Effects of Prolactin on Innate Immunity of Infectious Diseases

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Abstract: Prolactin (PRL) hormone has been considered as a cytokine able to modulate immune response in mammals. In addition, particular effects of this hormone on inflammatory response have been documented in autoimmune diseases, whereas its effects on innate immune response during infectious diseases are in general, unknown. The objective of this review is to present the state of art in the role of PRL on innate immune response during the establishment and progress of different infectious diseases caused by bacteria, fungi and protozoa in mammals. Most of the knowledge about actions of PRL is restricted to protozoa, where the hormone has mainly a protective role during infection. The effects of PRL on innate immune response to bacterial and fungal infections are poorly understood, but some evidences exist that demonstrate phagocytosis induction, which can be considered either as a protective role to kill pathogenic microorganisms, or as a factor that favors pathogens to persist intracellularly. Here, we will discuss that PRL effects on innate immune response to infection depend on the pathogenic microorganism, host, immunological state of organisms, PRL variant involved, as well as its concentration.

Keywords: Prolactin, infection, innate immunity.

INTRODUCTION

Prolactin (PRL) is a 23 kDa polypeptide hormone produced mostly by lactotrophs in anterior pituitary, whose main actions in mammals are related to lactation and reproduction. However, its production has been demonstrated by peripheral tissues, affecting more physiological processes than all other pituitary hormones combined, and is involved in more than 300 functions in vertebrates. The diverse functions of PRL can be a consequence of its multiple variants resulting from posttranslational modifications, such as enzymatic processing, phosphorylation, glycosilation, etc [1]. In the immune system, PRL acts as a cytokine playing an important role in mammal immune responses, including innate and adaptive immunity and autoimmune diseases. Accordingly, PRL has been considered as a cytokine and its receptor (PRLR) belongs to the class 1 superfamily cytokine receptors, this superfamily includes receptors for several interleukins, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), erythropoietin (EPO), thrombopoietin (TPO), etc. However, the development of animals with a targeted disruption of either the PRL or PRL-R gene suggest that PRL is not essential for immune system normal function [2, 3].

PROLACTIN AND INNATE IMMUNE RESPONSE

The immune system of vertebrates provides protection against a wide range of pathogens. There are two components in this system: nonspecific, constitutive (innate) defences, which act immediately against pathogens and a specific (adaptive) reaction, which generates immunological memory. Both systems consist of cellular and soluble components, which include cytokines, chemokines and complement factors. During an innate immunity response, macrophages and neutrophils engulf and destroy microbes after first encounter and together with natural killer (NK) cells secrete cytokines that regulate innate and adaptive immunity. The dendritic cells (DCs) present antigens to lymphocytes to stimulate adaptive immunity [4, 5].

Innate immune recognition is based on the detection of constitutive and conserved microbial products (also named pathogen associated molecular patterns, PAMPs), which include lipopolysaccharide (LPS), lipotheicoic acid (LTA), etc, that are recognized through pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). Binding to TLRs activates macrophages and DCs, causing them to release pro-inflammatory cytokines and chemokines and triggering functional maturation of DCs by upregulating the receptors CD80 and CD86. This leads to the initiation of antigen-specific adaptive immune responses and the functional differentiation of T cells. Innate immunity therefore acts to focus and control acquired immunity. During this orchestation of responses, PRL stimulates T cells, B cells, NK, macrophages, neutrophils, CD34 hematopoietic cells, and DC [5-8]. All these effects can be achieved by circulating PRL or PRL produced by immune cells. PRL expression and PRL-receptors have been demonstrated in immune cells, suggesting that the hormone may act by an auto- or paracrine way [1].

The role of PRL during pathogenic inflammatory conditions such as autoimmune diseases has been strongly studied and documented. To date, is well recognized that PRL enhances the progression of immune process in autoimmune

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diseases such as Rheumatoid arthritis, Multiple sclerosis, Systemic lupus erythematosus, Celiac disease, Systemic sclerosis, Type 1 Diabetes mellitus, Sjögren's syndrome, Graves' disease, Hashimoto's thyroiditis, Addison's disease or Lymphocytic adenohypophysitis [9, 10]. The immunomodulatory activities of PRL may arise from increasing nuclear transcription factors such as IRF-1 and NF-κB, which play a pivotal role in many immune functions. Pro-inflammatory mediators such as production of cytokines, chemokines or nitric oxide (NO) release, have been associated to PRL; however, its role during microbial pathogen infection has been poorly studied. Besides PRL function on immune cells, this hormone is able to regulate innate immune response of several types of cells including pulmonary epithelium, mammary epithelium or fibroblasts through NF-κB activation [11-13]. The purpose of this review is to discuss and describe the state of art of PRL role during innate immune response to infection by pathogenic microorganisms.

PHAGOCYTOSIS REGULATION

Microorganisms have developed different strategies to invade their host. Fungi, protozoa or bacteria can live within or outside host cells, which are ready to attack invading microorganisms without a previous stimulation, involving the innate immune system. Among the components on this system macrophages and neutrophils play an important function during the early phases of this kind of response [14]. Additionally, surface epithelium such as dermal, respiratory or gastrointestinal, constitute a natural barrier to infectious agents. These kind of cells are able to produce antimicrobial peptides, NO, enzymes, fatty acids, low pH, etc, in order to reduce infection, if pathogens cross this barrier, epithelial and endothelial cells can produce cytokines to activate cellular effector mechanisms, which include the phagocytosis of the invading microorganisms by granulocytes and mononuclear phagocytes. Neutrophils phagocytose microbes and can subsequently kill them, whereas mononuclear phagocytes once activated, can produce toxic effector molecules to kill intracellular pathogens [4, 15].

PRL has been demonstrated to induce the phagocytosis of macrophages; however, there are few reports that associate this activity with phagocytosis of invading microbes by phagocytes and non-professional phagocytes. The effect of PRL on phagocytosis of different microbes is summarized in Table **1**. Briefly, PRL is able to induce the phagocytosis of *Candida albicans*, *Staphylococcus epidermidis*, *S. aureus*, *Toxoplasma gondii* and *Acanthamoeba castellani*, and the intracellular replication of *Mycobacterium avium* once internalized [12, 16-20]. It remains to be demonstrated if PRL also stimulates invading properties of microorganisms. In addition, the molecular mechanisms by which PRL regulates phagocytosis are unknown.

ROLE OF PRL ON PRODUCTION OF PRO-INFLAMMATORY CYTOKINES AND INFLAMMATORY MEDIATORS

The inflammatory response following infection consists in the sequential release of mediators and the recruitment of circulating leukocytes, which become activated at the inflammatory site and release further mediators. Pro-inflammatory signaling pathways induced by PRRs activate innate immune system [5]. However, in most cases, the inflammatory response is resolved by the release of endogenous anti-inflammatory mediators (anti-inflammatory cytokines) as well as the accumulation of intracellular negative regulatory factors, such as NO or antimicrobial peptides. Activation of NF-κB factors plays a central role in inflammation through the regulation of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, antimicrobial peptides and inducible enzymes such as cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) [4].

As we mentioned above, PRL is able to stimulate the secretion of several pro-inflammatory and regulatory cytokines as well as other regulatory factors in different cell types [11-15]. However, the role of PRL in the innate immune response to infection has been poorly studied and is restricted to describe the hormone effect on infection of few pathogenic microbes or their products. PRL effects on inflammatory mediators following infection depend on the host, pathogen, immunological state of organisms, PRL variant involved, as well as the kind of experimental approach. Some of these effects are described below, and are related to bacterial, fungal, and protozoal infections in mammals. A summary of all effects of PRL reported on innate immune response to infection is shown in Fig. (**1**).

INNATE IMMUNITY REGULATION BY PRL DURING BACTERIAL INFECTION

Meli *et al.* [17] have reported a protective role of PRL during the *in vivo* infection with *Salmonella thyphimurium* in

Table 1. Role of PRL on Phagocytosis of Different Microorganisms

Effect	Pathogen	Phagocytic Cell
Stimulation of phagocytosis	*Staphylococcus aureus*[a]	Bovine mammary epithelial cells
Stimulation of phagocytosis	*Candida albicans*[b]	Mouse peritoneal macrophages
Stimulation of phagocytosis	*Staphylococcus epidermidis*[c]	Mouse peritoneal macrophages
Stimulation of phagocytosis	*Toxoplasma gondii*[d]	Murine microglia
Stimulation of phagocytosis	*Acanthamoeba castellani*[e]	Murine microglia
Stimulation of intracellular bacterial replication	*Mycobacterium avium*[f]	Bovine alveolar macrophages

[a][12], [b][16] [c][17], [d][18], [e][19], [f][20].

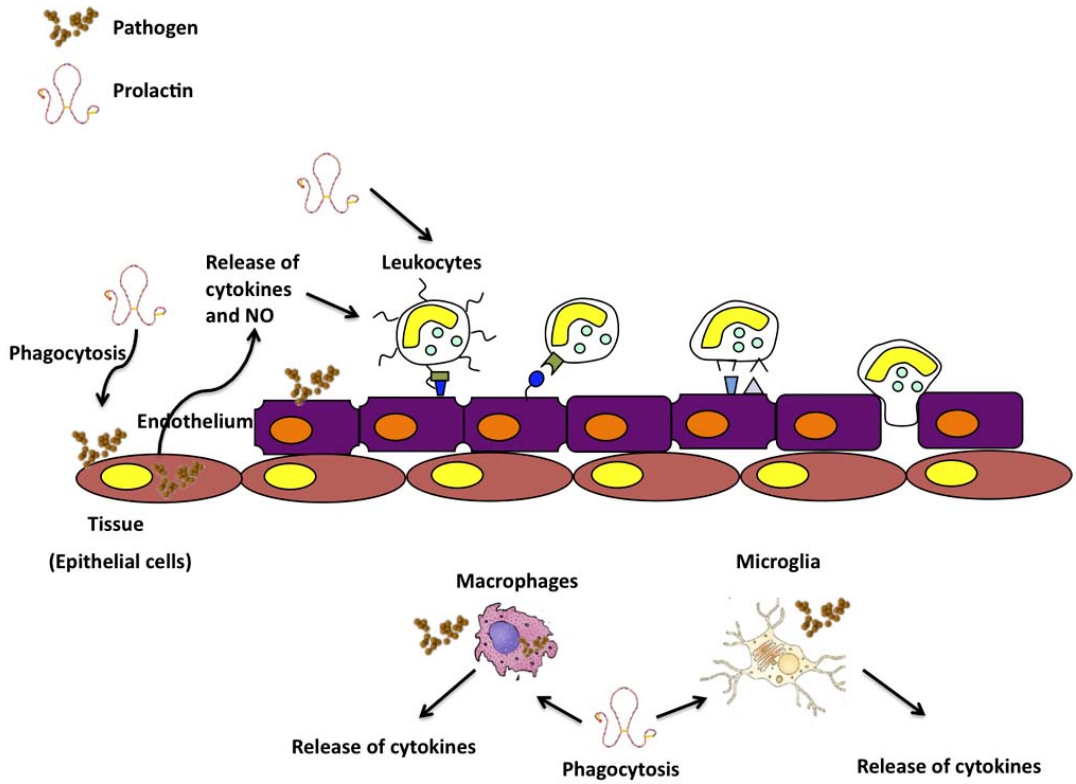

Fig. (1). Summary of PRL effects on innate immunity during infection. Briefly, PRL is able to induce phagocytosis of different microorganisms in both phagocytic (macrophages and microglia) and non-phagocytic cells (epithelial cells). In response, these cells will produce pro-inflammatory cytokines and NO, necessary for leukocyte recruitment to the infection site. PRL also can modulate leukocyte adhesion to endothelium [32].

a murine model. Mice treated with PRL reduced their mortality, and increased macrophage phagocytic activity by means of an induction in NO production as well as other reactive oxygen species (ROS). When animals were concomitantly treated with an inhibitor of NO-synthase (NOS), PRL activity on lethality, phagocytosis and intracellular killing by macrophages was abolished. In addition, Meli *et al.* [21] have demonstrated that hyperprolactinemia induced by pituitary graft in mice, reduces *S. thyphimurium* number in the spleen compared to control mice. Authors suggest that high levels of PRL can direct T cells toward a defined Th1 cytokine profile, up-regulating IFN-γ and IL-12 secretion in response to *Salmonella* infection.

Other reports suggest that PRL does not modify NO production by bovine macrophages infected with *Mycobacterium avium* ss. *paratuberculosis*. However, the cytokine profile regulated by this hormone has not been studied in this model [20].

Brand *et al.* [22] have demonstrated that PRL within physiological concentrations may increase TNF-α and IL-12 release from human peripheral blood mononuclear cells following LPS-stimulation, a Gram-negative cell wall component. TNF-α and IL-12 are considered as essential cytokine mediators of inflammation, septic complications and autoimmunity. Their production arises from the activation of nuclear transcription factors such as IRF-1 and NF-κB in response to LPS and PRL.

Using an *in vitro* infection model with *Staphylococcus aureus* and bovine mammary epithelial cells, Gutiérrez-Barroso *et al.* [12] have demonstrated that PRL together with *S. aureus* do not modify TNF-α and iNOS mRNA expression, while both down-regulate β-defensin and IL-1β mRNA expression, as well as NO production, suggesting that infection together with PRL can inhibit host innate immune response elements.

INNATE IMMUNITY REGULATION BY PRL DURING FUNGAL INFECTION

The roles of PRL on innate immune response during fungal infections are unknown. In spite of lack of information, Collins *et al.* [23] have shown that PRL serum levels were up-regulated in parturient rats upon feeding with zearalenone, an estrogenic mycotoxin produced by *Fusarium sp.* Previously, Milano *et al.* [24] have demonstrated that zearalenone increased PRL circulating levels in male rats.

Other approaches correlate PRL with delayed type hypersensitivity (DTH) response to *Candida albicans* infection in mice [25]. Although DTH does not involve a rapid (innate) response, it comprises inflammatory elements such as pro-inflammatory cytokine production. In this work, authors have shown a down-regulation of cytokines in mice sensitized and challenged with *C. albicans* in response to a molecular mimic of phosphorylated PRL. Further research is necessary in order to establish the role of PRL in the regulation of innate immune response to fungal infections.

INNATE IMMUNITY REGULATION BY PRL DURING PROTOZOAL INFECTION

The effect of PRL on protozoal infections has been studied more extensively than for bacteria or fungi. In general, a

protective effect of PRL *in vivo* has been associated to the infection with several protozoa. However, there is still a lack of information concerning the role of this hormone during innate immune response subsequent to this kind of infections. Particular interest has been raised out during pregnancy, where PRL levels are high. The following works described some PRL actions on inflammatory or innate immune response to several protozoa. A summary of these effects is shown in Table **2**.

Experiments conducted by Benedetto *et al.* [18] using a mouse toxoplasmosis model (infected with *Toxoplasma gondii*) suggest that both TNF-α and PRL may induce microglia cells to the anti-toxoplasma response such as intracellular killing of parasites and IL-1β, IL-3 and IL-6 release. Apparently, NO is not directly involved in anti-toxoplasmatic activity. Recently, Dzitko *et al.* [26] studied the seroprevalence of *T. gondii* woman antibodies with hyperprolactinemia, hypoprolactinemia and control group (normal PRL). Women with hyperprolactinemia showed lower seroprevalence than those with normal PRL, suggesting that a high level of PRL may be one of the important factors preventing *T. gondii* infection in women.

Possible participation of PRL in Leishmaniasis has not been fully accomplished. Work from Gómez-Ochoa *et al.* [27] has established that Syrian hamster lactating females infected with *Leishmania infantum* did not develop the illness, in relation to infected non-lactating females, suggesting a protective role of PRL during *Leishmania* infection. However, these data need further research in order to determine the specific functions of PRL to induce protection.

Innate immunity modulation following *Trypanosoma cruzi* infection has been extensively studied in humans and experimental models [28]. However, the implication of PRL in the establishment and progress of Chagas disease has been recently considered. Correa-de-Santana *et al.* [29] have shown that in rat mammosomatotrophic cell line GH3, PRL production was down-regulated following *T. cruzi* infection through inhibition of Pit-1 transcriptional factor. Further studies are necessary in order to determine if high levels of PRL have a protective role during *T. cruzi* infection.

Plasmodium falciparum malaria is a major health problem among pregnant women in endemic areas, which are more susceptible than their non-pregnant counterparts. A study of Bayoumi *et al.* [30] found lower levels of PRL in infected pregnant women compared with controls, and significant negative correlations between PRL and both IL-4 and IL-10, but no significant correlation between PRL and cortisol, suggesting that low hormone levels may increase susceptibility to infection, but further research on this subject is needed in order to clarify if higher levels of PRL can protect women from *P. falciparum* infection.

In spite of some Amoebae species cause important diseases to humans, the correlation of this kind of infections with PRL has not been explored enough. Results from Bennedeto *et al.* [19] have established a role of PRL during murine microglia infection with *Acanthamoeba castellani*. In this work, authors showed that treatment of murine microglia with PRL and IFN-γ synergistically triggered, in a dose-dependent manner, amebastatic activity and release of endogenous IL-1α, IL-1β, IL-6 and TNF-α. Additionally, microglia cells showed anti-parasitic activity in presence of PRL and IFN-γ, which is not dependent of NO production.

In addition to protozoal infection relevance to human health, there is also a worldwide concern in relation to domestic animal health. In this sense, protozoal infections affecting cattle causing many economic loses, particularly those relating to milk production or breeding problems. *Neospora caninum* is an obligate intracellular protozoa being recognized as a major cause of abortion and congenital infection in cattle worldwide. Bovine neosporosis appears either as the result of maternal infection during gestation or following a persistent infection during gestation. *Neospora*-seropositive cows are more likely to abort than seronegative cows. Recently, a work from García-Ispierto *et al.* [31] shows that non-aborting *Neospora*-seropositive cows had higher plasma PRL concentrations than seronegative animals, while the concentration of PRL decreased in aborting cows, suggesting a protective hormone effect against this kind of infection, probably due to its pro-inflammatory action.

CONCLUDING REMARKS

Most of the knowledge of PRL functions on immune system focuses on regulation of adaptive immunity and less is recognized about its regulation on innate immune response to infection by protozoa, bacteria and fungi. Accordingly,

Table 2. PRL Effects on Innate Immune Response to Protozoal Infection

Protozoa	Host	Effect/Correlation with PRL Levels
Toxoplasma gondii	Murine microglia[a]	PRL together with TNF-α, intracellular killing of parasites and IL-1β, IL-3 and IL-6 release.
Acanthamoeba castellani	Murine microglia[b]	PRL together with IFN-γ, anti-parasitic activity and IL-1β, IL-1α, IL-6 and TNF-α release.
Toxoplasma gondii	Pregnant women[c]	Hyperprolactinemia reduces seroprevalence.
Trypanosoma cruzi	Rat mammosomatotrophic GH3 cell line[d]	Reduction in PRL production.
Plasmodium falciparum	Pregnant women[e]	Lower levels of PRL in infected pregnant women, and negative correlations between PRL and cytokines IL-4 and IL-10.
Neospora caninum	Pregnant cows[f]	High levels of PRL in non-aborting infected cows.

[a][18], [b][19], [c][26], [d][29], [e][30], [f][31].

several reports show evidence that PRL is able to modulate phagocytosis of pathogenic bacteria, fungi and protozoa by different cell types, opening a new research area concerning PRL actions. Furthermore, PRL regulates production of several cytokines and inflammatory mediators in different models and cells, which constitutes a relevant response to infection. However, many of PRL actions here described, comprise correlations *in vivo* with PRL circulating levels; thus, is necessary to develop experimental approaches. In general, PRL shows an immunoprotective role during infection by protozoa, effect that is worth of further research.

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