ROLE OF ASSESSMENT OF SERUM LEVELS OF INTERLEUKIN-23 AND POLYMORPHISM OF INTERLEUKIN-12 RECEPTOR Β1 IN PATIENTS WITH ALLERGIC RHINITIS

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ABSTRACT

Background: Allergic rhinitis (AR) is a worldwide health problem that affect nearly 10–20% of whole population, therefore AR can be the most prevalent chronic non contagious disease. Several factors in the environment contribute to allergic diseases (for example, antibiotics, immunizations, cigarette smoke, pets, and diet); however, it is hard to understand the way of changing the surroundings to decrease the potential risks. Th17 cells might be involved in the process of neutrophil infiltration that occurs during the acute phase of the allergic reaction. The action of the proinflammatory cytokine IL-23 is dependent mainly on IL-12RB1, a type 1 transmembrane receptor that is associated with the p40-domain of IL-23 and promotes its signaling in complex with IL-23R. IL-23 was suggested to be a pivotal cytokine involved in the pathogenesis of AR and may become a novel target in the treatment of AR.

Keywords: Allergic Rhinitis, Interleukin-23, Polymorphism of Interleukin-12 Receptor B1.

I. ALLERGIC RHINITIS:

Allergic rhinitis (AR) is an immunoglobulin E (IgE) mediated inflammatory chronic disorder of the nasal mucosa caused by contact to allergens which affects a significant percentage of population among different countries with an increasing trend over the last years. Nasal symptoms such as nasal congestion, watery rhinorrhea, nasal itching, sneezing and post-nasal drip, are primarily observed in patients with AR. Snoring or mouth breathing due to nasal obstruction, ocular itching, pain or tearing, cough, loss of taste or smell, or even hearing dysfunction might be secondary chronic or recurrent symptoms of allergic rhinitis which can lead to sleep disturbance, irritability or sleepiness affecting emotional, physical, and social aspects of quality of life. AR is a very prevalent disease, but it is frequently underdiagnosed. The prevalence of AR is globally increased. A report from the World Health Organization (WHO) indicates that 40% of the population suffers from one or more allergic disease (1).

In adults, the prevalence of AR varies from 10% to 30%, while in children it is nearly 40% (2).

II. IMMUNOPATHOGENESIS OF ALLERGIC RHINITIS

1- Sensitization to allergens:

AR is an IgE-mediated disease, caused by environmental allergen exposure in genetically susceptible individuals, and is due to alterations in their immune system. Common allergens implicated in AR are mainly proteins and glycoproteins found in airborne particles. Following inhalation, allergen particles are deposited on the surface of the nasal epithelium with consequent elution of soluble allergenic proteins and their diffusion into the nasal mucosa (3).

During the initial sensitization process in AR, numerous aeroallergens facilitate allergen entrance to antigen presenting cells (APCs) over their protease activities which cleave tight junctions in the airway epithelium and stimulate epithelial cells. Activated nasal epithelial cells secrete thymic stromal lymphopoietin (TSLP), IL-33,
IL-25 and other cytokines and chemokines that affect group 2 innate lymphoid cells (ILC2s) and Th2 T lymphocytes directly or via contact with APCs located within and below the nasal epithelium (4).

The epithelial cytokines (TSLP, IL-33 and IL-25) have been shown to be vital for the activation of ILC2s that lack T cell receptors and do not express T cell nor other cell lineage markers (5).

APCs that include dendritic cells (DCs) (expressing CD1a, CD11c) and macrophages arrest allergens, mature and transfer to the draining lymph nodes, where they present processed allergen to naive T cells that are afterward tilted in favour of Th2 T cell expansion (3).

It has been shown that ILC2s may also moderate and differentiate naïve T cells into Th2 cells by producing IL-13 which is essential for dendritic cells to be operated into lymph nodes and subsequently leads to Th2 cell preparing (6).

Allergen-stimulated Th2 cells produce IL-4 that sustains Th2 cells. Th2 cells also produce IL-13 and prompt CD40 ligand (CD40L), which with IL-4 promotes heavy-chain class switching in B lymphocytes and also IgE production. IgE bind to the high-affinity receptor (FcεRI) on mast cells, basophils and APCs and sensitize these cells to allergens (3).

On allergen re-exposure, cross-linking of IgE–FcεRI complexes on APCs facilitates allergen uptake by APCs for processing and presentation. While IgE–FcεRI interaction on mast cells and basophils with allergen induces the classic early-phase allergic reaction (EPR), a proportion of subjects subsequently develop a late-phase inflammatory response (3).

**Figure1:** Activated epithelial cells secrete TSLP and IL-33 that activates dendritic cells directly or through ILC2s which captures antigens, migrates to the draining lymph nodes and presents to naive T cells inducing effector Th2 cells. Activated Th2 cells in local lymph nodes secrete IL-4 that promotes class switching to IgE production by B cells. Re-exposure of the sensitized allergen leads to cross-linking of IgE–FcεRI complexes on DCs, mast cells and basophils activating these cells to release of inflammatory mediators causing classic allergic reactions. Other Th2 cytokines such as IL-5, IL-9 and IL-13 are responsible for propagation and maintaining late-phase allergic inflammation (3).
AR is a perfect example for understanding allergic inflammation, where the generating factors can obviously be identified, mainly in patients with seasonal AR who can be observed through and out of the pollen season. Nasal secretions and mucosal response are easily accessible for procedures to study clinical and immunological responses in AR (7).

The prior hypothesis of Th1/Th2 imbalance promoted AR is expanded by identification of novel T-helper families, such as regulatory T cell (Treg), Th17 and Th9 (8).

Tregs play central roles in blocking the T helper 2 (Th2) differentiation, controlling airway allergic inflammation, and inhibiting inappropriate Th2 responses to environmental aeroallergens (8).

Tregs help by their effective contact with immune cells or by secretion of anti-inflammatory cytokines, for example interleukin IL-10 and transforming growth factor (TGF)-beta (9).

2-Immediate (Early) phase reactions (Mast cells activation):

Allergen challenge in sensitized individuals results within minutes in early-phase symptoms such as sneezing and itching followed by rhinorrhea and nasal obstruction which then incline to resolve probably within one hour. As shown in, allergen cross-linking complexes of sensitized IgE with FceRI at the surface of mast cells and basophils lead to degranulation of these cells and the discharge of preformed mediators such as histamine and tryptase, and the de novo generation of mediators from the membrane lipid such as cysteinyl leukotrienes (leukotrienes C4, D4 and E4) and prostaglandins D2 (10).

Itching as a common symptom of AR is elicited by histamine through H1 receptors that acts on sensory nerve endings, leading to a systemic response, such as outburst of sneezing. Also oedema and nasal congestion are due to secretion of leukotrienes, prostaglandin D2 and vascular endothelial growth factors which cause plasma leakage from blood vessels, pooling of blood in venous sinusoids and an increase in glandular mucus secretion (11).

Nasal fluid tryptase and histamine levels have been demonstrated to peak at 5 min indicating immediate activation of local mast cells after allergen contact followed by subsequent increases in the expression of surface activation markers such as CD63 on circulating basophils (3).

The essential role of mast cells and basophils has been underlined by Thurmond et al (12). After activation of tissue mast cells through FcεRI and the release of histamines, basophils are enrolled through H4 receptors to the nasal mucosa which are afterward stimulated through FcεRI in an allergen specific manner.

Additionally, prostaglandin D2 has been recognized as a vital mediator during the EPR that signals through its receptor, CRTh2 (Chemoattractant Receptor-homologous molecule expressed on T-Helper type 2 cells). Blockade of CRTh2 led to the prevention of development of both early and late phase responses to intranasal allergen challenge (12).

3- Late phase of allergen-induced airway inflammation:

Major Late-phase symptoms are nasal obstruction and watery nasal discharge. Depending on patient vulnerability and the dose of allergen, allergic individuals will manifest a late-phase nasal allergic response. In divergence to the lung, nasal late responses manifest largely as continuous symptoms and falls in peak nasal inspiratory flow at 4–12 h as shown in. Mediators released during the early phase response induce the activation of various inflammatory cells leading to late-phase response symptoms (13).

Adhesion molecules such as vascular cell adhesion molecule 1, E-selectin and intercellular adhesion molecule 1 facilitate flood of inflammatory cells towards the nasal mucosa, this promotes adherence of circulating eosinophils to endothelial cells. Also chemoattractants and cytokines such as IL-5 stimulate the infiltration of eosinophils, basophils and T cells from the systemic circulation into the nasal submucosa (13).

It has been shown that circulating ILC2s increase following nasal allergen provocation and during allergen exposure in AR patients. ILC2s denote alternative Th2 cytokine-producing cells besides mast cells, basophils and T cells that may contribute to constant nasal allergic inflammation response (13).
Nasal turbinate biopsies from AR patient obtained 6 h after allergen challenge and examined by immunohistochemistry revealed increased expression of the lymphocyte chemokine receptors CCR3, CCR4, infiltration by eosinophils and elevated levels of cells expressing mRNA for IL-4 and IL-5 (14).

Moreover, induction of rhinitis symptoms after grass pollen nasal allergen challenge displayed an increase in activation markers expressed on peripheral blood basophils, plasmacytoid dendritic cells and memory T cells at 6 h indicating induction of local and also systemic events indicating stimulation of Th2 cells (15).

Cytokines released from basophils, mast cells and Th2 cells such as IL-4, IL-5, IL-9 and IL-13 play an important role in the late-phase response. It has been demonstrated that there is an inverse correlation between both IL-5 and IL-13 with nasal patency post-challenge (7).

Both IL-4 and IL-5 play essential roles in eosinophil activation leading to release of major basic protein, eosinophil cationic protein and eosinophil peroxidase. These are known to be harmful to respiratory epithelium encouraging increased oxidative stress leading to epithelial damage. This leads to the release of epithelial-derived chemokines, cytokines and growth factors that ease persistence of late-phase responses and subsequent allergic inflammation (13)

IL-13 shares with IL-4 many activities including usage of a common receptor subunit (IL-4Rα-chain). IL-13 is released from mast cells, basophils and ILC2s and promotes B cells to switch to IgE synthesis (16).

III. IL-23 AND IL-12RB1 BIOLOGY AND FUNCTION

IL-23:
Interleukin-23 (IL-23) is a newly discovered cytokine that resemble IL-12 structurally and functionally. IL-23 is a composite cytokine composed of subunits IL-23p19 and p40 linked through a disulfide bond, its receptor complex is composed of IL-12RB1 (which is also shared with IL-12) and IL-23R. IL-23 is produced by various innate immune cells including DCs, macrophages, B cells and endothelial cells. IL-23 is a proinflammatory cytokine, its action is dependent mainly on IL-12RB1, a type I transmembrane receptor that physically associates with the p40-domain and promotes its signaling (17).

IL-23 is chiefly involved in promoting Th17 differentiation and proliferation via stimulation of the naive CD4 T cells to differentiate into Th17. It plays a pivotal role in the pathogenesis of many autoimmune and inflammatory diseases (18).

Homology search of the human DNA sequence database with a probe of the highly conserved C-terminal D helix segment of IL-6 related cytokines led to the identification and cloning of human IL-23p19; the IL-23 subunit p19 gene is located on the human chromosome 12 and on the murine chromosome 10 (19).

IL-23p19 is a non-glycosylated protein of 18.7 kDa and has the typical four-helix bundle structure characteristic for all IL-6/IL-12-type cytokines, made up of four long helices (A, B, C, D) arranged in an up-up-down-down topology (19).

Similar to IL-12, co-expression of IL-23p19 and p40 is required for secretion of the bioactive heterodimer IL-23 because p40 leads to enhanced secretion of IL-23p19. The p40 subunit enables IL-23 to interact with the IL-12RB1 (19).

Additionally, a physiological role for p40 domain is chemoattraction for macrophages which is mediated by intracellular domain of IL-12RB1. Upon recruitment of IL-23R and IL-12RB1 by IL-23 intracellular receptor-associated Janus kinases, IL-23 become activated through phosphorylation of several tyrosine residues within the intracellular domain of the IL-23R (19). Tyrosine residues within the intracellular IL-23R domain induce downstream effector molecules for STAT, MAPK and PI3K signaling. IL-23 signaling is dependent on the activation of Tyrosine kinase (Tyk) 2, which phosphorylates majorly STAT3, and to a lesser extent STAT1, STAT4 and STAT5 (20).

IL-12RB1:
IL-12RB1 is a glycosylated type I membrane protein of 70.5 kDa with 5 extracellular domains, a single transmembrane domain and a cytoplasmic domain. IL-12RB1 has two extracellular cytokine binding domains (CBD,
D2 and 3) and three fibronectin-type III domains. Encoded by the gene IL-12RB1, IL-12RB1 physically associates with IL-12 and IL-23 and signals in complex with IL-12RB2 or IL-23R, respectively (19).

The extracellular portion of IL-12RB1 contains the cytokine-binding region essential for physical association with IL-23, whereas the cytoplasmic portion acts in concert with IL-12RB2/IL-23R to transmit intracellular signals via the pre-associated kinases TYK2 and JAK2. Complicating IL-12RB1’s reputation as a promoter of human health is its association with IL-23 signaling” (20).

**Figure 2:** IL-12RB1 contributes to both the IL-12 and IL-23 signaling pathways. (A and B) The IL-12 signaling pathway comprises the cytokine IL-12 (a disulfide-linked heterodimer of the proteins p40 and p35), the type 1 transmembrane proteins IL-12RB1 and IL-12RB2, the intracellular pre-associated kinases Tyk2 (which associates with IL-12RB1) and Jak2 (which associates with IL-12RB2), and intracellular STAT4. (A) Prior to IL-12’s engagement with IL-12RB1 and IL-12RB2, STAT4 exists in monomeric form and is inactive as a transcription factor. (B) After IL-12’s engagement with IL-12RB1 (via its p40 domain) and IL-12RB2 (via its p35 domain), Tyk2 and Jak2 phosphorylate STAT4, resulting in STAT4/STAT4 homodimer formation. This homodimer translocates to the nucleus, where it serves as a transcription factor for multiple genes associated with Th1 development. (C and D) The IL-23 signaling pathway comprises the cytokine IL-23 (a disulfide-linked heterodimer of the proteins p40 and p19), IL-12RB1 and IL-23 (also a type 1 transmembrane protein), Tyk2 (which associates with IL-12RB1) and Jak2 (which associates with IL-23R), STAT4 and STAT3. (C) Prior to IL-23’s engagement with IL-12RB1 and IL-23R, STAT4 and STAT3 exists in monomeric forms and are inactive as a transcription factors. (D) After IL-23’s engagement with IL-12RB1 (via its p40 domain) and IL-12RB2 (via its p19 domain), Tyk2 and Jak2 phosphorylate STAT4 and STAT3, resulting in STAT4/STAT3 heterodimer formation. This heterodimer translocates to the nucleus, where it promotes the transcriptional signature associated with Th17 development (20).

**Development and Role of Th17 in Inflammation and Allergy**

Upon antigenic stimulation, naive CD4 T cells differentiate into 2 subsets, Th1 and Th2 cells, characterized by different cytokine production and effector functions. Th1 cells produce IFN-γ and regulate cell mediated immunity while Th2 cells involved in humoral immunity, produce IL-4, IL-5, and IL-13. IL-12 induces the
differentiation of naive CD4 T cells into IFN-γ producing Th1 cells through activation of STAT4. IFN-γ signals are transduced by STAT1, which activates a downstream transcription factor, T-bet, that enhances the expression of genes specific to Th1 cells. In contrast, IL-4 induces STAT6 activation, promoting the expression of GATA-3, a transcriptional factor essential for both IL-4 production and Th2 cell differentiation (19).

**Figure 3:** IL-23 promotes the development of Th17. IL-23 induces the differentiation of naive CD4 T cells into IL-17 producing helper T cells via mechanisms that are distinct from the Th1 and Th2 differentiation pathways. The transcriptional factors critical for the development of Th1 (STAT1, STAT4, and T-bet) and Th2 (STAT6) cells are not required for the induction of Th17 cells. The transcriptional factor(s) essential for the development of Th17 cells remain unknown. IFN-γ and IL-4 antagonize each other in the differentiation of Th1 and Th2 cells and the promotion of their function. IFN-γ also suppresses the differentiation of Th17 cells by reducing IL-23R expression on CD4 T cells. IL-4 also inhibits the development of Th17 cells. Studies suggest that Treg derived TGF-β induces the differentiation of Th17 cells from naive CD4 T cells (19).

Th17 cells are characterized by the production of various cytokines, including IL-17, IL-6, TNF-a, and IL-22. IL-17 is involved in tissue pathology in autoimmune models, anti-IL-17 reduced neutrophil infiltration in an experimental murine asthma model and increased eosinophil infiltration, whereas IL-17 induced recruitment and was identified as a survival factor for airway macrophages. These facts seem to suggest a regulatory role for IL-17. However, the role of Th17 cells in allergic inflammation is still obscure. Experimental studies seem to suggest that Th17 cells might be involved in the process of neutrophil infiltration that occurs during the acute phase of the allergic reaction (21).

IL-23 drives Th17, which produce IL-17 family cytokines IL-17A and IL-17F. IL-17 producing T helper cells (Th17 cells) and regulatory T cell (Treg cells) provided new understanding of the molecular mechanisms convoluted with immunological disorders, predominantly Th17 cells, have been contributed to the pathogenesis of classically recognized Th2-mediated allergic disorders (22).

**IL-12RB1 Gene Polymorphism**

The biological activities of IL-12 and IL-23 are mediated through high affinity binding to IL-12 receptor (IL-12R), which is composed of two subunits, IL-12Rβ1, encoded by IL-12RB1, and IL-12Rβ2, encoded by IL-12RB2 gene (24).

Previous studies showed that IL-12Rβ1/β2 genes were related to atopic dermatitis and IL-12Rβ2 gene was also associated with primary biliary cirrhosis (24).

The possible associations of IL-12Rβ1/β2 genes polymorphisms with AR have not yet been well studied (25).

**Conflict of Interest:** No conflict of interest.
REFERENCES


