impaired neutrophils activation and phenotypic modulation facilitates the establishment and exacerbation of *Candida* infections.[63]

Pattern Recognition Receptors (PRRs)

Fungal phagocytosis is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) through PRRs such as TLRs, C-type lectin receptors (CLRs) and galactin family proteins.[64] Fungal cell wall components, such as β-glucans, chitin and mannans, are the main source of PAMPs that are recognized by PRRs, which can be stimulated in different combinations, depending on the fungus and on the host cell types.[2] Related to TLRs, their individual activation may depend on fungus and fungal morphotypes itself, route of infection and receptor cooperativity. Also, TLRs have a major role in controlling pathogen antigen processing and presentation during phagocytosis.[65] The principal TLRs involved in sensing fungal components are TLR2, TLR4 and TLR9 and it is demonstrated that deficiencies in these are associated with some fungal diseases.[1] Chronic hyperplasic candidiasis (CHC) is accompanied by low levels of TLR2 expression,[66] as well as polymorphisms in TLR4 increase the susceptibility to pulmonary aspergillosis and bloodstream candidiasis.[1] It was already shown that TLRs functions and cellular expression have some age-related changes and, due to their importance to fungi recognition and clearance, this could constitute a way for fungi to take advantage in immune system.

Gasparoto et al.[67] described low expressing TLR2 neutrophils in blood and low expressing TLR4 neutrophils in saliva of elderly individuals. TLR2 can be downregulated by cytokines, such as IL-4 and IL-6, which were elevated in the elderly group. This is, in part, consistent with the described innate immunosenescence alterations, especially the downregulation of TLR4 in neutrophils and macrophages.

Age-related TLRs impairment can also result from biological activity of receptor for advanced glycation end-products (RAGE), since it has been described to play a relevant role

in innate immunity and in chronic inflammation diseases.[2] TLRs and RAGE signal integration can constitute a mechanism which discriminates between fungi and danger-induced immune responses, modulating inflammation.[68] In fact, RAGE activation through danger molecules, can inhibit TLRs (with bigger focus on TLR2 and TLR3) leading to a presumable innate response impairment, due to the implications of these receptors in fungal immunity. This becomes more important when it is noticed that RAGE expression is increased with ageing, as much as RAGE can accelerate ageing by interacting with AGEs accumulated through life.[69] AGEs, the products of nonenzymatic glycation and oxidation of proteins and lipids, are formed in varied settings common in aged individuals, such as DM, inflammation and neurodegeneration.[70] This RAGE upregulation is connected with perpetuated pro-inflammatory phenotype in elderly and consecutively with shorten lifespan.[69]

CLRs have a main role in fungal recognition and in initiation of both innate and adaptive immunity. For example, dectin-2 pairs with FcγR to produce pro-inflammatory cytokines when it identifies high-mannose structures, such as fungal hyphae cell walls.[71] Also, mannose receptor (MR) is involved in the phagocytosis of *C. albicans* and promotion of antifungal Th17 response through DCs antigen presentation.[1] Thus, ageing may affect CLRs function, through decreased expression of Fc-gamma receptor I (FcγRI), Fc-gamma receptor III (FcγRI) and mannose receptor (CD206).[67]

Antigen-Presenting Cells

DCs are specialized cells in antigen processing and presentation, becoming intermediaries between innate and adaptive immune systems, playing a major role in fungal diseases by differentiating commensal fungi from pathogenic ones. Recognition of pathogens by DCs includes receptors for several components of the complement system (CR), FcγR and PRRs, like MR,[62] and its differential activation will initiate different adaptive anti-fungal immune responses. This gives to DCs the capacity to distinguish between different fungi morphotypes,

a switch that is essential to maintain infection.[72] For example, phagocytosis of *C. albicans* hyphae involve FcγR and CR3, resulting in free hyphae in cytoplasm. In other way, internalization of *Candida* yeast cells mediated through MR will successfully clear the pathogen. However, if this process is mediated by CR3, single yeast cells can survive inside DCs, modeling the immune response.[73]

Despite of this, two main DCs subsets can be identified: inflammatory DCs initiate antifungal T_h17 and T_h2 responses; tolerogenic DCs activates T_h1 and T_{reg} cells.[74] This dichotomic system enables the host to differentiate between commensalism and infection.

Thus, senescent DCs, characterized by decreased antigen presentation, TLR-mediated signaling, chemotaxis and endocytosis, may have an important role in the increased susceptibility to fungal diseases in elderly, since the induction of adaptive immunity seems deregulated, allowing fungi to evade and survive to immune surveillance.

T-cells

In one hand, protective immunity against fungi is provided by T_h1 cells response, characterized by IFN-γ and TNFα secretion and promotion of opsonizing antibodies production, achieving optimal activation of phagocytes.[75] On the other hand, activated T_h2 cells attenuate T_h1 cells response through IL-4 production, and promote an alternative pathway of macrophages activation, fostering fungal infections, fungus-associated allergic responses and disease relapse.[1] Also, elevated IL-4 levels were suggested to compromise immune responses to *Candida* in recurrent vulvovaginal candidiasis since IL-4 blocks macrophage-mediated anti-*Candida* responses.[76] However, it seems that T_h1 inhibitory cytokines are needed to maintain an already established T_h1 reactivity against the fungus.[77]

Therefore, the age-related increased T_h2/T_h1 ratio can have a major role in development of fungal diseases in elderly, predisposing the organism to fungal persistence and colonization.

Albeit this has been the classical involvement of T_h-cells in fungal immunity, the interest in T_h17 and Tregs functions is growing. T_h17 cells are present in anti-fungal specific T-cell memory repertoire and its pathway has an important regulatory function, by promoting T_h1 and restraining T_h2 responses.[1] However, T_h17 pathway is associated with defective pathogen clearance, failure to resolve inflammation and to initiate protective immune responses, prevailing over the T_h1 pathway.[78] Since T_h17 memory cells are reduced in elderly and its differentiation from naïve $CD4^+$ T-cells is increased, this may mean that recognition of fungi already exposed to immune system can lead to T_h17 differentiation and increased production of IL-17, resulting in pathological inflammation. Even more, fungi have the ability to activate T_h17 cells and to subvert host's inflammatory response.[78] Also, genetic diseases that result in susceptibility to candida show impaired Th17 cell function.[79] IL-17 has been implicated in the progression of localized chronic infections as in serious systemic pathologies such as DM, chronic obstructive pulmonary disease, and cardiovascular diseases.[80]

T_{regs} are capable of fine-tuning protective anti-microbial immunity in order to minimize harmful immune pathology, by inhibiting aspects of innate and adaptive antifungal immunity, including functional T_h17, resulting in protective tolerance to fungi.[78] Furthermore, fungal growth, inflammatory immunity and tolerance to *C. albicans* and *Aspergillus fumigatus* were controlled in animal models by T_{regs} which have limited the early inflammation at sites of infection and have regulated the expression of T helper immunity in secondary lymphoid organs.[81,82] However, T_{regs} responses may limit the efficacy of protective immune responses, resulting in reduced damaged to the host but also fungal persistence and, eventually, immunosuppression.[83] When it is verified that with ageing regulatory T-cells population increase, inhibiting effector T-cells and DCs, it can be concluded that these changes can lead to a marked immunosuppression, aggravating the age-related immunological impairment and the development of fungal infections.

Inflammation

Clinical evidence indicate that fungal diseases also occur in the setting of heightened inflammatory response, in which immunity occurs at the expense of host damage and pathogen eradication.[84] For example, IL-17A resulted in increased adhesion and filamentous growth, by inducing artificial nutrient starvation conditions in *C. albicans* and *A. fumigatus*, corresponding in a dramatic increment of biofilm formation and fungal virulence.[85] The inflammatory response to fungi may serve to limit infection, but an heightened inflammatory response may contribute not only to chronic diseases and autoimmunity but also to fungal virulence.[78] Also, increased innate inflammatory response may, paradoxically, predispose to fungal infections or deregulated immune responses against fungi.[2]

Hereupon, immunosenescence is characterized by the accrual of pro-inflammatory cytokines, not only from the innate immunity cells but also from adaptive, resulting in a pro-inflammatory environment described as ‘‘inflamm-ageing’’. Thus, ‘‘inflamm-ageing’’ may be considered as another mechanism that increases elderly susceptibility to fungal infections.

Which are the clinical consequences?

The immunological environment provided by immunosenescence, characterized by impaired cell-mediated immunity and chronic inflammation, plus physiological and anatomical changes in aged-adults result in specific spectrum of pathogens as well as different manifestations and symptoms of infection from those of younger patients (Figure5).[12] This must aware physicians to diseases that are not accustomed to diagnose and successfully treat, such as arising endemic mycoses and opportunistic fungal infections.

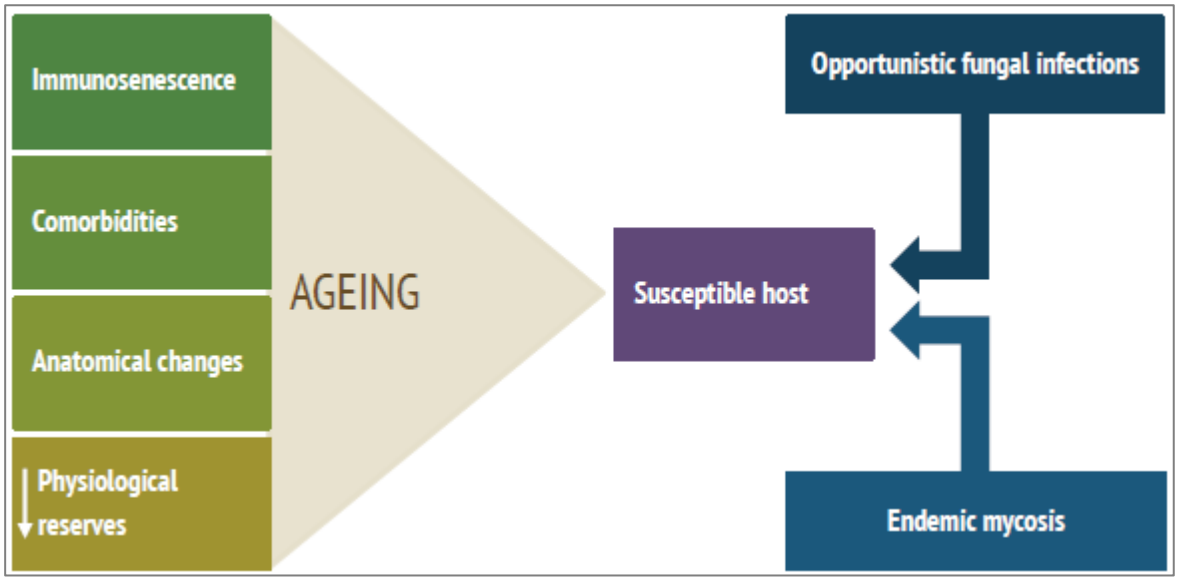

Figure 5: **Elderly susceptibility to fungal infections**
Age-related comorbidities, anatomical changes and decreased physiological reserves along with immunosenescence may turn the aged adults more susceptible to both endemic and opportunistic fungal infections, with worse outcomes.

Endemic mycoses

The main endemic mycoses in elderly are coccidioidomycosis, blastomycosis and, most importantly, histoplasmosis. Also, chronic obstructive pulmonary disease was the most common underlying disease for these endemic mycosis, with histoplasmosis getting the main role.[11] Kauffman[19] described histoplasmosis as having several manifestations of infection that are almost entirely found in elderly, probably due to the reactivation of a latent infection

acquired years before. Chronic progressive disseminated histoplasmosis seems to be more related to selective defect in cell-mediated immunity to *H. capsulatum* then to overtly immunosuppression, being consistent with age-related impairment of immune system. This disease is characterized by overwhelming parasitation of the reticuloendothelial system, with macrophages containing large numbers of yeasts. Clinically, older patients have fever, night sweats, anorexia, weight loss, fatigue and may develop Addison's disease due to diffuse adrenal involvement.

In spite of coccidioidomycosis symptoms have no differences between aged and younger patients, the immunosenescence and the comorbidities, as DM, are risk factors for more severe pulmonary coccidioidomycosis in aged adults.[19] In the same way, no clinical differences are noted between younger and older patients with blastomycosis.[19]

Opportunistic fungal infections

Opportunistic fungal infections may be due either to exogenous or endogenous fungi. Colonization by fungi constitutes a risk factor for many fungal infections, since when the host becomes vulnerable, they can take advantage of it, resulting in proliferation and invasion of tissues.[86] Concerning to cutaneous infections, ageing is one of the most predisposing conditions to develop mycotic nails. Thus, onychomycosis prevalence is high in elderly patients mainly due to dermatophytes, such as *Trichophyton rubrum*.[87] Infected fingernails if are not treated can serve as sources for satellite infections and lead to physical impairment and pain due to secondary bacterial infections and cellulitis.[12] Also, *Candida* species infection appeared to be the main cause of diaper dermatitis in the elderly hospitalized patients, some of them associated with initial irritant dermatitis.[88]

About oropharyngeal diseases, oral candidiasis takes the leading role by constituting the most common opportunistic oral infection in humans, especially in aged adults. This may be due to dentures usage and age-related reduced levels of anti-candidal agents and saliva flow, causing

the higher colonization by fungi and other microorganisms.[89] Concerning to digestive diseases, candida overgrowth and gut flora imbalance may have a major role. Candida esophagitis seems to rarely present the classical clinical symptoms, such as dysphagia or epigastric discomfort, being more associated with unexplained anemia, anorexia and malnutrition. Furthermore, candida esophagitis were also considered a marker of poor survival in older patients irrespective of their functional status.[18] The same was noticed in patients over 85 years with candiduria, principally linked to advanced age and frailty than to the impact of *Candida* species infection.[90]

Adults over than 65 years are also more susceptible to candidemia, since *Candida albicans* is the most common fungal agent in nosocomial invasive fungal infections.[20] Candidemia is not only associated with mortality rates around 30% to 40% but also with extended hospitalization rates.[91] These patients usually present acute septic syndrome, characterized by fever, chills, pustular skin lesions, and signals suggesting sepsis, indistinguishable from bacteremia.[19] Sometimes, more indolent candidemia is the cause of fever of unknown origin.[20] Candidemia by *C. glabrata* is more often seen in older patients (≥ 60 years) and is associated with a higher mortality rate, may be due to lower susceptibility to azole antifungals.[92] Furthermore, elderly patients under antibiotic treatment, especially broad-spectrum antibiotics as imipenem and cefoperazone-sulbactam, were a high-risk population for invasive fungal infections.[10] Although rare, aspergillosis and cryptococcosis may also occur more frequently in older persons, with higher mortality.[12] Besides this, emerging opportunistic fungal infections caused by unusual yeasts have been described in immunosuppressed individuals and may constitute a problem in the frail elderly. Fungi as *Saccharomyces*, *Geotrichum*, *Hansenula* and *Trichosporon* have been identified, with their diagnosis being difficult and treatment suboptimal.[93]

Treatment in the elderly

Treatment of fungal infections in the elderly is also compromised due to changes that occur during ageing process. Besides the reduced physiologic reserves, also the higher prevalence of chronic diseases and the use of multiple medications result in lower response rates and/or more adverse drug effects than in younger individuals.[94] Firstly, azoles are one of the most prescribed anti-fungals and can lead to potentially dangerous drug interactions, especially itraconazole and ketoconazole, due to the interaction with cytochrome P450 system, mainly by inhibiting CYP3A4.[95] Also, the wide-range use of azoles may lead to the higher prevalence of azole-resistant species, such as *C. glabrata* and *C. krusei*, turning the successful treatment more difficult.[20] Amphotericin B has been the gold standard therapy for invasive fungal infections management, but is highly associated with nephrotoxicity, mostly in the aged adults. However, the lipid formulations can overcome these adverse reactions.[95] Also, echinocandins seem efficacious and well tolerated in elderly patients.[94]

Conclusion

This review attempted to answer the initial question if elderly's defenses are ready for fungal infections. Along with three more questions, was described the state of the art about imunosenescence, anti-fungal immunity and fungal diseases in the elderly. The aim of those questions was not to provide absolute answers but rather offer the reader the most important data and share some considerations about this topic.

Along with all the other physiological, anatomical and psychological alterations, the immunosenescence is a consequence of ageing that can lead itself to aggravation of other elderly's diseases. The decreased cell-mediated immunity and the chronic inflammatory environment characteristic of ageing appear to provide conditions that favor the development and the establishment of fungal infections. Indeed, fungal diseases represent an important paradigm in immunology, since they can result from either the lack of recognition or over-activation of the inflammatory response.[2] Firstly, the lack of recognition of fungi in aged adults may be due to alterations in PRR expression and activation in phagocytes and DCs. Secondly, over-activation of inflammatory response may result from the secretion of pro-inflammatory cytokines of innate cells and T-cells subsets, especially T_h2 and T_h17. Furthermore, the increased population of T_{regs} in the elderly may lead to a locally immunosuppression, by inhibiting DCs and effector T-cells, which contributes to persistence of pathological fungi in the tissues. Thus, these immunological flaws along with the age-related comorbidities appear to be the reason why fungal diseases are increasing among the elderly, some of them with different clinical manifestations and outcomes.

But one question remains: Is it possible to ameliorate anti-fungal immune response in the elderly? Simpson et al[96] reviewed that habitual exercise is capable of regulating the immune system and delaying the onset of immunosenescence, by reducing senescent T-cells, increasing T-cells proliferation, lowering the levels of inflammatory cytokines and increasing

neutrophil phagocytic activity. Along with correct nutritional and hygienic measures, the elderly might avoid and solve the fungal diseases more effectively.

To finish, more research about anti-fungal immunity in the elderly is needed to better understand these mechanisms, as well as to develop improved approaches to fungal diseases in the aged group. Also a personalized and a holistic approach to fungal diseases in elderly are necessary to successfully reconstitute healthy status.

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