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Interleukin-4 Signaling Pathway and Effects in Allergic Diseases

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Abstract: Background: Allergic diseases, such as atopic dermatitis and allergic asthma, are associated with increased inflammation and interleukin-4 (IL-4) signaling. An inhibitor of the IL-4 receptor, dupimulab, was approved recently for dermatitis. This goal of this review is to elucidate the mechanism and effects of IL-4 signaling.

Methods: We reviewed information available in immunology and molecular biology textbooks, and research and review articles to accomplish our goal.

Results: The increased inflammation, in allergic diseases, is due to inflammatory cytokines released from innate leukocytes and local tissue. The increased IL-4 signaling activates the helper T(Th2) cells to release IL-4, and the allergic effects. The IL-4 binds to its receptors to activate JAK1/JAK3 mediated nuclear translocation of the phosphorylated STAT6 dimer, which stimulates the expression of IgE antibodies in B-cells. The released IgE stimulates the release of histamines from mast cells, alters the expression of genes associated with fibrosis, and induces apoptosis of epidermal or epithelial cells. The resultant IL-4 effects in allergic diseases include pruritus or wheezing, fibrosis and/or altered expression of extracellular matrix proteins, and loss of epidermal or epithelial barrier function.

Conclusion: The specific inhibition of the IL-4 signaling, through dupimulab that binds the IL-4 receptor subunit, would be effective in the specific inhibition of the allergic response in patients with allergic dermatitis or asthma.

Keywords: Dermatitis, asthma, IL-4, STAT6, apoptosis, fibrosis, lymphocytes.

1. INTRODUCTION

Allergic diseases are associated with inflammation and IL-4 signaling effects. We review the immune response, IL-4 signaling, cell death signaling, and IL-4 effects in dermatitis and allergic asthma.

2. ALLERGIC DISEASES: IMMUNE RESPONSE

Allergic diseases are associated with the altered immune response. Genetic and/or environmental factors cause induction of non-specific and specific immune responses, and associated signal transduction pathways that mediate inflammation and Th2 cell response in these diseases [1-5]. The non-specific immune response is mediated by the innate leukocytes and local cells, and involves the inflammatory cytokines (interleukin-1, interleukin-6, and tumor necrosis factor-α) that activate the nuclear factor-kappa B (NF-kB) pathway, and the lipid mediators (prostaglandins, leukotrienes) [1, 2, 4, 5]. The specific immune response is mediated by the T-lymphocytes and the B-lymphocytes. The succumbing of a cell to pathogens or antigens activates the cytotoxic T-lymphocytes to destroy the damaged cell [1, 2, 5]. The exposure of a cell to antigens activates the helper T-lymphocytes to defend cells [1, 2]. The helper T-lymphocytes consist of the Th1, Th2 and the Treg lymphocytes [1, 2, 5]. The Th1 cytokines, such as interferon γ, cause delayed type hypersensitivity reaction, the Th2 cytokines, such as IL-4, cause humoral or allergic response through antibodies, and the Treg cytokines, such as IL-10, modulate the inflammatory response [1, 2, 5]. The antigen presenting cells, primarily the dendritic cells, activate the helper T cells to interact with B cells displaying the antigen [2]. Initially, the helper T cells, activated by dendritic cells presenting the antigen, activate the B cells displaying the antigen to secrete IgM antibodies [2]. Subsequently, the activated helper T cells express CD40L that binds CD40 on B cells to activate “class switch recombination” and the secretion of other immunoglobulin subtypes [2, 3]. The Th2 cells induce allergic phenotype through eosinophil activation, and “class switch recombination” in B cells to produce IgE antibodies [1-5]. The IgE antibodies bind to basophils and mast cells to cause the release of histamines and other inflammatory substances [1-5].
IL-4 is key to the T\(_h2\) cell differentiation and the stimulation of IgE expression in B cells in the pathology of atopic dermatitis or allergic asthma [1-5].

3. IL-4 SIGNALING

The IL-4 binds to its receptors to activate JAK1/JAK3 that allows for STAT6 phosphorylation, dimerization and translocation to the nucleus to activate eosinophils, B-cells, and local cells (Fig. 1) [1-5]. The activated eosinophils release eosinophilic products, B-cells mediate the release of histamines, and the local cells undergo apoptosis and/or altered expression of genes associated with fibrosis (Fig. 1) [1-5].

![Diagram of IL-4 signaling](image)

**Fig. (1).** Mechanism of interleukin-4 signaling.

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**Cytokines/IL-4 and STAT pathway:** Cytokines, secreted by lymphoid, inflammatory, and hematopoietic cells, mediate the immune response [1]. The cytokines are classified into four families, which are the hematopoietin, interferons, chemokines and TNF-\(\alpha\) families [1]. They bind five families of receptors, which are the immunoglobulin superfamily receptors, class I cytokine receptor (hematopoietin receptor family), class II cytokine receptor (interferon receptor family), TNF-\(\alpha\) receptor, and chemokine receptor families [1]. The cytokine receptors are heterodimers or heterotrimers, with \(\alpha\), \(\beta\) and/or \(\gamma\) chains [1]. The specificity of cytokine binding is due to the \(\alpha\) subunit, and the signaling is via \(\beta\) and/or \(\gamma\) subunits [1].

IL-4 belongs to the hematopoietin family, and binds to class I cytokine receptor family, specifically the IL-2 receptor subfamily [1]. The cytoplasmic regions of the \(\alpha\) and \(\beta\) subunits of the receptor are associated with tyrosine kinase proteins, called JAK, with the IL-4 receptor associating with JAK1 and JAK3 [1]. IL-4 shares its \(\alpha\) receptor subunit with IL-13, resulting in the “commonality” of IL-4/IL-13 signaling in allergic diseases [2, 3]. The binding of the cytokine to its receptor causes the cross-phosphorylation of associated JAK kinases and subsequently the tyrosine residues in the cytoplasmic portion of the receptor [2]. The phospho-tyrosine residues on the receptor serve as docking regions for the specific binding of cognate STAT proteins via its complementary SH2 (src homology 2) domains [2]. The STAT proteins contain the central SH2 domain, the C terminal phosphorylation domain, and the N terminal DNA binding domain [2]. The docking of the receptor-specific STAT allows for the phosphorylation of the tyrosine residue in its C-terminal domain by JAK kinase, and the dissociation of STAT6 from the receptor [2]. The STAT6 dimerizes with another STAT by cross-binding to SH2 domains with the phospho-tyrosine in the C-terminal domains [2]. The dimerization of the STAT allows for the exposure of the nuclear localization signal (NLS), translocation to the nucleus and binding to specific regulatory DNA sequences through its N-terminal DNA binding domain [2]. There are six STAT proteins, with IL-4 specifically activating STAT6 [1]. STAT6 stimulates GATA-3 transcription factor and thereby T\(_h2\) response while inhibiting T\(_h1\) phenotype [1].

4. CELL DEATH SIGNALING

Cell loss occurs in allergic diseases through the activation of extrinsic and intrinsic apoptotic pathway, as well as induction of cell cycle arrest.

Inflammatory cytokines and FAS ligands activate extrinsic apoptotic pathway by activating Fas associated death domain (FADD) and TNF receptor associated death domain (TRADD), which activate the conversion of procaspase-8 to active caspase-8 (initiator caspase) that activates several effector caspases (-3, -6, -7) [2]. In addition, caspase-8 converts BH (Bcl-2 homology domain)3-interacting-domain death agonist (Bid) to truncated Bid (tBid) fragment, which binds to Bcl-2 (anti-apoptotic protein) in mitochondrial membrane to release Bax (pro-apoptotic protein) that forms an oligomeric pore for the release of cytochrome c to the cytoplasm [2]. The cytochrome c activates caspase-9 that activates capase-3, which cleaves cell proteins and causes cell death [2].

The intrinsic apoptotic pathway is activated by several BH3 only proteins, Bad, Bim and Puma, which bind Bcl-2 and thereby release Bax to induce apoptosis [2]. Bad is released, in the absence of trophic factors and active phospho-nositol (PI)-3kinase/protein kinase (PK)B pathway, from its 14-3-3, phosphoserine binding protein inhibitor; Bin is released from altered integrin signaling and cytoskeletal arrangement; and Puma is released following DNA damage [2]. DNA damage also activates ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related protein), which activate p53 [2]. The p53 activates pro-apoptotic Bax [2]. In addition, p53 activates p21, which binds to cyclin-dependent kinase (CDK) to cause cell cycle arrest [2]. While the STAT6, additively with p38 mitogen-activated protein kinase (MAPK), induces senescence in renal carcinoma cells, STAT6 promotes lymphocytic leukemia, through the overexpression of IL-9 [6, 7].
5. IL-4 SIGNALING EFFECTS

5.1. Dermatitis

Increased inflammation and T<sub>h</sub>2 response result in pruritus, fibrosis, loss of epidermal barrier, and spongiosis and edema (Fig. 2) [8-15]. The pruritus is from the histamine release from mast cells, fibrosis from the increased collagen expression by fibroblasts, loss of epidermal barrier and spongiosis/edema from the reduced filaggrin and keratin and increased proteolytic enzymes in keratinocytes, increased apoptosis of epidermal keratinocytes and expression of carbonic anhydrase II (CAII) by skin cells (Fig. 2) [8-15].

Atopic dermatitis or eczema is characterized by increased IL-4 signaling. The T<sub>h</sub>2 cells from patients with atopic dermatitis express increased levels of thymic stromal lymphopoietin (TSLP) receptors, which interact with TSLP overexpressed in the atopic dermatitis skin lesions, to induce release of IL-4 and promote T<sub>h</sub>2 response [9]. The skin transcriptome, assessed by microarray and qPCR, of acute canine atopic dermatitis lesion shows upregulation T<sub>h</sub>2 and pruritogenic pathways [10]. IL-4R<sup>α</sup> and STAT 6 gene polymorphisms are observed in patients with atopic dermatitis [11].

The IL-4R<sup>α</sup> signaling is important to skin repair, but leads to fibrosis and scar formation through the stimulation of collagen fibril assembly [12]. The mechanism involves the activation of macrophages, through the IL-4R<sup>α</sup> signaling, to release RELM-α, which acts of fibroblasts to induce lysyl hydroxylase 2 that directs collagen cross-linking and thereby collagen architecture [12]. The IL-4/IL-13 signaling inhibits filaggrin expression, while stimulating processing proteases such as matriptase and kallikreins-5, 7, in keratinocytes, which hinders epidermal barrier function [13]. In addition, IL-4/IL-13 signaling downregulates the expression of keratin and desmosomal cadherins associated with keratinocyte differentiation, and thereby epidermal barrier function [14].

IL-4/IL-13 signaling in skin equivalents leads to induction of DNA fragmentation/apoptosis, assessed by TUNEL assay, through the up-regulation of death receptor FAS mRNA, and intercellular edema/spongiosis, through upregulation of CAII that is associated with the regulation of metallo enzymes and thereby pH, water transport and ion homeostasis [15].

The available treatments for dermatitis had been corticosteroids, calcineurin inhibitor, phototherapy, or general immunotherapy; and recently (2017) has been through the specific inhibition of IL-4<sup>α</sup> receptor chain by dupilumab (Dupixent<sup>®</sup>, Sanofi and Regeneron Pharmaceuticals, Inc.) [5, 16-18]. Dupilumab received priority review and approval, by FDA, and is the first biologic in the treatment of moderate to severe atopic dermatitis in patients who are not responsive to the other options [5, 16-18]. The clinical trials of dupilumab for dermatitis demonstrates increased skin clearing and epidermal barrier function, and reduced pruritus [8, 11, 19-21]. The skin clearing was determined as Eczema Area and Severity Index (EASI) score, which is calculated by measuring the body area with eczema and the intensity of the symptoms [8]. The proportion of patients with a 50% reduction in EASI score (EASI-50) increased after 29 days of treatment with dupilumab, and further after 85 days of treatment [8].

5.2. Asthma

The effects of increased inflammation and T<sub>h</sub>2 response include wheezing, fibrosis, and hyperplasia, apoptosis and mucus secretion (Fig. 3) [22-25]. The wheezing is from eosinophilic products and histamine released from eosinophils and mast cells, respectively, fibrosis from increased collagen expression by fibroblasts, and hyperplasia, apoptosis and mucus secretion involving smooth muscle and epithelial cells (Fig. 3) [22-25].

The inhibition of JAK-STAT6 pathway has potential to inhibit subepithelial fibrosis and inflammation in esophageal eosinophilia patients [23]. The JAK-STAT6 pathway stimulates eotaxin-3, an eosinophil chemoattractant, which stimulates eosinophil infiltration, tissue remodeling and fibrosis [23]. Three inhibitors of the JAK-STAT6 pathway, AS1517499, leflunomide, and ruxolitinib inhibit eotaxin-3 expression in epithelial cells and fibroblasts from esophageal...
eosinophilia patients; and could thereby inhibit eotaxin-3 associated inflammation and fibrosis [23]. A monoclonal antibody against IgE, omalizumab, is effective in the management of asthma [24]. STAT6 mediates the upregulation of genes associated with airway smooth muscle cells hyperplasia [25].

The loss of bronchial epithelial cells and injury in asthma is from inflammation following the infiltration of T-cells and eosinophils [26]. The mechanism is apoptosis through the activation of peroxisome proliferator-activated receptor γ (PPAR γ) by IL-4 [27, 28]. The stimulation of 5-hydroxyeicosatetraenoic acid (5-HETE) by IL-4, by activating lipooxygenase, results in the activation of PPAR γ promoter activity, which induces apoptosis through the induction of death domain receptors, activation of caspase-8 and Bax, and downregulation of Bcl-Xl in lung cells [27]. The extrinsic and intrinsic apoptosis pathways are stimulated by PPAR γ. The PPAR γ and its ligands stimulate the extrinsic apoptosis pathway, by stimulating death receptor and inhibiting cellular FLICE inhibitory protein (cFLIP) that prevents caspase-8 activation; and the intrinsic apoptosis pathway, by inducing apoptosis inhibitors such as survivin that inhibits caspase-3 and caspase-9 activation [28]. The activation of PPAR γ by IL-4 is responsible for the apoptosis, loss of lung epithelial surface, and the infiltration of leukocytes that mediate the inflammatory process [27, 28]. The mechanism is similar to the apoptosis of epidermal keratinocytes in atopic dermatitis, following the infiltration of activated T-cells [29].

The drugs approved by FDA for allergies and asthma and their molecular targets are tabulated on the FDA website [30]. They include Dymista (Meda Pharmaceuticals), composed of azelastine hydrochloride (histamine H1-receptor antagonist) and fluticasone propionate (corticosteroid) for anti-allergy/inflammation, Qnasl (Teva Pharmaceuticals), composed of beclomethasone dipropionate (steroid) for anti-inflammation, and Grastek (Merck), Oralair (Greer Labs), and Ragwitek (Merck) composed of allergens for allergen immunotherapy [30]. The ongoing therapies targeting IL-4, in the form of antibodies, IL-4 variant or soluble IL-4 receptor, and their development status are listed in a recent publication [31]. The active ones include dupilumab (IL-4Rα inhibitor) for asthma, and SAR-156S97 (Sanofi) (Bi-specific antibody for IL-4 and IL-13) for idiopathic pulmonary fibrosis [31]. Dupilumab, the inhibitor of IL-4 α receptor subunit, has not yet been approved for asthma. However, the clinical trials of dupilumab for asthma demonstrate increased lung functioning as measured by forced expiratory volume (FEV), and reduced serum levels of IgE [22].

CONCLUSION

Inflammation and TH 2 response, through IL-4 signaling, are responsible for the clinical symptoms of allergic diseases such as atopic dermatitis and allergic asthma. Inflammation is largely mediated by the inflammatory cytokines that activate the NF-kB pathway and the extrinsic apoptosis pathway. The TH2 response is largely mediated by IL-4, which binds to its receptors to activate STAT6 transcription factor, which binds to responsive genes to induce IgE expression, which mediates the release of histamines, pruritogenic pathways, apoptosis, fibrosis, and extracellular matrix processing genes. The consequence is the clinical manifestation of allergic diseases, which includes pruritus (itching) or wheezing, impaired epidermal or epithelial barrier and fibrosis. The specific inhibition of the IL-4 signaling, through dupilumab that binds the IL-4 α receptor subunit, is effective in the inhibition of the allergic response in patients with allergic dermatitis or asthma.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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