Cytokine & Growth Factor Reviews xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Mini review

Interleukin 35: Critical regulator of immunity and lymphocyte-mediated diseases

Charles E. Egwuagu [a,*], Cheng-Rong Yu [a], Lin Sun [a], Renxi Wang [b]

[a] Molecular Immunology Section, Laboratory of Immunology, National Eye Institute (NEI), National Institutes of Health (NIH), Bethesda, MD 20892, USA
[b] Laboratory of Immunology, Beijing Institute of Basic Medical Sciences, Beijing 100850, China

ARTICLE INFO

Article history:
Received 8 July 2015
Accepted 8 July 2015

Keywords:
Interleukin 35
IL-12 family cytokines
Autoimmune diseases
Cytokine therapy
Regulatory B cells
Breg
i35-Breg
iTR35
Adoptive B cell therapy

ABSTRACT

Cytokines coordinate the activities of innate and adaptive immune systems and the Interleukin 12 (IL-12) family of cytokines has emerged as critical regulators of immunity in infectious and autoimmune diseases. While some members (IL-12 and IL-23) are associated with the pathogenesis of chronic inflammatory diseases, others (IL-27 and IL-35) mitigate autoimmune diseases. Unlike IL-12, IL-23 and IL-27 that are produced mainly by antigen presenting cells, IL-35 is predominantly secreted by regulatory B (i35-Bregs) and T (iTR35) cells. The discovery that IL-35 can induce the conversion or expansion of lymphocytes to regulatory B and T cells has considerable implications for therapeutic use of autologous regulatory B and T cells in human diseases. Although our current understanding of the immunobiology of IL-35 or its subunits (p35 and Ebi3) is still rudimentary, our goal in this review is to summarize what we know about this enigmatic cytokine and its potential clinical use, particularly in the treatment of CNS autoimmune diseases.

Published by Elsevier Ltd.

1. Introduction

Detection and eventual elimination of pathogens derive from productive interactions between the innate and adaptive immune systems. Cytokines produced by innate immune cells provide instructional signals for the differentiation of naïve lymphocytes into appropriate effector subsets while the differentiated effector lymphocytes in turn produce cytokines that orchestrate adaptive immune responses that eventually eliminate the pathogen and re-establish immune homeostasis. Cytokines are a broad group of soluble factors that function in an autocrine or paracrine manner. They play pivotal roles in coordinating activities of diverse immune cell types by coupling extracellular stimuli to intracellular signal transduction networks that mediate multiple physiological processes including differentiation, cell growth and development of target cells [1]. More than 100 cytokines have been described and classified into distinct families on the basis of their structure or receptor composition and include interleukins, interferons, hematopoietins, TNF family, adipokines and chemokines [1,2]. In this review, our focus is on Interleukin 35 (IL-35), a member of the enigmatic IL-12 family of cytokines that have profound influence on cell-fate decision of differentiating lymphocytes and play crucial roles in shaping and regulating host immunity. In contrast to the other members of the family (IL-12, IL-23) that are pro-inflammatory, IL-35 is an immune-suppressive cytokine and a critical regulator of immunity during autoimmune and infectious diseases [3–5]. Here, we discuss the immunobiology of IL-35 and its considerable appeal as an important therapeutic target. We also highlight proof-of-principle studies which have established that IL-35 and IL-35-producing regulatory lymphocytes are effective in suppressing mouse CNS (Central Nervous System) autoimmune diseases and suggest that autologous i35-Breg therapy may be effective in the treatment of human autoimmune diseases such as uveitis and multiple sclerosis.

2. The Interleukin-12 (IL-12) family cytokines

Studies over the past decade have revealed that the quality and nature of the immune response is influenced by the predominant cytokines secreted by APC (antigen-presenting cells) and the cytokines found to play critical roles in lymphocyte differentiation and/or cell-fate decisions are mostly members of the Interleukin 12 (IL-12) family of heterodimeric cytokines.

The IL-12 family of cytokines is comprised of IL-12, IL-23, IL-27 and IL-35 and belongs to the Type 1 family of hematopoietic cytokines [6]. Unlike most cytokines that function as monomeric,

* Corresponding author at: Molecular Immunology Section, National Eye Institute, National Institutes of Health, Building 10, Room 10N109A, 10 Center Dr., Bethesda, MD 20892-1857, USA.
E-mail address: egwuaguc@nei.nih.gov (C.E. Egwuagu).

http://dx.doi.org/10.1016/j.cytogfr.2015.07.013
1359-6101/Published by Elsevier Ltd.

homodimeric, or homotrimeric proteins, IL-12 cytokines are one of a few cytokines that function as heteromers. Each member comprises of heterodimeric subunits; an α-subunit with a helical structure similar to classic 4-helix bundle type 1 cytokines such as IL-6 and a β-subunit structurally related to the soluble IL-6 receptor (IL-6Rα) [6]. As the three α-subunits (IL-12p35, IL-23p19 and IL-27p28) are structurally related, each can conceivably pair with either of the structurally homologous β subunits (IL-12p40 and Ebi3) and this is indeed the basis for the shared usage of IL-12p40 by IL-12 and IL-23 and similar sharing of Ebi3 by IL-27 and IL-35 [7,8]. Although there are currently four known members in the family, the predictable range of combinations is six and it is conceivable that additional IL-12 members would soon be discovered. It is however interesting that pairing of an alpha chain with IL-12p40 appears to generate IL-12 cytokines that promote inflammation while dimerization with Ebi3 gives rise to members that suppress inflammation and autoimmune diseases (Fig. 1). Mechanistically, IL-12 cytokines mediate their biological activities through high-affinity receptors composed of heterodimeric or homodimeric subunits, each characterized by presence of cytokine-receptor homology domains, fibronectin-like domains and immunoglobulin-like domains [6,9]. Upon engaging their cognate receptor, receptor-associated Janus kinases (JAKs) are activated by transphosphorylation, providing phosphotyrosine-docking sites that recruit specific members of the STAT (signal transducers and activators of transcription) family of transcription factors [6,9]. STATs recruited to the receptor complex are phosphorylated at critical tyrosine residues present at the transcription activation domain, form homo- or hetero-dimers and translocate into the nucleus where they bind to specific DNA sequences and modulate gene expression [10,11].

Interleukin 12 (IL-12) was the first member of the family described and was identified and purified from the cell culture media of Epstein–Barr virus (EBV)-transformed B lymphoblastoid cell lines [12]. It is comprised of the IL-12p35 and IL-12p40 subunits [6] and several transcription factors including IFN-regulatory factor-1 (IRF-1) and IRF-8 have been implicated in regulation of the gene coding for IL-12p35 or IL-12p40, respectively [13,14]. IL-12p35 is ubiquitously expressed, while IL-12p40 expression is inducible in some hematopoietic cell types and co-expression of both subunits in the same cell is required to produce the disulfide-linked bioactive IL-12p70 cytokine [15,16]. Although it can be secreted by a variety of hematopoietic cell types and EBV-transformed B cells, the major physiological producers of the IL-12 cytokine are dendritic cells (DCs) and macrophages. High-affinity IL-12 receptor (IL-12R), comprised of IL-12Rβ1 and IL-12Rβ2 subunits, is expressed mainly by activated T cells, NK cells and detectable on DCs and EBV-transformed B cells lines. It is however, undetectable on most resting T cells. Naïve T cells are unresponsive to IL-12 signal because they express only the IL-12Rβ1 subunit while antigen-stimulation Th1 cells express both subunits and are responsive to IL-12. The IL-12 signal is transduced by TYK2 (tyrosine kinase 2) that constitutively associates with IL-12Rβ1 subunit and JAK2 with the IL-12Rβ2 subunit [6,17]. Although STAT1, STAT3 and STAT4 are all activated to varying extents by IL-12 *in vitro*, physiological responses of IL-12 are mediated mainly through activation of STAT4 [6,17,18].

Interleukin-23 (IL-23) was discovered in 2003 and shares the IL-12p40 subunit with IL-12 but differs from IL-12 because of its unique IL-23p19 subunit [19–21]. Similar to IL-12, co-expression of IL-12p40 and IL-23p19 subunits in the same cell is required to secrete the disulfide-linked bioactive IL-23 cytokine. Sharing the IL-12p40 subunit enables IL-12 and IL-23 to interact with the IL-12Rβ1 receptor subunit. The high affinity IL-23 receptor derives from the combination of IL-12Rβ1 with a unique IL-23 receptor subunit (IL-23R) and biological effects of IL-23 on its target cells are mediated through activation of TYK2, JAK2, STAT3 and STAT4 [20,21]. Many innate immune cells including DCs, macrophages, B cells and endothelial cells produce IL-23 and the high affinity IL-23 receptor is expressed on activated T cells and other immune cells including Th17 cells, γδ T cells, natural killer T (NKT) cells and innate lymphoid cells (ILCs) [19]. IL-23 prolongs the expression of type 17 signature cytokines (such as IL-17, IL-22 and GM-CSF) that induce tissue pathology and mediate chronic inflammation by promoting the survival and maintenance of Type 17 cells [19,20].

Interleukin 27 (IL-27) was first identified in 1996 and it is comprised of EBV-induced gene 3 (Ebi3) and IL-27p28 subunits [22]. The Ebi3 subunit is a soluble type I cytokine receptor-like molecule that shares homologies with IL-12p40 and CNTFR while IL-27p28 is similar to the IL-12p35 or IL-23p19 subunits of IL-12 and IL-23 cytokines, respectively [21,23]. In contrast to IL-12 and IL-23, IL-27p28 and Ebi3 are not secreted as disulfide-linked dimer and the nature of the association between IL-27p28 and Ebi3 *in vivo* is uncertain [24]. Thus, co-expression of Ebi3 and IL-27p28 subunits in the same cell may not be required for production of the bioactive IL-27 cytokine and may instead be secreted

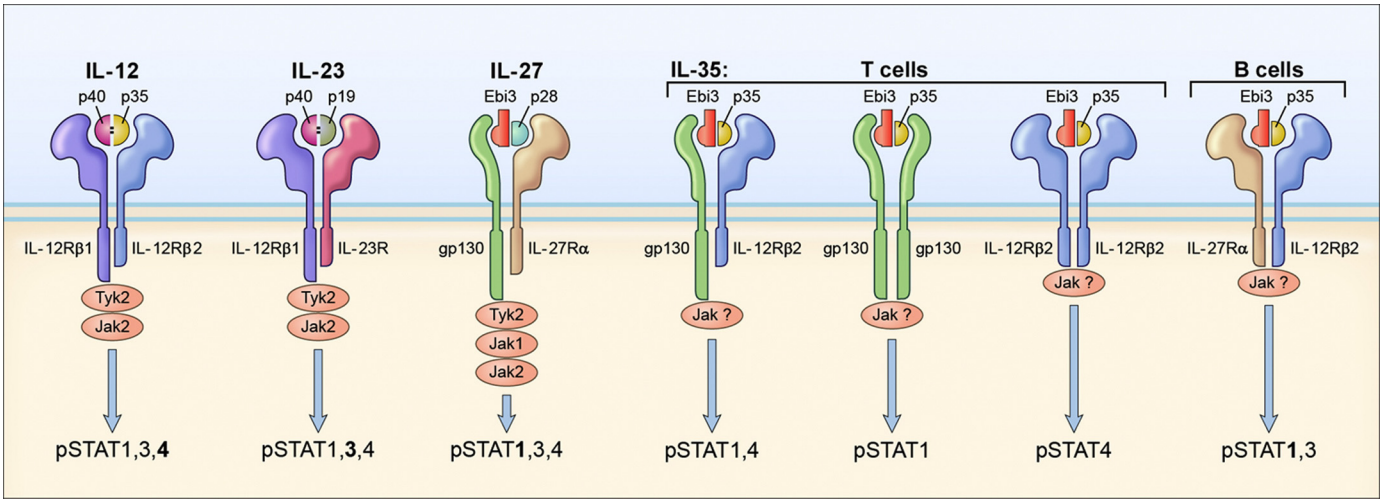

Fig. 1. The IL-12 family of heterodimeric cytokines. Each member is comprised of an α-subunit (IL-12p19, IL-12 p35, IL-27p28) homologous to classic 4-helix bundle type 1 cytokines (*e.g.* IL-6) and a β-subunit (IL-12p40, Ebi3) structurally related to the soluble IL-6 receptor (IL-6Rα). Upon binding to cognate receptors (IL-12Rβ1, IL-12Rβ2, IL-23R, IL-27Rα, or gp130), receptor-associated Janus kinases (Jak1, Jak2, Tyk2)) are activated, providing phosphotyrosine-docking sites that recruit specific members of the STAT (signal transducers and activators of transcription) family of transcription factors.

Please cite this article in press as: Egwuagu CE, et al. Interleukin 35: Critical regulator of immunity and lymphocyte-mediated diseases. Cytokine Growth Factor Rev (2015), http://dx.doi.org/10.1016/j.cytogfr.2015.07.013