Impact of Cytokines in Mast cells Allergic Inflammation

By Pio Conti *

Abstract—These instructions give you guidelines for preparing papers for IEEE Transactions and Journals. Use this document as a template if you are using Microsoft Word 6.0 or later. Otherwise, use this document as an instruction set. The electronic file of your paper will be formatted further at IEEE. Paper titles should be written in uppercase and lowercase letters, not all uppercase. Avoid writing long formulas with subscripts in the title; short formulas that identify the elements are fine (e.g., "Nd–Fe–B"). Do not write “(Invited)” in the title. Full names of authors are preferred in the author field, but are not required. Put a space between authors’ initials. Define all symbols used in the abstract. Do not cite references in the abstract. Do not delete the blank line immediately above the abstract; it sets the footnote at the bottom of this column.

Index Terms—Enter key words or phrases in alphabetical order, separated by commas. For a list of suggested keywords, send a blank e-mail to keywords@ieee.org or visit http://www.ieee.org/organizations/pubs/ani_prod/keyrd98.txt

I. INTRODUCTION
Mast cells (MCs): Mast cells are prominent in inflammatory diseases and have been implicated in the pathophysiology of asthma and allergy (Fig. 1). The ability of mast cells to generate or release the vasoactive/spasmogenic mediators histamine, PGD2, sulfidopeptide leukotrienes, platelet-activating factor, cytokines/chemokines and other factors is thought to be relevant to immediate inflammation [1]. Mast cells which express c-Kit and high-affinity IgE receptors (FceRI) and are predominantly localized in mucosal and connective tissues, can be activated by bacterial or viral antigens, cytokines, growth factors, and hormones, leading to differential release of distinct mediators without degranulation [2]. This process appears to involve de novo synthesis of mediators, such as interleukin(IL)-6 and vascular endothelial growth factor, with release through secretory vesicles (50 nm), similar to those in synaptic transmission. In addition, mast cells accumulate in the stroma of a variety of inflamed and transformed tissues in response to locally produced chemotactic factors for monocytes/mast cells, such as RANTES and MCP-1 [3]. Recent evidence indicates that in addition to direct effect of these mast cell products some mast cell mediators themselves modulate inflammatory mediator production. Recent studies also demonstrate a convincing role for IL-18 in atopic responses. Moreover, IL-18 directly stimulates basophils and mast cells to produce Th2 cytokines and histamine independently of IgE. IL-18 also induces IL-13 and/or IL-4 production by NK cells, mast cells and basophils. However, it has been proven that some other cytokines such as IL-10 and cytokines from the IL-10 family, and TGF-beta have anti-inflammatory properties in allergy [4]. Human mast cell is known to be a rich source of prostaglandins, and they are crucial effector cells evoking immune responses against bacterial pathogens. The interaction of mast cells with other immune cells, including macrophages, dendritic cells and T cells, to induce protective immunity is very important and not well understood. Several cytokines are believed to be involved in Th2-mediated inflammatory responses in allergic diseases such as asthma, anaphylaxis, and atopic dermatitis, and in host defense against parasites, and stimulate allergic cell superoxide production and degranulation. These cytokines are expressed and act on many cells participating in allergic inflammation such as antigen-experienced Th2 cells, mast cells, eosinophils, basophils, dendritic cells, and CD34⁺ stem cells, and

Received May 20, 2013, accepted May 28, 2013
*Professor of Immunology Division, Medical School, University of Chieti-Pescara, Via dei Vestini, Chieti, Italy (pconti@unich.it); and Affiliated Professor, Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA.
induce Th2-type cytokine production by different cells [5]. Mast cells through ST2 receptor recognize IL-33, and signaling pathways are started with subsequent activation of NFκB and transcription of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-13, TNF, chemokines, and prostaglandins. Allergic asthma is associated with a substantial increase in the mucus content of the airway epithelium [6]. Mucus hyperproduction in asthma results from Th2-induced airway inflammation. Although an increased Th2 immune response is evidently associated with asthma, the decreased Th1 immune response also favors the outcome of the pathogenesis. Mucus could be induced in mice lacking IL-4 and IL-5 and corresponding inflammatory responses devoid of eosinophils or mast cells. Th2 type is characterized by high levels of IL-4 and IL-13. IL-13 itself can directly induce airways hyperresponsiveness. Therefore, IL-13 plays an important role in initiating and generating the physiological abnormalities that come to play in the asthmatic diathesis. Mast cell disorders are defined by an abnormal accumulation of tissue mast cells (MCs) in one or more organ systems. Symptoms in mastocytosis result from MC-derived mediators and, less frequently, from destructive infiltration of MCs. Cutaneous mastocytosis is a benign disease of the skin and may regress spontaneously [7, 8]. Systemic mastocytosis is a persistent disease in which a somatic c-kit mutation at codon 816 is usually detectable in MCs and their progenitors [8, 9].

Cytokines are soluble factors that bind to cell surface receptors and regulate intercellular communications playing an essential role in the innate and adaptive immune responses [10]. Cytokines play a central role in the pathogenesis of allergic diseases and allergic inflammation. Several pro-inflammatory cytokines including interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are known to stimulate a number of cells to produce inflammatory mediators such as prostaglandins. Cytokines of the (IL-1) family, such as IL-1 alpha/beta, IL-18, IL-32, IL-36 and IL-38 have important functions in host defense, immune regulation, and inflammation. IL-1 consistently induces COX-2 gene expression and prostaglandin synthesis in several cell lines [3, 11, 12]. IL-1α and IL-1β are pro-inflammatory cytokines which act through the binding of type I IL-1 receptor (IL-1R1). The IL-1 family of cytokines is composed of 11 different ligands.

IL-36 is a family member of IL-1, is produced by many different cells, activates MAPK and NF-kB pathways, and is a common mediator of both innate and adaptive immune responses [13]. IL-36 is involved in psoriasis mediated by mast cells which is overexpressed in human plaque skin (Table I). The inhibition of IL-36 with IL-36RA in human psoriatic skin has been found to ameliorate the inflammatory reactions. IL-36(-α-β-γ) is highly expressed in epithelial tissues and skin which are exposed to pathogens and are also expressed abundantly in psoriasis. In addition, IL-36α and IL-36β, but not IL-36γ, directly induce TNF-α mRNA and protein synthesis in keratinocytes, demonstrating that IL-36α and IL-36β could regulate TNF-α directly [13]. IL-36 also regulates the production of pro-inflammatory cytokines, including IL-12, IL-1β, IL-6, TNF-α, and IL-23 by dendritic cells with a more potent stimulatory effect than that of other IL-1. Moreover, IL-36 induces the production of IFN-γ, IL-4, and IL-17 by CD4(+) T cells and cultured splenocytes [14]. In experimental animal, overexpression of IL-36α in keratinocytes exhibit inflammatory skin lesions, this effect was less severe after IL-36RA treatment in vivo. Taken together, these observations indicate that IL-36R ligands, including IL-36α, IL-36β, and IL-36γ, exert proinflammatory effects in vitro and in vivo and that IL-36RA acts as a natural antagonist [13].

IL-38 is the most recent addition to the ever-growing family of cytokines and is also a member of the IL-1 family (Table II). IL-38 mRNA is expressed in several tissues such as heart, placenta, fetal liver, spleen, thymus, and activated B cells of the human tonsil. The expression in a variety of immune and similarity to IL-1RA (IL-1 receptor antagonist) suggest a role of IL-38 in the inflammatory response, allergy and host defense [15]. IL-38 acts as an IL-1RA, and binds the same IL-1 receptor type I. However, the binding affinity of recombinant IL-38 is significantly lower than that of IL-1RA and IL-1β. This newly cytokine seems to act more as an immuno-regulatory
than as a pro-inflammatory cytokine. Therefore, IL-38 plays a role in the pathogenesis of inflammatory diseases such as psoriatic arthritis and ankylosing spondylitis, exerting protective function. However, some effects of IL-38 remain unclear, for instance, why it decreases pro-inflammatory cytokines in peripheral blood mononuclear cells whereas it increases pro-inflammatory cytokines in dendritic cells [13].

VEGF (Vascular Endothelial Growth Factor) is an angiogenic and inflammatory protein expressed in response to soluble mediators such as cytokines and growth factors (Table III). VEGF receptors are located on endothelial cells, but also in many non-endothelial cells, and act through autocrine pathways to regulate cell survival and function. VEGF is an endothelial cell-specific mitogenic peptide which plays a key role in vasculogenesis, and also stimulates vascular permeability, which leads to inflammation [16]. Compounds that selectively inhibit VEGF function and block angiogenesis have led to great promise in the treatment of various cancers and inflammatory diseases [17].

VEGF was first discovered for its ability to regulate vascular endothelial cell permeability, and is an important regulator of physiological angiogenesis, and vascular development in all human tissue. Therefore, VEGF is a mediator of angiogenesis and inflammation which are closely integrated processes in a number of physiological and pathological conditions, including psoriasis, rheumatoid arthritis, arteriosclerosis, and tumor [16, 18].

Vascular endothelial growth factor is an important cytokine in the physiological development of blood vessels as well as the development of vessels in tumors and other tissues. VEGF is also important in animals for the regulation of stem cell and monocyte/macrophage recruitment, maintenance of tissue barrier functions and neuroprotection.

In addition to its role in regulating endothelial cell proliferation, migration, and cell survival, VEGF receptors are located on many different cells and act through autocrine pathways to regulate cell survival and function.

VEGF has many important biological effects and exerts its cellular function mainly by interacting with two high-affinity trans-membrane tyrosine kinase receptors: VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). It has been proven that inhibition of VEGF receptor activity reduces angiogenesis. The most studied and developed inhibitors are monoclonal antibodies that neutralize VEGF, and small molecule VEGFR kinase inhibitors [16].

VEGF can also be inhibited by endostatin, a proteolytic fragment of the vascular and epithelial basement membrane collagen type XVIII, which is an efficient antiangiogenic and antitumor molecule [19]. Recombinant endostatin inhibits the proliferation and migration of endothelial cells and induces their apoptosis [19]. For these reasons, the inhibition of VEGF or its receptor signaling system is an attractive target for therapeutic intervention.

TGF-β (Transforming growth factor-beta) is a potent regulatory cytokine with diverse effects on hemopoietic cells [4]. The pivotal function of TGF-β in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation, and survival. In addition, TGF-β controls the initiation and resolution of inflammatory responses through the regulation of chemotaxis, activation, and survival of mast cells, lymphocytes, natural killer cells, dendritic cells, macrophages, and granulocytes. The regulatory activity of TGF-β is modulated by the cell differentiation state and by the presence of inflammatory cytokines and costimulatory molecules. Collectively, TGF-β inhibits the development of immunopathology to self or non-harmful antigens without compromising immune responses to pathogens [20].

IL-33 is a member of the IL-1 family that signals through the IL-1 receptor-related protein ST2 [17] and was found to be a potent inducer of T helper 2 (Th2) responses and Th2-associated cytokines IL-4, IL-5, and IL-13, but not Th1, in contrast to other closely related family members. This cytokine is the newest inflammatory interleukin identified as a novel member of the IL-1 family and synthesized as a 31 kDa peptide precursor cleaved by caspase-1 to generate mature cytokine IL-33 and it is more similar to IL-1α than to IL-1β [17, 21].

IL-33 is a heterochromatin-associated nuclear factor in vivo and is associated with heterochromatin and
mitotic chromosomes in living cells [22, 23]. This protein acts by binding its receptor T1/ST2 (Toll-IL-1 receptor), an orphan member of IL-1 receptor family, activating NF-kB pathway and MAP kinases driving the production of Th2 cytokines. IL-33 is known to enhance experimental allergic inflammation by directly stimulating mast cells to produce inflammatory cytokines and it is abundantly expressed by high endothelial venules in human tonsils [24]. We recently showed that Substance P (SP) can induce VEGF secretion from mast cells and IL-33 augments the effect of SP in VEGF transcription and translation protein [16, 25]. We also found that IL-33 is a strong activator of mast cell lines (LAD II cells) capable of inducing MCP-1 (a C-C chemokine family member) release at transcriptional and translational level, IL-8 and VEGF (unpublished data), suggesting a possible role of this new cytokine in the inflammatory response and recruitment of inflammatory cells [16].

**Substance P** (SP): There is much evidence to support the hypothesis that SP is one of the most important neurotransmitters and neuromodulators present in the human brain [26-28]. Its biological effect has been intimately linked to the pathophysiology of several relevant neurological and psychiatric disorders, such as migraine, asthma, nausea, inflammatory bowel syndrome, anxiety, depression and stress [29, 30]. SP was first discovered in 1931 and was isolated by Leeman et al. [31] in the 70’s and it was shown to be one-decapeptide. Its biological effect has been intimately linked to the pathophysiology of several relevant neuropsychiatric diseases, such as migraine, asthma, nausea, inflammatory bowel syndrome, anxiety, depression and stress [30, 32]. During the past 30 years SP has been identified as an important mediator in the development and progress of inflammation by binding to its high-affinity neurokinin-1 receptor (NK-1R). Several common skin diseases are now acknowledged to be worsened by psychological stress, particularly immuno-dermatoses such as atopic dermatitis, psoriasis, seborrheic eczema, prurigo nodularis, lichen planus, chronic urticaria, alopecia areata and pruritus sine materia [33]. A wealth of mediators released systemically or locally in the skin in response to stress increase sensory innervation upregulate the production of other pruritogenic agents, perpetuate (neurogenic) inflammation and lower the itch threshold [34]. This demonstrates that pathogenesis of atopic dermatitis involves the interactions of immune and neuroendocrine systems with SP participation. Stress primarily exacerbates allergic dermatitis via SP-dependent cutaneous neurogenic inflammation and subsequent local cytokine shifting and should be considered as a therapeutic target. Recently, high levels of neurotrophic factors have been found in bronchial asthma; these factors include nerve growth factor, brain-derived neurotrophic factor, and leukemia inhibitory factor, among others [35]. Siebenharr et al. demonstrate that SP fibers are increased in early lesions, and that SP treatment induces catagen follicles along with activated CD8+ T cells [36-38]. Several data demonstrate lesion grade dependence of below-level pain development and suggest chemokines as potential candidates for integrating inflammation and central neuropathic pain after spinal cord injury.

SP enhances LPS-induced macrophage TNF-α production from stressed animals and stimulates the production of IL-8, a CXC chemokine, in response to stress. Stress induces cytokine alteration and contributes to inflammation, an effect mediated by SP involving immuno cells. Inhibition of the SP activity, using SP receptor antagonists, has consistently resulted in profound decreases in edema formation and marked improvements in functional outcome [39]. It has been shown that substance P by itself causes an increase of synthesis of CC and CXC chemokines in inflammatory cells. CXCL8 (IL-8) is a chemokine with chemotactic and inflammatory properties. We recently found that SP is capable of stimulating the release of CXCL8 in human mast cell cord blood [40]. This observation supports the concept that the neurogenic system modulates inflammatory events by substance P-mediated chemokine CXCL8 release in human mast cell [40].

**Autism:** Inflammatory mediators in autism usually involve activation of astrocytes and microglial cells. In addition, pro-inflammatory chemokines such as MCP-1 and modulatory cytokines, such as TGF-beta-1, are consistently elevated in autistic brains. However, the role of the immune system in the development of autism is controversial. A strong
connection is believed to be the disease mastocytosis in which the skin and the intestines contain more mast cells than average. These mast cells are very sensitive to various allergenic triggers. Authors reported that cytokine imbalances in autism have a pathological role and have important interaction with the nervous system, contributing to the dysfunction in autism spectrum disorders (ASD) [41]. The major cytokines such as IL-1-α, IL-1-β, IL-4, IL-6, IL-10, IL-11, IL-13, IL-18, TNF-α, IL1-RA, TGF-β, and CCL2 are all expressed in healthy CNS [42]. Many of these cytokines and their receptors have differential expression patterns across the CNS. IL-1, IL-6 and TNF-α are pro-inflammatory cytokines and they represent the key mediators of neuroimmune interactions, leading to severe neurological and mental diseases. IL-6 mRNAs and its receptor (IL-6R) are developmentally regulated in the rat brain [43, 44] and the adult hippocampus has the highest detectable levels of both transcripts; while IFN-γ is found at neuronal synapses [45] suggesting that it may act at the level of the synapse to influence brain function. It has been reported that many cytokines, such as IL-1-beta, IL-6, IL-4, IFN-γ, and TGF-beta, are implicated in the nervous system and therefore in autism. Other authors reported that increased pro-inflammatory cytokine levels in the brain (TNF-α, IFN-γ, and IL-8); and NF-κB, may also contribute to autism spectrum disorders [46].

In addition, other authors confirmed that several cytokines and chemokines, including IL-1β, IL-6, IL-8 and IL-12p40, are elevated in the ASD plasma of very young children (ages 2–5 years), and that these increases are associated with more impaired communication and aberrant behaviors [46]. It has been also reported that mast cells express leptin and leptin receptors, a finding implicating paracrine or autocrine immunomodulatory effects of leptin on mast cells [47]. Leptin may play a role in ASD. In fact, leptin is higher in obese subjects [48, 49], and elevated plasma leptin levels during pregnancy are indicative of placental dysfunction [50], and plasma levels of leptin are significantly higher in patients with autistic disorder. Acute stress may have a role in this disease and leads to high serum level IL-6 which is mast cell-dependent [51]. Mast cells have been implicated in inflammatory conditions that worsen by stress [52]. Mast cell-derived cytokines, such as IL-6, can increase blood-brain-barrier (BBB) permeability [51, 53]. Corticotropin-releasing hormone (CRH) may have an immunomodulatory role and has been associated with intestinal inflammation. CRH can also be secreted from immune cells [54], mast cells [55], skin [56, 57] and post-anglionic nerve endings [58], leading to pro-inflammatory effects [59, 60-65]. When mast cells are activated with CRH they release several pro-inflammatory cytokines [66-69]. Patients with stress, result in secretion of CRH from the hypothalamus which regulates the hypothalamic-pituitary-adrenal (HPA) axis. Increased plasma levels of CRH have been linked to preterm labor [70-78] and in mothers with anxiety during that period of pregnancy. A number of cytokines, including IL-1 and IL-6, can trigger secretion of CRH in vitro [79-82]. Moreover, CRH stimulates IL-6 release from human peripheral blood mononuclear cells that infiltrate the fetal membranes and the placenta during intrauterine infection [83-85]. Other cytokines may also be implicated in ASD. For instance, TNF increases approximately 50-fold in the cerebrospinal fluid, and IL-6 gene expression also increases in the brain of ADS patients. Since mast cells are surely involved in this disease, macrophage chemoattractant-protein-1 (MCP-1), a potent chemo-attractant for mast cells, is also important. Acting on mast cells, TGF-β1 along with IL-9 may also have a role in worsening ASD symptoms. Recently it was reported that IL-9 induces mast cell release of VEGF which also inhibits gut mast cell function [86-87]. In addition to cytokines, essential components of the complement cascade, including C1q and C3, are also expressed in the CNS.

Neurotensin (NT) is a brain and gut peptide that contributes to gut inflammation due to acute stress [89]. This is an important molecule augmented and secreted in serum of children with autism [90], which can stimulate mast cell and mediate ASD. A common link among the neurobehavioral disorders associated with intrauterine inflammation appears to be the evidence for immune dysregulation in the developing brain. Other immune parameters, including maternal infection and dysregulated cytokine signaling, have been found to be associated with ASD. However, the role played by cytokines in the pathogenesis of ADS remain to be determined [90-91].
References


Castagliuolo I, Leeman SE, Bartolak-Suki E, Nikulasson S, Qua B, Carraway RE, Pothoulakis C. A neurotensin antagonist, SR 48692, inhibits colonic responses to immobilization stress in rats, *Proc Natl Acad Sci USA* 93 (22), 12611-5, 1996.
Figure 1. Mast cells in different culture medium and at different magnification: A, B, C, and D.
**Biological effects of IL-36**

- Activates the MAPK and NF-kappaB pathways
- An important player in innate and adaptive immune response
- Pathophysiology of several diseases
- Initiation or regulation of immune responses and inflammation
- IL-1F5 (renamed IL-36RA) exerts receptor antagonist activities
- IL-1F5 inhibits NF-κB in certain cell types
- Mediate lesional psoriasis skin
- Mediate epidermis and dermis inflammation
- Mediate the proliferation of keratinocytes and in activate epithelial tissues
- Strongly increased in psoriatic-like mouse skin plaques
- IL-36α and IL-36β, but not IL-36γ, directly induced TNF-α
- IL-36 can be induced by Th17 cytokines
- Direct regulates IL-8 and IL-6
- Regulates the expression and enhance the function of Th17
- Interrelation with IL-22 in skin inflammation
- Mediate chronic kidney disease, and human rheumatoid synovial tissues

---

**Table I.**


**Biological effects of IL-38**

- Member of the IL-1 family of ligands
- Gene located on human chromosome 2q13-14.1 near the IL-1RA gene
- Encodes 152-amino acid protein
- Shares between 41% and 43% amino acid identity with human IL-1RA
- Shares characteristics of the IL-1RA family
- It is expressed in heart, placenta, fetal liver, spleen, thymus, and human tonsil
- Role in inflammatory response and host defense
- Acts as an IL-1RA, and binds the same IL-1 receptor type I
- Binds to the IL-36R, as does IL-36RA
- Binding affinity of IL-38 is lower than that of IL-1RA and IL-1β
- It is involved in the regulation of immune responses
- It plays a role in psoriatic arthritis and ankylosing spondylitis
- It is protective in psoriasis
- Involved in increased number of lymphocytes Th17
- Involved in the high production of IL-17A, IL-21, and IL-22
- Plays a role in MAPK activation
- Reduces the production of *Candida*-induced IL-17 and IL-22
- Do not affect the production of Th1 cytokine IFN-γ
- Similar biological effects with IL-36RA
- Shares primary amino acid homology with IL-1RA and IL-36RA
- Do not bind IL-18R
- Decreases pro-inflammatory cytokines in PBMCs
- Increases pro-inflammatory cytokines in dendritic cells
- Has an inhibitory effect on Th17 cytokines

**Table II.**
Some biological effects of VEGF

- VEGF/Flt-1 signaling plays a significant role in vascular inflammation and neo-intima formation.
- The rising levels of VEGF and IL-6 may be good and specific biomarkers for transplant (GVHD).
- Vascular endothelial growth factor (VEGF) plays a pro-inflammatory mediator as well as a vascular permeability factor.
- Vascular endothelial growth factor (VEGF) is produced by cancer cells, while monocyte chemotactic protein-1 (MCP-1) is produced mainly by tumor-infiltrating macrophages and regulatory T cells.
- Angiogenesis is dependent upon VEGF ability to co-ordinately regulate multiple endothelial functions.
- VEGF stimulates endothelial cell growth, survival, and vascular permeability.
- VEGF is responsible for vascular proliferation and blood vessel invasion of the synovial lining membrane in RA.
- VEGF functions: survival, proliferation, migration, vascular permeability, tubulogenesis, NO and prostanoid synthesis, and gene expression.

Table III.