Interleukin 18 (IL-18) is a pleiotropic cytokine involved in the regulation of innate and acquired immune response. In the milieu of IL-12 or IL-15, IL-18 is a potent inducer of IFN-gamma in natural killer (NK) cells and CD4 T helper (Th) 1 lymphocytes. However, IL-18 also modulates Th2 and Th17 cell responses, as well as the activity of CD8 cytotoxic cells and neutrophils, in a host microenvironment-dependent manner. It is produced by various hematopoietic and nonhematopoietic cells, including dendritic cells and macrophages. In an organism, the activity of the cytokine depends on the intensity of IL-18 production, the level of its natural inhibitory protein — IL-18BP (IL-18 binding protein) and the surface expression of IL-18 receptors (IL-18R) on the responding cells. This review summarizes the biology of the IL-18/IL-18BP/IL-18R system and its role in the host defense against infections. The prospects for IL-18 application in immunotherapeutic or prophylactic interventions in infectious and non-infectious diseases are discussed.

**Key words:** interleukin-18 (IL-18), IL-18 receptor, IL-18 binding protein

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### INTERLEUKIN-18

Interleukin 18 (IL-18) was first described in the serum of mice inoculated intraperitoneally with endotoxin and was called the “IFN-gamma inducing factor” (Nakamura et al., 1989). The name was changed to IL-18 after isolation of this cytokine from the liver extracts of mice treated with Propionibacterium acnes and subsequently challenged with lipopolysaccharide, after molecular cloning (Okamura et al., 1995). Although originally described as a factor capable of inducing IFN-gamma production by murine splenocytes, the effector role of IL-18 rapidly expanded. IL-18 is currently classified as one of the members of the IL-1 cytokine superfamily— that acts as an important regulator of innate and acquired immune responses (García et al., 2003; Dinarello et al., 2013). This cytokine is a potent activator of polarized T helper 1 (Th1) cells for IFN-gamma production and lymphocyte proliferation (Lebel-Binay et al., 2000). Some studies have shown a functionally pleiotropic and complex functioning of IL-18, depending on the host environment. This cytokine plays effector and regulatory roles in a variety of early inflammatory responses. It is also expressed at the sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in the context of numerous infectious diseases (Lebel-Binay et al., 2000; Diakowska et al., 2006; Kinjo et al., 2002; Fabbi et al., 2015).

### THE PRODUCTION AND ACTIVATION OF IL-18

In the body, IL-18 is constitutively expressed by several cell types, including macrophages, Kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, intestinal epithelial cells, microglial cells and synovial fibroblasts (García et al., 2003). This cytokine is produced by activated immune cells, dendritic cells, monocytes and macrophages, T and B lymphocytes, natural killer cells (NK) and neutrophils. IL-18 is produced as a 24 kDa inactive precursor (pro IL-18) lacking a signal peptide required for secretion (Okamura et al., 1995). In order to be activated, it must be processed by the intracellular cysteine protease caspase-1, which cleaves the precursor into an active mature molecule of 17,200 Da (Dinarello et al., 2013; Wei et al., 2014). Cleavage of pro IL-18 into mature IL-18 allows this molecule to be released from the cell, although a significant amount of the IL-18 precursor remains unprocessed inside the cell. A signal, which is supplied by IL-18 to the interior of the cell, needs binding of the mature cytokine to its ligand, which is the IL-18 receptor alpha chain (IL-18Rα). However, the low affinity of binding between IL-18 and IL-18Rα prevents initialization of the signal transduction pathway and immune cell activation (Schneider et al., 2010). Full activation of cells by IL-18 requires interaction between the interleukin 18Rα receptor and the IL-18 beta chain co-receptor (IL-18Rβ). This complex is functionally and structurally similar to other members of the IL-1 family, with the IL-1RaCp co-receptor (IL-1 receptor accessory protein). The cytoplasmic fragment of the IL-18 receptor and other receptors of the IL-1 family have a TIR domain (Toll-IL1 receptor), belonging to the Toll-like (TLR) receptors. The activation of IL-18 results in a cascade of reactions in which the Toll-IL-1 receptor (TIR) recruits and binds to the myeloid differentiation factor 88 (MyD88), which mediates signal transduction to the TNF receptor associated factor 6 (TRAF6) and IL-1 receptor associated kinases (IRAKs). That reaction causes activation of the NF-κB transcription factor, which stimulates gene transcription leading to the production of pro-inflammatory cytokines (Dinarello et al., 2013; Kal-
na et al., 2000; Wei et al., 2014). The IL-18 signal transduction pathway is illustrated in Fig. 1. IL-18 modulates numerous immune reactions mainly by stimulating the IFN-γ production and its modulatory effects depend on the co-existence of IL-18 with IL-12 or IL-15 in the microenvironment (Robinson et al., 2012). These cytokines can increase the expression of the IL-18Rβ receptor, which is crucial for IL-18 signal transduction.

The proinflammatory activity of IL-18 is balanced by a constitutively secreted IL-18 binding protein (IL-18BP) with an extremely high affinity to IL-18, which is significantly higher than that of IL-18Ra. IL-18BP is a member of the Ig superfamily (Novick et al., 2013). By binding IL-18, IL-18BP diminishes the production of IFN-γ and other proinflammatory cytokines in order to reduce triggering autoimmune responses to infections (Nakanishi et al., 2001). In humans, an increase in disease severity can be associated with an imbalance between IL-18 and IL-18BP, which yields to elevation of the levels of free IL-18 in the circulation (Dinarello et al., 2013). The increase in the levels of IL-18 and/or IL-18BP has been implicated in severity of systemic juvenile idiopathic arthritis, systemic lupus erythematosus, myocardial infarction, Crohn’s disease, acute kidney injury, inflammatory bowel disease, sepsis and other diseases. The IL-18BP as well as IL-18 neutralizing antibodies, have been used safely in clinical trials in humans. However, it cannot be forgotten that in some models of disease, IL-18 plays a protective role. The broad spectrum of IL-18 functions, as well as the differing levels of the cytokine and IL-18BP that occur in numerous diseases, indicate that both, IL-18 and IL-18BP, can also be useful as the biomarkers in diagnostics (Dinarello et al., 2013).

IL-18 is regarded as a potent regulator of innate and acquired immune responses (García et al., 2003; Dinarello et al., 2013). With the participation of IL-12 or IL-15, IL-18 induces NK activity and directs immunity towards Th1 cell response, characterized by the profound IFN-γamma production. Without IL-12 or IL-15, IL-18 does not induce IFN-γamma production because these two cytokines increase the expression of IL-18Rβ, which is essential for the IL-18 signal transduction (Dinarello et al., 2013). It has been also shown that IL-18 promotes IFN-γamma production in synergy with other Th1-related cytokines, IL-2 and IL-23 (Okamoto et al., 2002, Nakahira et al., 2002, Okazawa et al., 2004). In the absence of IL-12, IL-18 can induce the Th2 response. In E. coli-infected mice, IL-18 promoted both, Th1 and Th2 responses (Kinosita, Kurana et al., 2006). Studies performed in double knockout mice of IL-12p40 and IL-18, have shown that IL-18 plays a role in the induction of Th17 cell responses (Lim et al., 2013). It has been suggested that IL-18 activates and enhances IL-17 production in already polarized Th17 cells, in a TCR-independent manner in synergy with IL-23 (Weaver et al., 2006). The IL-18-driven increase in IFN-γamma production is accompanied by the enhancement in T cell proliferation and production of various cytokines (IFN-γamma, TNF-α, GM-CSF, IL-14, IL-5, IL-13) by T helper (CD4+) lymphocytes and in activation of cytotoxic T (CD8+) lymphocytes. Multiple intraperitoneal IL-18 injections, but not just a single injection, enhanced both Th1 and Th2 response, humoral immunity, as well as neutrophil phagocytic activity in immunocompromised mice infected with pathogens, such as E. coli, Listeria monocytogenes, Staphylococcus aureus, Cryptococcus neoformans (Kinosita et al., 2013). However, exogenous IL-18 may sometimes induce exaggerated inflammatory reactions that are harmful to the host, because of its potent IFN-γamma inducing capability. The excessive IL-18-driven reaction sometimes causes multiorgan injuries and lethality.

**PROSPECTS FOR THE IL-18 APPLICATION IN IMMUNE INTERVENTIONS**

The IL-18 driven intensification of IFN-γamma production is accompanied by an increase in nitrogen oxide synthase and killing ability of macrophages. It suggests an important role of IL-18 in the resistance to intracellular pathogens- which are able to develop inside immune cells, including macrophages. M.tb, the causative agent of tuberculosis (TB), belongs to this group of bacterial pathogens. Recent epidemiological data clearly indicates that TB remains one of the most deadly infectious diseases. According to the WHO data from 2013, this disease was diagnosed in more than 9 million cases worldwide, and up to 2 million people die annually because of it. The control of TB is still difficult because of not fully effective diagnosis and insufficient protective effectiveness of the only currently used anti-tuberculosis BCG (Bacillus Calmette-Guerin) vaccine (WHO, Global Tuberculosis Report, 2014). A crucial role of IL-18 in the host protection against M.tb infection was shown by Kinjo et al. in studies using IL-18 knockout and IL-18 transgenic mice. IL-18 deficient mice were more prone to an M.tb infection and their sera, spleens, lungs and livers contained less IFN-γ than those of wild-type mice (Kinjo et al., 2002). The IFN-γ production by spleen cells stimulated with mycobacterial antigens was also impaired in IL-18 knockout mice. In contrast, IL-18 transgenic mice were more resistant to an M.tb infection than control wild mice, and the levels of IFN-γ in their serum and its production by mycobacterial antigen-stimulated spleen cells were increased. These data suggested a significant contribution of IL-18 to the development of Th1 immunity (Kinjo et al., 2002). The pronounced
role of IL-18 in the defense against TB was confirmed by Schneider et al., (Schneider et al., 2010). The protective Th1 response to M.tb was decreased in IL-18 deficient mice, which constituted a privilege for mycobacterial propagation. Neutrophil driven lung immunopathology, concomitant with unrestricted growth of M.tb bacteria, was most probably responsible for the premature death of IL-18 knockout mice infected with M.tb. In humans, IL-18 promoter gene –607C/A polymorphism was found to be a risk factor for TB in the Chinese population, but not for the south Indian population (Li et al., 2013, Harishankar et al., 2007). A large case-control study revealed that polymorphisms in the IL-18 receptor alpha chain gene IL-18R1 were associated with the risk of TB in older Chinese people (over 46 years old) (Zhang et al., 2014). In addition, SNPs (Single Nucleotide Polymorphism) in the IL-18R1 promoter were associated with the genotype-specific methylation status and genotype-specific IL-18R1 expression, which suggests that increased DNA methylation and decreased mRNA expression of IL-18R1 might partially mediate the increased susceptibility to TB.

Extraordinary susceptibility to infections’ complications, such as sepsis in patients with severe surgical stress, i.e. trauma injury, burn injury or major surgery, is a frequent and unresolved problem (Kinoshita et al., 2013). The loss of the physical skin barrier, as well as bacterial translocation from the gut, can cause sepsis in such patients. Bacterial infection can lead to lethal multi-organ injuries, as the host defense system is significantly weakened, which promotes microbial growth. Mice studies suggest a possible medical application for IL-18 in the treatment of post-burn E. coli infection. Multiple injections of IL-18 to the burn injured mice remarkably increased the IFN-γ production by mononuclear liver cells, thus improving bacterial clearance and mouse survival after E. coli infection (Kinoshita et al., 2004; Kinoshita, Kuranaga et al., 2006). Small doses of IL-18 also restored the development of specific antibacterial immune responses, preventing infections with Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus, the most common bacteria in post-burn infections (Kinoshita et al., 2004; Kinoshita, Shimomiva et al., 2006; Kinoshita et al., 2011). IL-18 driven activation of neutrophils was mostly responsible for improved elimination of these pathogens. Therapy involving administration of IL-18 also results in an up-regulation of IFN-γ production by NK cells and recruitment of neutrophils, monocytes and macrophages into infectious foci. IL-18 treatment for burn-injured mice strengthened the host defense against P. aeruginosa infection by the up-regulation of natural IgM production in the liver B1 cells, which were characterized as CD43+CD5−CD23−B220+IgM− cells (Kinoshita, Shimomiva et al., 2006). Such antibodies may opsonize bacteria and facilitate their ingestion by phagocytes before specific antibacterial antibodies are produced. Multiple IL-18 injections activate natural IgM-producing B-1 cells in the liver and restore the humoral immunity against bacterial infections after a burn injury. Such activity of IL-18 may also be helpful in preventing the serious complications in pneumococcal respiratory infections in immunocompromised patients (Kinoshita et al., 2013). Altogether, these data suggest that IL-18 treatment may be recognized as an alternative and useful therapeutic tool against infections caused by intracellular and extracellular pathogens, even in individuals with an immunodeficiency.

In some preclinical models IL-18 has been found to have an antitumor activity (Srivastava et al., 2013, Fabb et al., 2015). It has been shown that systemic administration of IL-18 enhances the regression of a well-established primary tumor by a mechanism that depends on CD8+ T cells, Fas (CD95)/Fasl. (Fas ligand) interaction and endogenous IFN-γ, particularly in a combination with other cytokines (Robertson et al., 2008). In a combination with monoclonal antibodies (mAB) recognizing the CD20 antigen on B lymphocytes, IL-18 co-stimulates IFN-γ production and antibody-dependent cellular cytotoxicity (ADCC) of NK cells, which are activated through the surface receptors for Fc fragments of the antibody molecules. In this way, IL-18 augments the activity of mAB against B cell leukemias and lymphomas. In other experimental studies, synergistic effects of IL-18 and M. bovis BCG bacteria on the IFN-γ Th1 responses were observed in a mouse model of bladder cancer (Luo et al., 2004). BCG has been applied in the treatment of superficial bladder cancer for years, however, 30–50% of patients did not respond to the BCG therapy. To improve the therapeutic efficacy of BCG, a recombinant BCG strain that functionally secretes murine IL-18 (rBCGmIL-18) was developed. BCG bacteria themselves are strong Th1 inducers. Small amounts of IL-18 released by rBCGmIL-18 augmented Th1 immunity in mice, which led to: a) reduced mycobacterial growth in spleen after infection, b) increased production of IFN-γ, TNF-α (tumor necrosis factor-α) and GM-CSF (granulocyte macrophage colony stimulating factor), and decreased secretion of IL-10, by spleen cells stimulated with BCG, c) augmented macrophage cytotoxicity against bladder cancer MBT-2 cells. It can be expected that this feature of recombinant BCG strains, capable of expressing IL-18, might be useful in immunotherapy and prophylaxis of diseases in which Th1 response is desirable (Dinarello et al., 2013; Luo et al., 2004; Novick et al., 2013). This expectation seems to be confirmed by our recent demonstration of a remarkable advantage of recombinant rBCGhIL-18 producing human IL-18 over nonrecombinant BCG in the stimulation of dendritic cells to preferentially trigger strong IFN-γ secretion by naïve CD4(+) T cells in healthy humans vaccinated with BCG (Szpakowski et al., 2015). Previously, the rBCGmIL-18 strain producing murine IL-18 had been found to modify the Th2 type responses in a murine model of the ovalbumin-dependent allergic reaction. Following in vivo treatment stimulation with an ovalbumin, lymph node cells from rBCGmIL-18-treated mice produced less IL-5 and more IFN-γ than those of mice injected with nonrecombinant BCG (Biet et al., 2005). After a challenge with ovalbumin, a strong reduction of bronchoalveolar eosinophilia was observed in rBCGmIL-18-injected mice. This activity of the rBCGhIL-18 strain might be helpful in alleviating the symptoms of allergic reactions. The polarized response of Th2 lymphocytes to an allergen is considered to be the main cause of the pathogenesis of asthma (Kowalski et al., 2015).

Some data point to the crucial role of IL-18 in maintaining the homeostasis. A study group of IL-18 deficient mice indicated a predisposition of mice to obesity and other metabolic disorders. These mice were characterized with a significantly higher weight (by 40%) and an increase in the body fat content (over 100%) compared to wild-type animals. Individuals with a defect in the expression of the surface IL-18Rα receptor also showed a predisposition to obesity, diabetes and other metabolic disorders. These disorders were due to the inefficient functioning of the central nervous system region responsible for the regulation of the appetite, which might affect the depo-
sition of fat in the key blood vessels (Dinarello et al., 2013; Novick et al., 2013).

The knowledge of the biology system including IL-18, its receptor IL-18R and inhibitor IL-18BP allows to suggest a possibility of alleviating the symptoms of diseases associated with the IFN-γ overproduction, such as systemic lupus erythematosus, Wagner’s disease or Crohn’s disease, by blocking the IL-18 activity. It is worth emphasizing that blocking the activity of IL-18 may also find applications in the treatment of multiple sclerosis, where IL-18 promotes the expression of a surface vascular cell adhesion molecule 1, attributed to play an important role in the development of the disease (Dinarello et al., 2013; Novick et al., 2013; Wei et al., 2014).

Despite all the potentially positive aspects of administration of exogenous IL-18 in preventing various complications in bacterial infections, immunostimulation of antitumor responses or diminishing allergic disorders, this cytokine can also cause an exaggerated inflammatory response due to its potent IFN-γ inducing capability. It limits a possibility of IL-18 therapy only to immunocompromised hosts, where this cytokine may effectively restore the host immune responses without evoking any exaggerated inflammatory processes. In order to overcome these limitations, new methods of IL-18 administration need to be developed to avoid the potential harmful effects of exogenous IL-18. Recombinant BCG mycobacteria producing IL-18 seem to be a good formula for the administration of IL-18 (Biet et al., 2002, Lazo et al., 2004).

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