Review paper

Natural T_H2 (nT_H2) cells, interleukin 36 and interleukin 37 – new elements of innate immunity

AGATA MĘKAL, ALICJA TRZECIAK-RYCZEK, BEATA TOKARZ-DEPTUŁA, WIESŁAW DEPTUŁA

Department of Microbiology and Immunology, Faculty of Natural Sciences, University of Szczecin, Szczecin, Poland

Abstract

This paper presents new elements of innate immunity, such as recently described natural T_H2 cells (nT_H2 or NHC), interleukin 36 (IL-36) and IL-37. nT_H2 cells are located in structures defined as fat-associated lymphoid clusters (FALC), and their role is principally associated with the production of cytokines that are characteristic for T_H2 lymphocytes participating in the course of allergic processes and in host defence during invasions of various parasites. The expression of IL-36 was mainly recorded in the thymus, testis and uterus cells. This interleukin indicates suppressive effect on innate immunity, principally by inhibition of pro-inflammatory cytokine production, as well as by creation of an active complex with signalling protein – Smad3 and cooperation with TGF-β. In turn, IL-37 is synthesized in the same places as IL-36, and additionally in macrophages and epithelial cells. It is also considered as an inhibitor of innate immunity.

Key words: *innate immunity, nT_H2 cells, IL-36, IL-37.*

(Centr Eur J Immunol 2011; 36 (2): 113-116)

Introduction

At present, immune system can be divided into two components, namely elements forming natural, innate immunity, and the ones forming adaptive – acquired immunity. It is assumed [1] that innate immunity, also defined as constitutive immunity, is the defensine-, cytokine- and chemokine-dependent immunity of many immune cells, perforin-dependent activity of NK cells, cytotoxic activity of antibodies-independent complement, phagocytosis and non-specific reactivity of T-cells to viruses. Such immunity plays fundamental role not only in fighting of infections, but it is also important in the course of diseases associated with the impact of environmental factors. The latest studies indicated that elements forming innate immunity also include newly described natural T_H2 cells (nT_H2), as well as interleukin 36 (IL-36) and the very similar IL-37 [2-7].

Natural T_H2 cells (nT_H2)

Natural nT_H2 cells, previously defined as natural helper cells (NHC), form the population located in previously unnoticed structures named fat-associated lymphoid clusters (FALC), which are scattered along the blood vessels in the peritoneal mesentery in mammals, including humans [3, 7]. These cells were also found in adipose tissue around the kidneys and genitalia, and in the subcutaneous fat tissue, but their number was fewer [3]. On the basis of Giemsa staining and observation in electron microscopy, it was determined that nT_H2 have morphology characteristic of lymphoid cells, namely a round shape, dark nucleus, scant content of cytoplasm, and poorly developed Golgi apparatus and endoplasmic reticulum (ER) [3]. The number of these cells is limited by the small size and number of FALCs, yet their strategic location and secretion of specific cytokines cause that the role of such natural helper cells is significant [3, 7]. It was evidenced that such cells principally produce cytokines characteristic of Th2 lymphocytes, such as IL-4, IL-5, IL-10, and IL-13, and that their main function is associated with participation in the course of allergic processes and in host defence during invasions of various parasites [3, 7]. However it was indicated that they haven't got the TCR receptor – typical T-cell surface receptor, therefore they do not respond to antigens specific for such

Correspondence: Wiesław Deptuła, Department of Microbiology and Immunology, Faculty of Natural Sciences, University of Szczecin, Felczaka 3 C, 71-412 Szczecin, Poland. Phone number: +48 91 444 16 05, fax: +48 91 444 16 06, e-mail: kurp13@univ.szczecin.pl

cells. They respond to IL-2 and indicate expression of the common γ-chain receptor, which functions as the signalling component of the IL-2 receptor [7]. In response to stimulation with IL-25, IL-2, as well as IL-33, which is an untypical cytokine, because it activates the cells via the ST2 receptor, belonging to the family of interleukin 1 receptors, nT_H2 reveal higher expression of IL-5 and IL-13, and low expression of IFN-γ [7-10]. Furthermore, IL-33 is secreted by non-lymphoid cells (endothelial cells, epithelial cells, fibroblasts, adipose cells – adipocytes), and it stimulates nT_H2 not only to synthesis of IL-5 and IL-13, but also activates certain progenitor cells to produce GM-CSF (granulocyte/macrophage colony stimulating factor) [7, 11]. It was evidenced that most of the synthesized IL-33 is targeted to the nucleus of the cells producing it, from where it is periodically released, exclusively after their death, hence IL-33 may act as "alarmin", namely the substance that sends signals after cell death, while its release may be a potential signal informing about the danger in the organism [7, 11]. Probably, the induction of nT_H2 by IL-33 is a form of immune response to danger signals released when e.g. the intestinal mucosa is attacked by various pathogens and parasites [7, 11]. It was indicated that as a result of stimulation of nT_H2 cells with phorbol ester (PMA – phorbol myristate acetate), they reveal high expression of IL-2, IL-4, IL-5, IL-6 and GM-CSF, and rather lower expression of IFN-γ, although much higher than the level of this cytokine synthesized by lymphocytes T $CD4^+$ from spleen or lymphocytes that are present in the mesenteric lymph nodes [3]. It was, however, determined that nT_H2 cells do not produce the aforementioned cytokines after stimulation with lipopolysaccharide (LPS) or concanavalin A [3]. According to Moro *et al*. [3], nT_H2 reveal similarity to NK cells (natural killer cells) in the activation process, but contrary to them, they lack NK-cell-lineage markers, yet they have receptors characteristic of their progenitor cells, such as receptor for IL-7, c-Kit receptor, and Sca-1 receptor [3, 7]. It was determined that as a result of parasite infections, nT_H2 induce proliferation of B lymphocytes in Peyer's patches, as well as stimulate goblet cells to mucus production. These cells also produce IL-13, which impacts on the increase in the number of goblet cells (hyperplasia), which, as a consequence, facilitates the removal of e.g. *Nippostrongylus brasiliensis* from the intestines [7, 9, 10, 12]. In turn, in mice deprived of nT_H2 cells, hyperplasia of goblet cells was not recorded. It must be added that their location creates and allows them for contact with the population of B1 lymphocytes present in the peritoneal cavity, which are capable of self-renewal and participate in the quick but poorly specific defence against pathogenic microbes [7, 13]. It was evidenced that B1 cells produce antibodies specific to components of microorganisms or self-antigens e.g. the ones produced during programmed cell death [7, 14]. Moro *et al*. [3] proved that nT_H2 cells enhance proliferation of B1 lymphocytes owing to IL-5 they produce, which is the key growth factor for B1 cells. Apart from than, via IL-5, but also via IL-6, nT_H2 cells regulate the production of antibodies, principally IgA, which act on the surface of the mucosa, conditioning local immunity of the gastrointestinal tract, which would confirm their role and participation in the immune response associated with the immunity of the mucosa [3].

Interleukin 36 and interleukin 37

So far, IL-36 had been classified as IL-1F7 within the IL-1 family [2, 5, 6], however the different biological activity of this cytokine and the differences in its structure as compared to other cytokines from the IL-1 family, have allowed for its separation as an individual interleukin [5]. It was evidenced that it is synthesized in many tissues, yet its strong expression was principally recorded in the thymus, testes and uterus cells [6]. It is generated as an inactive form of pro-interleukin, which is then activated under the influence of caspase 1 and 4 [2, 15]. So far, it had been assumed that this interleukin has the capacity of binding to the receptor for IL-18 (IL-18R) and IL-18 binding protein (IL-18BP), however until present the biological function of such complexes has not been described [6,16]. In turn, the latest molecular studies revealed that IL-36 forms active complex with signalling protein Smad3 (transcription factor the name of which derives from Sma and MAD – *Caenorhabditis elegans* and *Drosophila melanogaster* gene homologues), and cooperates with TGF-β (transforming growth factor β) [5, 15, 17]. Moreover, it has been evidenced that inhibition or silencing of the activity of endogenous protein Smad3 causes significant weakening of biological properties of this interleukin [5]. Interleukin 36 has a suppressive effect onto innate response by inhibiting the production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF [5, 18]. It was indicated that silencing of endogenous IL-36 in the peripheral blood mononuclear cell culture (PBMC) causes the level of pro-inflammatory cytokines such as IL-1α/β, IL-6, TNF-α, GM-CSF, grows 3-fold with the unchanged level of anti-inflammatory mediators [5, 15]. In turn, the introduction of IL-36 to the culture epithelial cells of the lungs and macrophages results in inhibition, even up to 99%, of the pro-inflammatory cytokines production, after stimulation with LPS. Furthermore, the studies of that team [5] indicated that transformed mice indicating overproduction of IL-36 are protected in bacterial infections against toxic effect of LPS, and therefore against endotoxic (septic) shock. Studies at the cellular level have also proved that the activity of IL-36 leads to the decrease in the activity of dendritic cells (DC) [5]. It was evidenced that with the overproduction of this interleukin, the number of $CD86^+$/$MHCII^+$ receptors decreases on DC cells, which are responsible for their activation during the infection [5].

In turn, IL-37 described by Nodl *et al.* [4] is also included in the family of interleukin 1, which was

previously defined as IL-1F7. The authors [4] proved that the expression of IL-37 occurs in the same places as IL-36, and additionally in macrophages and epithelial cells, but only after stimulation with e.g. IL-18, IL-1β, TNF, IFN-γ. It is assumed that the function of IL-37 is similar to the function of IL-36, and is principally related to inhibition of the production of pro-inflammatory mediators, such as IL-1α, IL-1β, IL-6, IL-12, G-CSF, GM-CSF and TNF [4]. It was recorded that the level of these substances increases due to silencing of endogenous IL-37 in human blood cells, whereas the level of pro-inflammatory cytokines such as IL-10 remains unchanged. Furthermore, just as in the case of IL-36 [5], transgenic mice with overproduction of IL-37 [4], are protected against the shock induced by LPS, and also indicate reduction to liver damage after administration of LPS. Apart from that, analogically as IL-36, IL-37 interacts with Smad3 protein [4]. Such reactions are significantly impacted upon by TGF-β_1, because its low concentrations induces endogenous IL-37. Furthermore, it was evidenced that IL-37 is the only cytokine binding Smad3 protein that is transferred to the nucleus after phosphorylation, which after binding to DNA causes inhibition to activation of DC cells, macrophages, including their cytotoxicity, and acquisition by T lymphocytes of the tolerance capacity to their own antigens [4, 19-21]. The studies proved that in the "asymptomatic conditions", in the case of Cytomegalovirus (CMV) infection, low level of IL-37 was recorded, while with the development of the infection and the inflammation, a clear increase in its level was observed, and as a consequence – reduction in expression of pro-inflammatory cytokines [4]. Therefore, Nodl *et al*. [4] conclude that inhibition of pro-inflammatory cytokine production by IL-37 is required in the later phases of immune response when the danger to the body increases, thus expression of this cytokine prevents the development of excess inflammatory response. Also, the studies performed on THP-1 (human acute monocytic leukaemia cell line) that generate IL-37, which were stimulated with LPS and IFN-γ, revealed that IL-37 inhibits activation of many kinases, e.g. STAT1-4 kinases (Signal Transducer and Activator of Transcription), involved in the signal transduction pathway for pro-inflammatory cytokines, such as IL-6, IL-12 and IFN-γ, as well as MAP p38α kinase (mitogen-activated protein kinase), involved in several signal cascades, principally pro-inflammatory ones [4, 22, 23]. On the basis of such observations, it is assumed that, similarly as IL-36, IL-37 is an inhibitor to immune response associated with innate immunity, principally owing to the suppression of pro-inflammatory cytokine production, which allows for its classification, aside e.g. IL-10 and TGF-β, into the group of anti-inflammatory cytokines [4]. It must also be added that the striking similarity between these two interleukins (IL-36 and IL-37), described by the same team of authors [4, 5], allows for suspecting that they may be one cytokine.

Conclusion

To conclude, one must determine that natural T_H2 cells (nT_H2, previously referred to as NHC) and IL-36 and IL-37, constitute new and important elements of innate immunity, as they have an important role in the correct functioning of the immune system in mammals. It is suggested that these cells, although recently discovered, may constitute evolutionary old mechanism that preceded the development of adaptive immunity, and prevents the auto-immunisation processes. In turn, IL-36 and the very similar IL-37, by revealing suppressive effect onto innate immune response, may be a very important element protecting the body e.g. against the septic shock caused by the very widespread particles of bacterial origin, such as LPS.

References

1. Deptuła W, Tokarz-Deptuła B, Stosik M (2008): Immunology for biologists – new edition. University of Szczecin, Szczecin (in Polish).
2. Kumar S, Hanning CR, Brigham-Burke MR et al. (2002): Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. Cytokine 18: 61-71.
3. Moro K, Yamada T, Tanabe M et al. (2010): Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature 463: 540-544.
4. Nold MF, Nold-Petry CA, Zepp JA et al. (2010a): IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol 11: 1014-1022.
5. Nold MF, Nold-Petry CA, Zepp JA et al. (2010b): IL-36 (Formerly IL-1F7) suppresses innate immunity by inhibiting inflammatory cytokines and dendritic cell activation by association with SMAD3. Cytokine 52: 3-4.
6. Pan G, Risser P, Mao W et al. (2001): IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. Cytokine 13: 1-7.
7. Strober W (2010): Immunology: The expanding T_H2 universe. Nature 463: 434-435.
8. Chackerian AA, Oldham ER, Murphy EE et al. (2007): IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. J Immunol 179: 2551-2555.
9. Fallon PG, Ballantyne SJ, Mangan NE et al. (2006): Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5 and IL-13 at the onset of helminth expulsion. J Exp Med 203: 1105-1116.
10. Knight PA, Brown JK, Pemberton AD (2008): Innate immune response mechanisms in the intestinal epithelium: potential roles for mast cells and goblet cells in the expulsion of adult Trichinella spiralis. Parasitology 135: 655-670.
11. Haraldsen G, Balogh J, Pollheimer J et al. (2009): Interleukin-33 – cytokine of dual function or novel alarmin? Trends Immunol 30: 227-233.
12. Humphreys NE, Xu D, Hepworth MR et al. (2008): IL-33, a potent inducer of adaptive immunity to intestinal nematodes. J Immunol 180: 2443-2449.
13. Hardy RR (2006): B-1 B cell development. J Immunol 177: 2749-2754.
14. Duan B, Morel L (2006): Role of B-1a cells in autoimmunity. Autoimmun Rev 5: 403-408.

15. Sims JE, Smith DE (2010): The IL-1 family: regulators of immunity. Nat Rev Immunol 10: 89-102.
16. Bufler P, Azam T, Gamboni-Robertson F et al. (2002): A complex of the IL-1 homologue IL-1F7b and IL-18 binding protein reduces IL-18 activity. Proc Natl Acad Sci USA 99: 13723-13728.
17. Grimsby S, Jaensson H, Dubrovska A et al. (2004): Proteomics-based identification of proteins interacting with Smad3: SREBP-2 forms a complex with Smad3 and inhibits its transcriptional activity. FEBS Lett 577: 93-100.
18. Sharma S, Kulk N, Nold MF et al. (2008): The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. J Immunol 180: 5477-5482.
19. Li MO, Flavell RA (2008): Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. Immunity 28: 468-476.
20. Trotta R, Col JD, Yu J et al. (2008): TGF-beta utilizes SMAD3 to inhibit CD16-mediated INF-gamma production and antibody-dependent cellular cytotoxicity in human NK cells. J Immunol 181: 3784-3792.
21. Werner F, Jain MK, Feinberg MW et al. (2000): Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. J Biol Chem 275: 36653-36658.
22. Ross JA, Nagy ZS, Cheng H et al. (2007): Regulation of T cell homeostasis by JAKs and STATs. Arch Immunol Ther Exp (Warsz) 55: 231-245.
23. Schieven GL (2005): The biology of p38 kinase: a central role in inflammation. Curr Top Med Chem 5: 921-928.