# THE MOLECULAR GENETICS OF CHRONIC GRANULOMATOUS DISEASE

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Phagocytes, such as macrophages and granulocytes, contain a membrane-associated NADPH oxidase that produces superoxide and other reactive oxygen intermediates responsible for microbicidal, tumoricidal, and inflammatory activities. Defects in oxidase activity in chronic granulomatous disease (CGD) lead to severe, life-threatening infections that demonstrate the prime importance of the oxygen-dependent microbicidal system in host defense. The estimate incidence of this disease is 1/250.000 live births per year. The molecular defects causing CGD are generally due to the absence, low expression or malfunctioning of one of the NADPH oxidase components. The X-linked form of the disease is caused by defects in the heavy chain of the cytochrome $b_{588}$ component (gp91-phox) and accounts 56% of the cases. The autosomal recessive forms are caused by defects in one of the cytosolic components of the NADPH oxidase (p47-phox or p67-phox, respectively 33% and 5% of the cases); or even the cytochrome $b_{588}$ light chain component (p22-phox , 6% of the cases) to date, no CGD patients have been reported with defects in p40-phox, rap1A, rac1, or GDI components. The diversity of these mutations and the multiple affected genes give an explanation for the clinical and genetic heterogeneity of CGD. Our aim is to show the current investigation about the molecular genetics of CGD patients in Brazil and Latin America in addition to new findings about the regulation of the NADPH oxidase by transcription factors and their connection with other immunodeficiencies.

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# ROLE OF PHAGOCYTIC CELLS IN PARACOCCIDIOIDOMYCOSIS

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Paracoccidioidomycosis is a deep mycosis caused by *Paracoccidioides brasiliensis*. Phagocytic cells play a critical role against this fungus with several papers showing macrophages and monocytes antifungal effects. However, despite neutrophils represent the first cells to migrate toward inflamed tissue, works focusing their antifungal functions are scarcer. These findings stimulated us to contribute for the better understanding of human neutrophils role in early infection stages, by studying their capacity to kill *P brasiliensis*. Nonactivated cells failed to exhibit antifungal activity. However, if they were IFN-$\gamma$, TNF-$\alpha$ or GM-CSF-activated, a significative fungicidal activity was detected. Killing process was inhibited in presence of catalase and superoxide dismutase showing the role of $H_2O_2$ and superoxide anion as effector molecules. Our results indicated that an activation process is essential for neutrophil antifungal activity. Therefore, we asked if IL-10, a suppressor cytokine, would have the capacity to deactivate these cells. This cytokine significantly inhibited *P. brasiliensis* killing by IFN-$\gamma$-activated neutrophils, reducing their capacity to release $H_2O_2$. The results showed the importance of early neutrophils exposure to activator or suppressor cytokines, for *P. brasiliensis* killing modulation. However, one question arises in relation to the cellular source of these cytokines in first stages of infection. It has been proposed that NK cells might be the potential source of IFN-$\gamma$ and GM-CSF. TNF-$\alpha$ and IL-10 production might occur by macrophages or even neutrophils. Works in our laboratory have been demonstrated that patient's monocytes produce high levels of TNF-$\alpha$ and IL-10. These cells also release these cytokines after challenge with fungus *in vitro.* These findings suggest that *in vivo* the potential sources for TNF-$\alpha$ and IL-10 might be alveolar macrophages activated with *P. brasiliensis* antigens.

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# INNATE IMMUNITY AND ORGAN TRANSPLANTATION: THE POTENCIAL ROLE OF TOLL-LIKE RECEPTORS

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Traditionally, the recognition and tolerance of transplanted grafts has been considered to be within the realm of the adaptive immune system. Innate immunity, on the other hand, as the first line of host defense, plays a role in fighting against invading microorganisms. Recently, with the discovery of the Toll-like receptors (TLRs), the role of innate immune responses in the control of adaptive immunity has become a new area of interest. Emerging evidence suggests that in addition to responding to pathogen-associated molecular patterns of microorganisms, TLRs can be activated by endogenous ligands, expressed by mammalian cells. These 'danger signals' may participate in ischemia-reperfusion related organ damage and subsequently influence function and survival of transplanted grafts. Furthermore, it has been suggested that adaptive immune responses can enhance the acute inflammatory responses controlled by innate immunity in organ transplantation. This review addresses the potential involvement of TLRs in different stages of organ transplantation. Intriguing and controversial findings are presented and discussed in order to stimulate more attention to this emerging and potentially important area of research in organ transplantation.

**KEY WORDS:** adaptive immunity, danger signals, ischemia-reperfusion, rejection

**FINANCIAL SUPPORT:** Ontario Thoracic Society, the Canadian Cystic Fibrosis Foundation, the Canadian Institutes of Health Research (MT-13270, MOP-42465), CAPES (Brazil).

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# HUMAN MILK AND INNATE IMMUNITY

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Breastfed infants have lower morbidity and mortality due to diarrhea than those fed artificially. This had been attributed primarily to the secretory antibodies and prebiotic factors in human milk. Oligosaccharides are the third largest component of human milk. They were initially considered to be functionless by-products of glycoprotein and glycolipid synthesis during milk production. However, in the past few decades it has become apparent that the human milk oligosaccharides are comprised of thousands of components, at least some of which protect against pathogens. Oligosaccharide protection against infectious agents may result in part from their prebiotic characteristics, but is thought to be primarily due to their inhibition of pathogen binding to host cell ligands. Most human milk oligosaccharides are fucosylated, and their production depends on enzymes encoded by the genes associated with expression of the Lewis blood group system. The expression of specific fucosylated oligosaccharides in milk thus varies in relation to maternal Lewis blood group type, and is significantly associated with the risk of infectious disease in breastfed infants. Specific fucosylated moieties of oligosaccharides and related glycoconjugates (glycans) are able to inhibit binding and disease by specific pathogens. This presentation will provide evidence that specific glycans, especially the oligosaccharides, are the major constituent of an innate immune system of human milk whereby the mother protects her infant from enteric and other pathogens through breastfeeding. Other immune components of human milk will be reviewed. The large input of energy expended by the mother in the synthesis of milk oligosaccharides is consistent with the human reproductive strategy of large parental input into rearing relatively few offspring through a prolonged period of maturation. These protective glycans may prove useful as a basis for the development of novel prophylactic and therapeutic agents that inhibit disease by mucosal pathogens.

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# TOLL-LIKE RECEPTOR SIGNALING AND FAILURE OF NEUTROPHIL MIGRATION IN SEVERE SEPSIS

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Sepsis is a systemic inflammatory response that results from the inability of the immune system to limit bacterial spread during an ongoing infection. Neutrophils are critical effectors cells of the innate immune system that protect the host by migrating to inflammatory sites and killing pathogenic microbes. Studies from our laboratory have demonstrated that lethal sepsis, induced by cecal ligation and puncture (CLP) or bacterial inoculation models, is associated with impaired neutrophil migration to sites of infection. This impairment of neutrophil migration resulted in augmented number of bacteria in peritoneal cavity and blood which was associated with high mortality. Conversely, in sub-lethal sepsis, the neutrophil migration was not suppressed and the bacterial infection was restricted to the peritoneal cavity, consequently no significant mortality was observed. It seems that an early and inappropriate systemic inflammatory response, characterized by elevated levels of plasma cytokines, chemokines and nitric oxide released by immune cells after stimulation through bacteria and/or their products, mediates the neutrophil migration impairment. Moreover, although signaling through the TLRs has been implicated as an important element of host defense during an infection, recently, we showed that TLR2 and TLR4 signaling are crucial to the establishment of the impairment of neutrophil migration in lethal polymicrobial sepsis. It was observed that TLR2- and TLR4-deficient mice subjected to lethal polymicrobial sepsis induced by CLP did not present failure of neutrophil migration. As consequence, these animals presented low bacteremia and a high survival rate and did not display systemic inflammation determined by high levels of circulating cytokines and lung neutrophil sequestration. In conclusion, during lethal polimicrobial sepsis, a high concentration of cytokines/chemokines in the circulation with consequent production of nitric oxide induced by TLR2 and TLR4 signaling is a critical event that results in impaired neutrophil migration to the infectious focus, leading in turn to a failure of circumscription of the infection and high mortality.

**KEY WORDS:** Sepsis, neutrophil migration, TLRs, nitric oxide.

**FINANCIAL SUPPORT:** FAPESP, CNPq, PRONEX and FAEPA.

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# MODULATION OF IMMUNE RESPONSE BY LEUKOTRIENES

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Leukotrienes are best known as bronchoconstrictor and vasoactive mediators released by mast cells that contribute to asthmatic responses (1). They are produced by all myeloid cell lineages in response to a different stimuli and their broader participation in a wide array of pathologic inflammatory and acquired immune responses is increasingly recognized (2, 3, 4, 5). Much less well appreciated is their role in innate immune responses. Leukotrienes can be generated in response to microbial stimuli and it mediate a variety of antimicrobial functions. Moreover, a variety of conditions associated with increased susceptibility to infection are characterized by a relative deficiency of leukotrienes production. Leukotrienes biosynthesis involves the release of AA from membrane phospholipids by $Ca^{2+}$ – dependent cytosolic phospholipase $A_2$a (cPLA$_2$a) and it´s conversion, by 5-lypoxigenase enzyme (5-LO), into leukotriene $A_4$ (LTA$_4$), LTA$_4$ is then enzymatically converted to LTB$_4$ by LTA$_4$–hydrolase or to leukotriene $C_4$ (LTC$_4$) by addition of a molecule of glutathione through the action of LTC$_4$–synthase (6, 7). We have been investigating the effects of leukotriene synthesis inhibition during the immune response in a murine model of histoplasmosis, tuberculosis and strongyloidiasis. Moreover, using 5-LO KO mice, we investigated the effects of abscence of leukotriene deficiency during the infection. The results reveal that leukotrienes play an intrinsic and essential role in eliminating histoplasmosis (4), tuberculosis, and strongyloidiasis infection (5). We continued this line of investigation by evaluating the participation of leukotrienes in the immune protection induced by Cell-Free Antigens (CFAgs) against *H. capsulatum* infection. Our data strongly suggest that leukotrienes are essential to the immune protection induced by CFAg immunization. These findings contribute to a greater understanding of the role that leukotrienes play in host defense.

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**FINANCIAL SUPPORT:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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# ACTIVATION OF THE INNATE IMMUNE RESPONSE MEDIATED BY TOLL-LIKE RECEPTORS IN A MURINE INTRANASAL MODEL OF HSV-1 INFECTION.

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Recently it is shown the importance of Toll-like receptors (TLRs) in the host response to different virus infections. MyD88 is an adaptor protein that is downstream to mediated TLR activation and is essential for the production of inflammatory cytokines. The Herpes simplex virus is related with different diseases, being one of the most frequently non-epidemic causes of encephalitis. Using *in vitro* techniques and knock-out mice in an intranasal model of infection we show the importance of MyD88 and other innate immunity molecules such as IFNγ, TNFarp55 and iNOS. First of all, using CHO transfected cells we show the activation of TLR2 by the HSV-1 in a dose dependent way. Despite this activation, macrophages of TLR2$^{-/-}$ mice induced with HSV-1 had the same TNFα production profile that WT mice, while MyD88$^{-/-}$ macrophages did not produce this cytokine. To study the role for TLRs *in vivo*, C57BL/6, TLR2$^{-/-}$, MyD88$^{-/-}$ and IFNγ$^{-/-}$ four week-old mice were infected with $10^4$p.f.u. of HSV-1. Histopathological and immunohistochemical analyses demonstrate a severe encephalitis and the presence of virus in the brain of MyD88$^{-/-}$ and IFNγ$^{-/-}$, on the contrary of WT and TLR2$^{-/-}$ mice, that showed no signs of encephalitis and no presence of virus in the brain. MyD88$^{-/-}$ and IFNγ$^{-/-}$ had 100% and 50% of lethality, respectively, while both the WT and TLR2$^{-/-}$ mice survive 100% to the infection. The HSV-1 was found in the trigeminal ganglia of all mice, which characterize effective infection of all mice with and without symptoms. To look for other molecules that may be important in the HSV-1 disease, TNFαrp55$^{-/-}$ and iNOS$^{-/-}$ mice were infected in the same way, which presents 75% and 100% lethality, respectively. ELISA analyses show no differences in the production of TNFα in the sixth day post infection in mice brain or between the 1-4$^{th}$ day in mice serum. Our data points to a very localized infection and probably the control of the infection might be in the ganglia and also suggest the importance of the TLRs pathway and other innate immunity molecules in the intranasal model for HSV-1 encephalitis.

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# B-1 CELLS IN INNATE AND ACQUIRED IMMUNITY

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The term B-1 cell was originally proposed to describe a subtype of B lymphocytes, which differs from B conventional cells by anatomical localization, developmental origin, surface markers expression, antibody repertoire and growth properties. B-1 cells express high levels of surface IgM, low levels of B220 (CD45R) and IgD, but not CD23, whereas conventional B-2 cells express high levels of B220 and IgD, CD23 and low levels of IgM. Besides, typical B-1 cells residing in peritoneal cavity also express low levels of Mac-1 (CD11b). Further, B-1 cells are sub classified in B-1a cells, which express CD5, and their phenotypic $CD5^-$ "twins", B-1b cells. Our laboratory has demonstrated that B-1b cells proliferate in cultures of adherent mouse peritoneal cells and differentiate into a mononuclear phagocyte, provisionally named "lymphophage". Yet, that these cells migrate from the peritoneal cavity to a non specific inflammatory lesion. From these observations the origin, differentiation and function of these cells in normal and pathological conditions has being intensively investigated in our laboratory. Results bringing evidence that B-1 cells participate in innate and acquired immunity will be presented and discussed.

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# C3, KEY COMPONENT OF THE COMPLEMENT SYSTEM INVOLVED IN NON SPECIFIC PATHOGEN RECOGNITION

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The complement system is one of the first lines of innate defense against pathogenic microorganisms. Its activation leads to the formation of inflammatory peptides, opsonic fragments, and the membrane attack protein. C3 contains an internal thioester bond which is formed by the association of the sulfhydryl group (Cys 1010) and a glutamyl carbonyl (Gln 1012) on the C3 a-chain. Proteolytic cleavage of a 77 – residue peptide from the N-terminus of the C3 a-chain generates C3a and C3b. Attachment of C3b is accomplished through a covalent link between the carbonyl group of the metastable thioester and either –NH2 or –OH groups of proteins or carbohydrate structures exposed on the pathogen cell surface. C3 molecules that do not bind in this way are inactivated by binding to water molecules. Covalent binding of C3 tags invading pathogens as foreign substances addressing them to be phagocytosed or lysed. C3, once it is deposited covalently as C3b on the pathogen cell surface it can be directed to either an amplification or inactivation step. Amplification requires intervention of factor B, a serine pro-enzyme, which is activated by factor D forming the C3 convertase C3bBb. Inactivation requires intervention of four distinct proteins, factor I, factor H, complement receptor type 1 (CD35) and the membrane cofactor protein (CD46), which by acting in concert cleave C3b into an inactive product, iC3b, releasing a small peptide, C3f. On host cell surfaces bearing polyanions such as sialic acid, factor H binds to C3b with a higher affinity than does factor B. On microbial surfaces that lack a polyanionic coating, factor B binds to C3b with a higher affinity than does factor H, leading to amplified cleavage of C3. Amplification leads to destruction of pathogens while inactivation allows pathogens multiplication.

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