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**Original Article**

# Human Umbilical Cord Blood-Derived Mast Cells

## *A Unique Model for the Study of Neuro-Immuno-Endocrine Interactions*

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## Abstract

Findings obtained using animal models have often failed to reflect the processes involved in human disease. Moreover, human cultured cells do not necessarily function as their actual tissue counterparts. Therefore, there is great demand for sources of human progenitor cells that may be directed to acquire specific tissue characteristics and be available in sufficient quantities to carry out functional and pharmacological studies. A case in point is the mast cell, well known for its involvement in allergic reactions, but also implicated in inflammatory diseases. Mast cells can be activated by allergens, anaphylatoxins, immunoglobulin-free light chains, superantigens, neuropeptides, and cytokines, leading to selective release of mediators. These could be involved in many inflammatory diseases, such as asthma and atopic dermatitis, which worsen by stress, through activation by local release of corticotropin-releasing hormone or related peptides. Umbilical cord blood and cord matrix-derived mast cell progenitors can be separated magnetically and grown in the presence of stem cell factor, interleukin-6, interleukin-4, and other cytokines to yield distinct mast cell populations. The recent use of live cell array, with its ability to study such interactions rapidly at the single-cell level, provides unique new opportunities for fast output screening of mast cell triggers and inhibitors.

**Index Entries:** Asthma; coronary artery disease; dermatoses; inflammation; mast cells; migraines; skin; stress.

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## Introduction

Mast cells are ubiquitous in the body and are well known for their role in hypersensitivity reactions *(1–5)*. They derive from a distinct precursor in the bone marrow *(6,7)* and "mature" under local microenvironmental factors into different types depending on the tissue *(8)*. Mast cells are mostly known for their role in allergic reactions, during which crosslinking of their surface receptors for IgE FcεRI *(9,10)* lead to degranulation and release of numerous (Table 1) vasoactive, proinflammatory, and nociceptive mediators that include histamine, interleukins (IL)-6, IL-8, prostaglandin $D_2$, tryptase, and vascular endothelial growth factor (VEGF; *8,11,12*). Mast cell chemoattractants include stem cell factor (SCF), nerve growth factor (NGF; *13*), RANTES, and MCP-1 *(14)*. In addition to allergic triggers, mast cells can be activated by anaphylatoxins, antibody light chains, bacterial and viral antigens, cytokines and neuropeptides *(15)*. Immunoglobulin-free light chains appear to elicit immediate hypersensitivity-like responses *(16,17)* through mast cell activation and subsequent induction of T-cell-mediated immune responses (Table 2; *18*). Mast cells could, therefore, be critical in innate or acquired immunity *(8,19,20)* and for the development of inflammatory diseases, especially arthritis, asthma, chronic dermatitis, coronary artery disease (CAD), and migraines (Table 3; *15*). These findings have led to a number of

Table 1
Mast Cell Mediators

| *Mediators* | *Main pathophysiological effects* |
|---|---|
| Prestored | |
| *Biogenic amines* | |
| Histamine | Vasodilation, angiogenesis, mitogenesis, pain |
| 5-Hydroxytryptamine (5-HT, serotonin) | Vasoconstriction, pain |
| *Chemokines* | |
| IL-8, MCP-1, MCP-3, MCP-4, RANTES | Chemoattraction, tissue infiltration of leukocytes |
| *Enzymes* | |
| Arylsulfatases | Lipid/proteoglycan hydrolysis |
| Carboxypeptidase A | Peptide processing |
| Chymase | Tissue damage, pain, angiotensin II synthesis |
| Kinogenases | Synthesis of vasodilatory kinins, pain |
| Phospholipases | Arachidonic acid generation |
| Tryptase | Tissue damage, activation of protease-activated receptors, inflammation, pain |
| *Peptides* | |
| CRH | Inflammation, vasodilation |
| Endorphins | Analgesia |
| Endothelin | Sepsis |
| Kinins (bradykinin) | Inflammation, pain, vasodilation |
| Somatostatin (SRIF) | Anti-inflammatory (?) |
| SP Inflammation, pain | |
| VIP | Vasodilation |
| UcnInflammation, vasodilation | |
| VGEF | Neovascularization ,vasodilation |
| *Proteoglycans* | |
| Chondroitin sulfate | Cartilage synthesis, anti-inflammatory |
| Heparin | Angiogenesis, NGF stabilization |
| Hyaluronic acid | Connective tissue, NGF stabilization |
| De novo synthesized | |
| *Cytokines* | |
| Interleukins (IL)-1–6, 9, 10, 13, 16 | Inflammation, leukocyte migration, pain |
| INF-γ; MIF; TNF-α | Inflammation, leukocyte proliferation/activation |
| *Growth factors* | |
| SCF, GM-CSF, b-FGF, NGF, VEGF | Growth of a variety of cells |
| *Phospholipid metabolites* | |
| Leukotriene $B_4$ $LTB_4$ | Leukocyte chemotaxis |
| Leukotriene $C_4$ ($LTC_4$) | Vasoconstriction, pain |
| Platelet activating factor | Platelet activation, vasodilation |
| Prostaglandin $D_2$ | Bronchonstriction, pain |
| *Nitric oxide (NO)* | Vasodilation |

CRH, corticotropin-releasing hormone; TGF-β, transforming growth factor-β; CSF, colony stimulating factor; TNF-α, tumor necrosis factor-α; INF-γ, Interferon-γ; SRIF, somatomedin release inhibitory factor, somatostatin; MIF, macrophage inflammatory factor; GM-CSF, granulocyte monocyte-colony stimulating factor; b-FGF, fibroblast growth factor; NGF, nerve growth factor; SCF, Stem cell factor; VEGF, vascular endothelial growth factor.

observations: (1) certain organs, most prominently the brain do not develop allergies; (2) not all tissues that respond in allergic reactions do so at any particular time or every patient; (3) mast cells in different tissues respond to different triggers, and (4) 50–60% of patients who experience asthma or rhinitis do not have elevated serum IgE levels; (5) mast cells must be able to regulate immune responses without causing anaphylactic shock. However, unlike allergic reactions, mast cells are rarely seen to degranulate during autoimmune *(21)* or inflammatory processes *(22)*.

## Selective Release of Mast Cell Mediators

The only way to explain these observations would be through the ability of mast cells to undergo "differential" or "selective" release of mediators *(23)* without degranulation *(24)*. This process involves ultrastructural alterations of the mast cell electron dense granular core indicative of secretion, but without degranulation, a process that has been termed "activation," *(25–27)* "intragranular activation" *(28)* or "piecemeal" degranulation *(29)*. During this process, mast cells can release many mediators *differentially* or *selectively* (Table 4;

Table 2
Mast Cell Triggers

| |
|---|
| Antigen + IgE |
| Anaphylatoxins (C3a, C5a) |
| CRH, Ucn |
| IL-1 |
| Immunoglobulin—free light chains |
| LPS |
| NGF |
| NT |
| SCF |
| SP |
| Superantigens |
| VIP |
| Viral DNA sequences |

during the diagnosis of mast cell sarcoma-leukemia *(43,44)*. Of these, the former does not need SCF and cannot be activated immunologically, while the latter require SCF and respond to FcεRI aggregation, but grow much slower than the HMC-1 cells *(43)*.

We have been using umbilical cord blood stem cell-derived cultured mast cells (hCBMCs) in order to have a pure population of normal HMC-1. HMC-1progenitors can be obtained from umbilical cord blood and grown into mast cells in about 10 wk *(45)*. Human umbilical cord blood is obtained from normal deliveries in accordance with established institutional guidelines. hCBMCs are derived by the culture of $CD34^+$ progenitor cells as previously described *(45)* with minor modifications (Fig. 1). Briefly, mononuclear cells are isolated by pipeting heparin-treated cord blood onto lymphocyte separation medium (INC Biomedical, Aurora, OH). $CD34^+$ progenitor cells are isolated from mononuclear cells by selection of cells positive for the AC133 antigen ($CD133^+/CD34^+$) by magnetic cell sorting (Miltenyi Biotec, Auburn, CA). For the first 4 wk, $CD34^+$ cells are cultured in IMDM (GIBCO BRL, Long Island, NY) supplemented with 0.55 μ*M* 2-mercaptoethanol, 100 mg/L Insulin–Transferin–Selenium supplement (ITS) from GIBCO, 0.1% bovine serum albumin (BSA; Sigma, St. Louis, MO), penicillin/streptomycin, 100 ng/mL SCF (Amgen), and 50 ng/mL IL-6 (Chemicon) at 37°C in 5% $CO_2$-balanced air. After 4 wk of culture, BSA and ITS in the culture medium are substituted with 10% fetal bovine serum (FBS; GIBCO). By 8 wk, >99% of the cells in the culture are identified as mast cells by immunostaining for tryptase.

The hCBMCs contain little chymase, thus resembling mucosal mast cells and do not respond to neuropeptides such as neurotension (NT) or substance P (SP; *46*). Addition of IL-4 for the last 3 wk of culture increases the content of chymase in >50% of cells *(47)*. Nevertheless, the possibility of developing "tissue-specific" mast cells is most likely if one were to start with cord matrix progenitors that are $CD34^-$.

In contrast to hCBMCs, which are $CD34^+$, umbilical cord-matrix-derived mast cells (hCMSCs) are 8-d $CD34^-$ embryonic

Table 3
Inflammatory Diseases Involving Mast Cells

| *Disease* | *Pathophysiological effects* |
|---|---|
| Asthma | Bronchonstriction, pulmonary inflammation |
| AD | Skin vasodilation, T-cell recruitment, inflammation, itching |
| CAD | Coronary inflammation, myocardial ischemia |
| Chronic prostatitis | Prostate inflammation |
| Chronic fatique syndrome | Diencephalic effects |
| Endometriosis | Peritoneal inflammation, pain |
| Fibromyalgia | Muscle inflammation, pain |
| Interstitial cystitis | Bladder mucosal damage, inflammation, pain |
| Migraines | Meningeal vasodilation, inflammation, pain |
| Multiple sclerosis | Increased BBB permeability, brain inflammation, demyelination |
| Neurofibromatosis | Skin nerve growth, fibrosis |
| Osteoarthritis | Articular erosion, inflammation, pain |
| RA | Joint inflammation, cartilage erosion |
| Rhinitis | Nasal inflammation |

*30–32*) as originally shown for serotonin *(23)* and eicosanoids *(33–35)*. More recently, IL-6 was shown to be released in response to stem cell factor (SCF; *36–39*) and to IL-1; through small vesicles (40–80 nm in diameter), unrelated to the secretory granules (800–1000 nm in diamerter; *40*). VEGF could also be released selectively without degranulation by corticotropin-releasing hormone (CRH; *41*).

## Sources of Human Mast Cells

The study of human mast cells (HMC-1) and their role in the diseases mentioned has been hampered by the lack of sufficient number of appropriate mast cells. Many observations were based on biopsy material that is understandably limited. Tissue mast cells have been disaggregated and studied from clear margins of excised breast or lung carcinoma or from foreskins following circumcision. These sources are also limited, variable, from different age groups, and subject to the considerable alterations inherent to tissue treatment by proteolytic enzymes.

Leukemic mast cells have also been employed, such as the human mast cell line (HMC)-1 obtained from the peripheral blood of a 52-yr-old woman with refractory mast cell leukemia *(42,43)*, and most recently the LAD lines established from different bone marrow aspirates obtained from a 44-yr-old man

Table 4
Selective Release of Mast Cell Mediators

| *Stimuli* | *MC type* | *Mediators released* | *Mediators not released* | *Physiological importance* | *Refs.* |
|---|---|---|---|---|---|
| Endogenous | | | | | |
| *IL-1*β | RPMC | NO | Platelet activating factor, H | Inflammation | *216* |
| $PGE_2$ | RPMC | IL-6 | H, TNF-α | Cytoprotection | *217* |
| SCF | BMMC | IL-6 | H, $LTC_4$, TNF-α | MC development | *38* |
| *IL-12* | RPMC | INF-γ | H | Th1 immunity | *218* |
| CD8 ligands | RPMC | TNF-α, NO | H | T-cell interaction | *219* |
| Thrombin | BMMC | IL-6 | 5-HT, TNF-α | Anticlotting | *220* |
| SDF | hCBMC | IL-8 | H, GM-CSF, INF-γ, IL-1β | Endothelial transmigration | *221* |
| Monomeric IgE | BMMC | IL-6 | H, $LTC_4$ | MC survival | *222* |
| *Endothelin-1, endothelin -3* | RMMC | TNF-α, IL-12↑ | IL-4, IL-10, IL-13↓* | Th1 immunity | *223* |
| $LTC_4/LTD_4$ | IL-4-primed hCBMC | TNF-α, MIP-1α, IL-5 | H | Non-IgE mediated inflammation | *224* |
| IL-1 | hCBMC | IL-6, IL-8, TNF | H, tryptase | Inflammation | *40* |
| CRHR-1 | hCBMC | VEGF | H, tryptase, IL-8 | Inflammation | *204* |
| CRHR-2 | hCBMC | IL-6 | H, tryptase, IL-8, VEGF | Inflammation | *225* |
| *Exogenous/pharmacological* | | | | | |
| Amitriptyline | RPMC | Serotonin | Histamine | Headaches | *23* |
| LPS | RPMC | IL-6 | HA | Bacterial infection | *36* |
| **CpG DNA** | BMMC | TNF-α, IL-6 | HA, IL-4, IL-12, GM-CSF, INF-γ | Host response to bacteria | *226* |
| Cholera toxin | RPMC | IL-6 | HA, TNF-α | Inflammation | *227* |
| PMA | BMMC | VEGF | 5-HT | Angiogenesis | *12* |
| *Clostridium difficile* toxin A | RPMC | TNF-α | HA | GI tract inflammation | *228* |
| *Hemophilius pylori* | | | | | |
| VacA toxin | BMMC | IL-6, IL-8, TNF-α | HA | Gastric injury | *125* |
| Suboptimal FcεRI stimulation | BMMC | MCP-1, HA low | IL-10, HA | Chemokines >>Cytokines/HA | *229* |
| *Staphylococcus aureus* peptidoglycan or zymosan | hCBMC | GM-CSF, IL-1β, RANTES, $LTC_4$ | β-hexosaminidase, IL-6 | Exacerbation of asthma by bacterial infections | *119* |

stem cells, but their potential has only recently been appreciated. They have the unique ability of being embryonic cells without the explicit creation of an embryo to obtain them. They are found in the Wharton's Jelly of the umbilical cord, have capabilities and characteristics that are very close to embryonic stem cells *(48–50)*. hCMSCs have been preliminarily characterized by some investigators, and shown to express receptors generally found on mesenchymal stem cells (CD44 and CD105), but not hematopoietic lineage markers (CD34 and CD45). The hCMSCs can give rise to differentiated cell types found in embryonic germ layers including nerve cells, bone, cartilage, fat, tendon, muscle, and marrow stroma. hCMSCs have been reported to be c-kit positive *(51)*, which means they can develop into cells of neuroectoderm origin, such as brain mast cells *(52)*.

Recent developments have allowed us to use single cultured mast cells for multiple measurements. The use of live cell array (Fig. 2) permits the study of growth factors, triggers and inhibitors in real time using multiple fluorescent probes. This system will allow us to create conditions for the investigation of the characteristics of tissue-specific mast cells and their potential role in the inflammatory diseases discussed next.

## Eczema

Eczema is divided into atopic and nonatopic, but they both involve skin mast cells *(53)*. Mast cells are located close to sensory nerve endings *(54)* and can be triggered by neuropeptides, *(55–58)* such as NT *(59)*, NGF *(60)*, SP, *(61)*, and pituitary adenylate cyclase activating polypeptide (PACAP), all of which can be released from dermal neurons *(62)*. In fact, skin mast

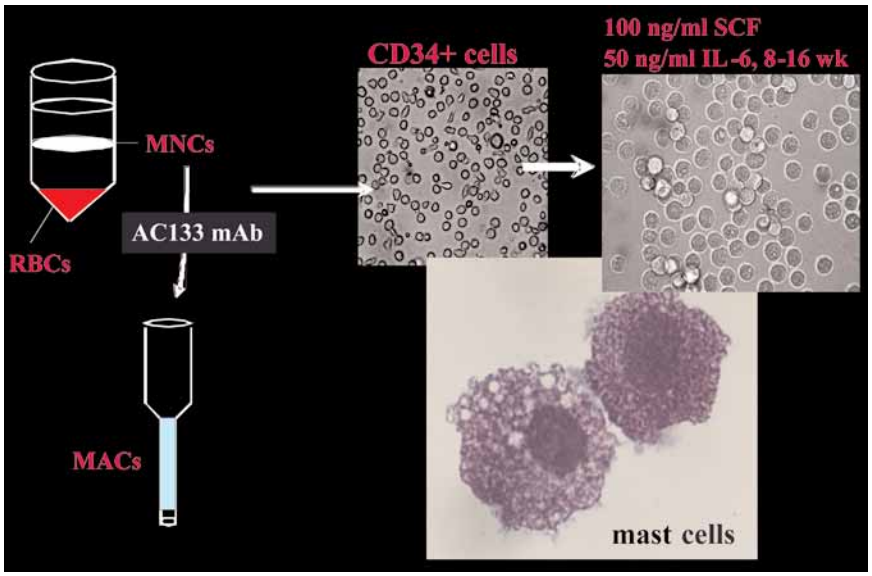


Fig. 1. Diagrammatic representation of the protocol for isolation and culture of mast cell progenitors from umbilical cord blood.

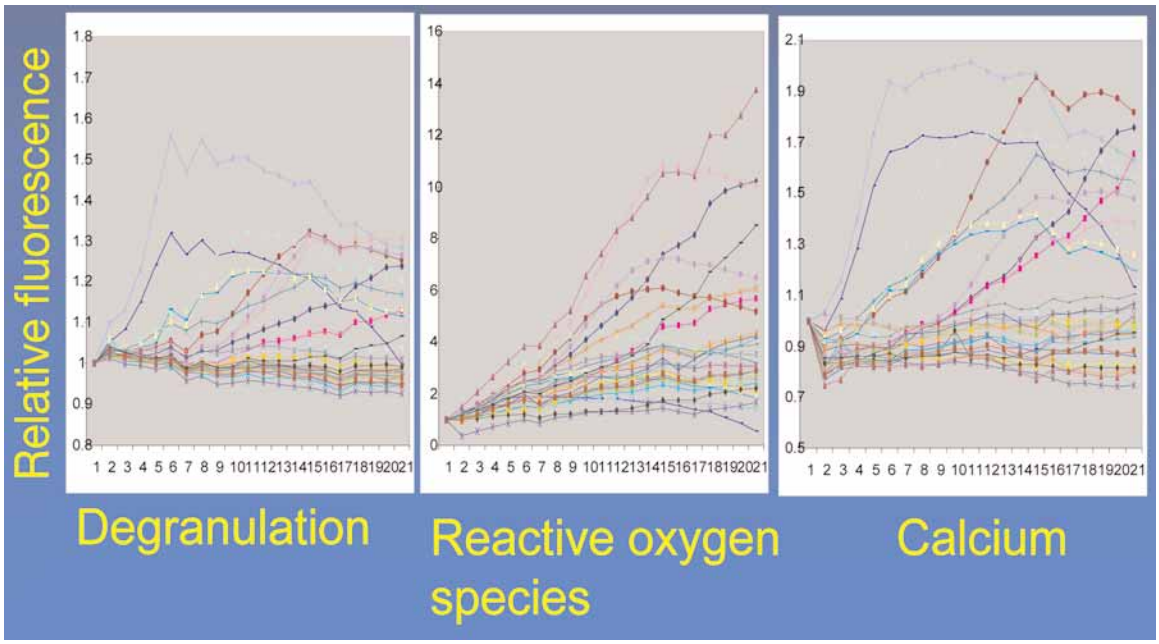


Fig. 2. Representative time curves of mast cells stimulated in cell array, showing degranulation, reactive oxygen species generation and intracellular calcium ion elevations, in individual mast cells (same color in all panels).

cells contain SP *(63)*, Although cultured mouse and HMC-1 contain and secrete NGF *(64)*. Proteases released from mast cells could act on plasma albumin to generate histamine releasing peptides *(65,66)* that could further propagate mast cell activation and inflammation. Proteases could also stimulate protease-activated receptors inducing microleakage and widespread inflammation *(67,68)*.

Many dermatoses, such as atopic dermatitis (AD), chronic urticaria, and psoriasis, are triggered or exacerbated by stress *(69)*, which also worsens eczema *(70)* and acne vulgaris *(71)*. Computer-induced stress enhanced allergen specific responses, with concomitant increase in plasma SP levels, in patients with AD *(72)*. Similar findings with increased plasma levels of SP, vasoactive intestinal peptide (VIP), and NGF, along with a switch to a TH2 cytokine pattern, were reported in patients with AD playing video games *(73)*. Exercise was also shown to increase the responsiveness of skin mast cells to morphine only in patients with exercise-induced asthma *(74)*.

Skin has its equivalent of the hypothalamic-pituitary-adrenal (HPA) axis *(75,76)*. Its main regulator, CRH and its receptors are present in the skin *(77)* from where CRH is released in response to stress *(78)*. Acute stress also exacerbates skin

delayed hypersensitivity reactions (79) and chronic contact dermatitis in rats, an effect dependent on mast cells and CRH-1 receptors; (80) acute stress also induces redistribution of leukocytes from the systemic circulation to the skin (81). Acute restraint stress induces rat skin vascular permeability (82), an effect inhibited by a CRH receptor antagonist and absent in mast cell-deficient mice (83,84). CRH (83) and it structurally related peptide, urocortin (Ucn; 84) can activate skin mast cells and induce mast cell-dependent vascular permeability in rodents. CRH also increases vascular permeability in human skin (85), a process dependent on mast cells. CRHR-2 receptor expression was shown to be upregulated in stress-induced alopecia in humans (86), Although CRHR-1 expression was increased in chronic urticaria (87). The immunoendocrine responses to stress in chronic skin inflammatory diseases have been reviewed (15,88) and it was proposed that mast cells constitute the "sensor" of a "brain–skin" connection (89).

## Asthma

Asthma is one of the most common chronic illnesses, affecting roughly 300 million people worldwide (90,91). Despite advances in both our scientific knowledge the morbidity and mortality owing to asthma continues to increase, as well as in hygiene and improved drugs for this disease (91). The World Health Organization has estimated that 1/250 deaths worldwide are owing to asthma, which highlights the need for an improved understanding of the cellular and molecular mechanisms that contribute to the pathogenesis of asthma.

The use of animal "models" has provided useful information about the mechanisms of airway inflammation and hyperreactivity characterizing asthma (92,93). Chronic exposure to aerosolized ovalbumin leads to airway inflammation, airway hyper-responsiveness (AHR; 94) as well as microvascular leakage in rodent airways (94–96). Microvascular leakage in the airway wall may also be important for the airway wall remodeling that characterizes most asthmatics (97,98). More recently, the house dust mite allergen model was shown to effectively induce chronic airway inflammation and AHR (97,99).

The role of mast cells in asthma is undisputed, but their role may have been underestimated, especially in viral infections (100–102). Viral infections commonly exacerbate asthma and contribute to >50% of asthma-associated deaths; moreover >80% of childhood asthma exacerbations are associated with viral airway infections (103). A number of studies have shown that viral infections increase AHR and antigen sensitization (104) as well as recruitment of inflammatory cells (105). Rhinovirus, adenovirus, as well as influenza, and parainfluenza viruses have been implicated in the pathogenesis of asthma (106–109). In fact, rhinovirus infections during infancy appear to predict childhood wheezing (110), while respiratory syncytial virus during the first 3 mo of life was shown to promote a TH2 response, especially significantly high levels of IL-4 (111). Such early infancy viral respiratory infections may also induce metalloproteinases that are involved in airway remodeling in asthma (112).

TLRs were shown to be important in recognition of ligands associated with bacterial or viral infections, and play a key role in the development of adaptive immune responses (113,114) especially in asthma (115). Ten human TLRs have been identified so far (114,116,117). Rodent mast cells express bacterial toll-like receptors (TLR) 2 and 4 (118,119). HMC-1 express viral TLR-9 (120), activation of which produced IL-6 (120), while TLR-3 activation produces IFN (121). Lipopolysaccharide (LPS) induces TNF release of through TLR-4, while peptidoglycan induces histamine release through TLR-2 from rodent mast cells. Fetal rat skin-derived mast cells express TLR 3, 7, and 9 and activation by CPG oligodeoxynucleotide induces release of TNF and IL-6, as well as RANTES and MIP, but without degranulation (122,123). LPS could not induce release of granulocyte monocyte-colony stimulating factor (GM-CSF), IL-1, or $LTC_4$ (119). However, LPS did induce secretion of TH2 cytokines, IL-5, IL-10 and IL-13 and increased their production by FcεRI cross-linking (124). Elsewhere, it was shown that TLR-2 activation produced IL-4, IL-6, and IL-13, but not IL-1 (125), whereas LPS produced TNF, IL-1, IL-6, and IL-13, but not IL-4 or IL-5, without degranulation (125).

Stress has long been postulated to negatively impact asthma, but the mechanisms by which this occurs remains poorly defined (126–131). Recent reports indicate that stress can induce asthma exacerbations (126–129, 132, 133). Maternal stress may be responsible for the subsequent cellular response in childhood asthma (134). It has been postulated that stress associated with urban living may contribute to poor asthma control (135). One study showed that adolescents with asthma in a low socioeconomic group, that reported more stressful and acute life events, had more asthma exacerbations and higher serum Th-2 cytokines than those in higher socioeconomic status (136). The Inner City Asthma Study showed a correlation between community violence and asthma morbidity (137). Post-traumatic psychological stress following the 9/11 attacks on the World Trade Center correlated with increased symptom severity in subjects with moderate-to-severe asthma and with utilization of urgent care in New York City (138,139). In an epidemiological study carried out among 10,667 Finnish first-year university students (18–25 yr old), excess of stressful events, such as concomitant severe disease or death of immediate family members or family conflicts, were associated with exacerbations of asthma (127). Moreover, stress associated with final, as compared to midsemester, examinations of college students with mild asthma increased sputum eosinophil counts, as well as eosinophil-derived neurotoxin and IL-5 once the eosinophils obtained were cultured for up to 24 h (130). It was suggested that a shift in cytokine generation to that of a Th2 type may be the defining parameter (133). In one longitudinal study (12 mo) of 92 adults with asthma, it was determined that subjects who reported more negative life events and had low levels of social support had more episodes of asthma exacerbations induced by upper respiratory tract infections (140). A prospective long-term follow-up community-based cohort study of young adults ($n$ = 591, 19–40 yr old) showed a dose-response relationship between panic and asthma (141). In fact, one study showed that greater levels of caregiver-perceived stress at 2–3 mo was associated with increased risk of subsequent repeated wheezing among children during the first 14 mo of life (142). Even though, the HPA axis apparently functions normally in asthmatic adult patients in response to stress (143); in one study, there was a significantly lower cortisol response to stress in asthmatic children (144) suggesting there may be age differences.

The timing of stress application appears to lead to different results. Short-term (3 d) stress before allergen challenge decreased the number of inflammatory cells, but increased IL-6, while long-term (7 d) stress evidently increased the number of inflammatory cells but did not alter IL-6 levels *(145)*.

## Inflammatory Arthritis

The presence of mast cells in the joints has been known *(22,146–152)* but the increasing role of mast cells in inflammatory arthritis was reviewed only recently *(153)*. The accumulation of mast cells in the joints may be owing to the local release of chemoattractants, such as RANTES and MCP-1 *(14)* found in joints of patients with arthrosynovitis *(154)*. Mast cells are required for autoimmune arthritis *(155)* and inflammatory arthritis *(156)* as knee involvement was absent in the joints of $W/W^v$ mast cell deficient mice as compared with their +/+ controls. Inflammatory arthritis was also significantly reduced in CRH knockout mice *(156)* and in mice treated with the CRH receptor-1 antagonist, antalarmin *(157)*.

Mast cells in the joints of rheumatoid arthritis (RA) patients express CRH receptors *(158)*. Moreover, both CRH *(158,159)* and Ucn *(160,161)*, as well as CRH receptors are increased in the joints of RA patients, the symptoms of which worsen by stress *(162,163)*.

## Coronary Inflammation

Mast cells are particularly prominent in coronary arteries during spasm *(164)* and accumulate in the shoulder region of human coronary plaque rupture *(165–167)*. Cardiac mast cells can participate in the development of atherosclerosis, coronary inflammation and cardiac ischemia *(168)*. The HMC-1 proteolytic enzyme chymase is the main cardiac source of converting enzyme that generates the coronary constrictor angiotensin II *(169)*; the chymase can also induce the removal of cholesterol from HDL particles and uptake by macrophages that become "foam" cells, major components of coronary atheromas *(170–173)*. Cardiac mast cell-derived histamine *(174)* can constrict the coronaries *(175)* and can sensitize nerve endings *(176)* this is particularly important since mast cells are localized close to nerve endings in atherosclerotic coronary arteries *(177)*. Patients with acute CAD have increased serum IL-6 elevations that derive primarily from the coronary sinus, suggesting a cardiac source *(178)*. Both histamine *(179)* and IL-6 *(180)* are significant independent factors of CAD morbility and mortality. There are also reports of anaphylactic CAD that has been termed the "Kounis" syndrome *(181,182)*.

Increasing evidence implicates acute psychological stress and cardiac mast cells in CAD, especially when occurring without angina that appears to involve a sizable portion of myocardial infections (MI; *183–186*). Acute stress induces rat cardiac mast cells activation, an effect blocked by the "mast cell stabilizer" disodium cromoglycate (cromolyn; *187*). Acute stress can also induce histamine release from mouse heart *(188)* as well as increase serum histamine and IL-6 *(188,189)*. These effects are dependent on mast cells and are greater in apolipoprotein E knockout mice that develop atherosclerosis *(188,189)*.

Recently, cryptogenic strokes originating from patent foramen ovale in the heart were associated with >50% of migraines with aura; closure of patent foramen ovale lead to migraine resolution *(190)*. Such migraines may develop from brain mast cell activation by an ischemic environment, CRH released in response to ischemic stress or endothelin released to counter ischemia *(191)*.

## Migraines

Migraine headaches are usually associated with meningeal and cerebral vasodilation, as well as "spreading" neuronal depression *(192)*. It was hypothesized, over 20 yr ago, that mast cells may be involved in the pathophysiology of migraines *(193)*. Mast cells are located in close apposition to neurons in the meninges *(194,195)* and can be activated by neuropeptides *(55)* by antidromic nerve stimulation *(26)* as well as by acute restraint stress *(27)*. Brain mast cells were also activated by acute stress leading to increased vascular permeability, *(196)* effects dependent on mast cells and CRH *(197)*.

Stress is known to precipitate or exacerbate migraines, raising the possibility of some underlying pathological mechanism. In one study of children migraines, the frequency and severity of migraines was reduced, along with the unique mast cell marker tryptase, when they were taught relaxation techniques *(198)*. Recent findings have led to a new model for the pathogenesis of intracranial neurogenic inflammation that calls for CRH acting on the sensory nucleus of the trigeminal nerve, which expresses CRH receptors *(199)* and to secrete mast cell stimulating peptides and/or a direct action of local CRH released from nerve endings or from mast cells acting directly on the vasculature *(15)*. Recent studies have shown that stress-induced neurogenic inflammation depends on NK-1 receptors, but does not require SP *(200)*, while it may involve a direct action of CRH on brain microvessels *(201)*. Yet, delayed responses may also involve IL-6 and nitric oxide (NO) elevations in dura macrophages *(202)*.

Mast cells are localized close to CRH-positive neurons in the median eminence *(203)* and express functional CRH receptors *(204)*. The median eminence is rich in mast cells *(205,206)* and contains most of the histamine in the brain *(207)*. Hypothalamic mast cell activation can, therefore, stimulate the HPA axis *(208–210)*. Histamine is a major regulator of the hypothalamus *(211)* and can increase its CRH mRNA expression *(212)*. Moreover, HMC-1 can synthesize and secrete large amounts of CRH *(213)* as well as IL-1 and IL-6, which are independent activators of the HPA axis *(214)*.

## Conclusion

Mast cells are unique bone marrow-derived cells that can be activated by many immune and nonimmune triggers, including acute stress through CRH. Inhibition of CRH-induced mast cell activation *(215)* is, therefore, a novel target for the development of new treatments for inflammatory and autoimmune disorders. Umbilical cord-derived blood or matrix progenitor cells offer unique opportunities for the development and study of tissue specific (i.e., skin, lungs, and brain) mast cells. The emerging use of cell array technology provides a powerful tool for the *fast output screening* of the effect of different growth factors on *individual* mast cell responses to growth factors, triggers and inhibitors Table 5.

Table 5
Advantages of Cell Array

| |
|---|
| 1. Fast |
| 2. Uses very few cells per assay |
| 3. Uses minimal quantity of reagents |
| 4. Easy to do time-course |
| 5. Can take multiple simultaneous measurements |
| 6. Obtains data from individual cells |

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## References

1 Leung DY, Diaz LA, DeLeo V, Soter NA. JAMA 1997;278: 914–1923.
2 Charlesworth EN. Chem Immunol 1995;62:84–107.
3 Church MK, Clough GF. Ann Allergy Asthma Immunol 1999;83:471–475.
4 Noli C, Miolo A. Vet Dermatol 2001;12:303–313.
5 Jarvikallio A, Harvima IT, Naukkarinen A. Arch Dermatol Res 2003;295:2–7.
6 Rodewald HR, Dessing M, Dvorak AM, Galli SJ. Science 1996;271:818–822.
7 Chen CC, Grimbaldeston MA, Tsai M, Weissman IL, Galli SJ. Proc Natl Acad Sci USA 2005;102:11,408–11,413.
8 Galli SJ, Nakae S, Tsai M. Nat Immunol 2005;6:135–142.
9 Blank U, Rivera J. Trends Immunol 2004;25:266–273.
10 Kraft S, Rana S, Jouvin MH, Kinet JP. Int Arch Allergy Immunol 2004;135:62–72.
11 Grutzkau A, Kruger-Krasagakes S, Baumeister H, et al. Mol Biol Cell 1998;9:875–884.
12 Boesiger J, Tsai M, Maurer M, et al. J Exp Med 1998;188:1135–1145.
13 Aloe L, Levi-Montalcini R. Brain Res 1977;133:358–366.
14 Conti P, Pang X, Boucher W, et al. Blood 1997;89:4120–4127.
15 Theoharides TC, Cochrane DE. J Neuroimmunol 2004;146:1–12.
16 Kraneveld AD, Kool M, van Houwelingen AH, et al. Proc Natl Acad Sci USA 2005;102:1578–1583.
17 Redegeld FA, van der Heijden MW, Kool M, et al. Nat Med 2002;8:694–701.
18 Redegeld FA, Nijkamp FP. Trends Immunol 2003;24:181–185.
19 Rottem M, Mekori YA. Autoimmun Rev 2005;4:21–27.
20 Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Annu Rev Immunol 2005;23:749–786.
21 Benoist C, Mathis D. Nature 2002;420:875–878.
22 Woolley DE. N Engl J Med 2003;348:1709–1711.
23 Theoharides TC, Bondy PK, Tsakalos ND, Askenase PW. Nature 1982;297:229–231.
24 Theoharides TC, Douglas WW. Endocrinology 1978;102: 1637–1640.
25 Dimitriadou V, Lambracht-Hall M, Reichler J, Theoharides TC. Neuroscience 1990;39:209–224.
26 Dimitriadou V, Buzzi MG, Moskowitz MA, Theoharides TC. Neuroscience 1991;44:97–112.
27 Theoharides TC, Sant GR, El-Mansoury M, Letourneau RJ, Ucci AA Jr, Meares EM Jr. J Urol 1995;153:629–636.
28 Letourneau R, Pang X, Sant GR, Theoharides TC. Br J Urol 1996;77:41–54.
29 Dvorak AM, McLeod RS, Onderdonk A, et al. Int Arch Allergy Immunol 1992;99:74–83.
30 Kops SK, Van Loveren H, Rosenstein RW, Ptak W, Askenase PW. Lab Invest 1984;50:421–434.
31 Van Loveren H, Kops SK, Askenase PW. Eur J Immunol 1984;14:40–47.
32 Kops SK, Theoharides TC, Cronin CT, Kashgarian MG, Askenase PW. Cell Tissue Res 1990;262:415–424.
33 Benyon R, Robinson C, Church MK. Br J Pharmacol 1989;97:898–904.
34 Levi-Schaffer F, Shalit M. Int Arch Allergy Appl Immunol 1989;90:352–357.
35 van Haaster CM, Engels W, Lemmens PJMR, Hornstra G, van der Vusse GJ, Heemskerk JWM. Biochim Biophys Acta 1995;1265: 79–88.
36 Leal-Berumen I, Conlon P, Marshall JS. J Immunol 152:5468–5476.
37 Marquardt DL, Alongi JL, Walker LL. J Immunol 1996;156:1942–1945.
38 Gagari E, Tsai M, Lantz CS, Fox LG, Galli SJ. Blood 1997;89:2654–2663.
39 Hojo H, Sun R, Ono Y, et al. Cancer Lett 1996;108:55–59.
40 Kandere-Grzybowska K, Letourneau R, Boucher W, et al. J Immunol 2003;171:4830–4836.
41 Cao J, Curtis CL, Theoharides TC. Mol Pharmacol 2006;69: 998–1006.
42 Alexandrakis MG, Letourneau R, Kempuraj D, et al. Eur J Haematol 2003;71:448–454.
43 Drexler HG, MacLeod RA. Leuk Res 2003;27:671–676.
44 Kirshenbaum AS, Akin C, Wu Y, et al. Leuk Res 2003;27:677–682.
45 Kempuraj D, Saito H, Kaneko A, et al. Blood 1999;93:3338–3346.
46 Amano H, Kurosawa M, Ishikawa O, Chihara J, Miyachi Y. J Dermatol Sci 2000;24:146–152.
47 Toru H, Eguchi M, Matsumoto R, Yanagida M, Yata J, Nakahata T. Blood 1998;91:187–195.
48 Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Stem Cells 2005;23:220–229.
49 Wang HS, Hung SC, Peng ST, et al. Stem Cells 2004;22:1330–1337.
50 Romanov YA, Svintsitskaya VA, Smirnov VN. Stem Cells 2003;21:105–110.
51 Mitchell KE, Weiss ML, Mitchell BM, et al. Stem Cells 2003;21:50–60.
52 Hirota S, Nomura S, Asada H, Ito A, Morii E, Kitamura Y. Arch Pathol Lab Med 1993;117:996–999.
53 Brown S, Reynolds NJ. BMJ 2006;332:584–588.
54 Wiesner-Menzel L, Schulz B, Vakilzadeh F, Czarnetzki BM. Acta Derm Venereol (Stockh) 1981;61:465–469.
55 Goetzl EJ, Chernov T, Renold F, Payan DG. J Immunol 1985;135:802S–805S.
56 Foreman JC. Allergy 1987;42:1–11.
57 Church MK, Lowman MA, Rees PH, Benyon RC. Agents Actions 1989;27:8–16.
58 Goetzl EJ, Cheng PPJ, Hassner A, Adelman DC, Frick OL, Speedharan SP. Clin Exp Allergy 1990;20:3–7.
59 Carraway R, Cochrane DE, Lansman JB, Leeman SE, Paterson BM, Welch HJ. J Physiol 1982;323:403–414.
60 Watt FM, FASEB J 1991;5:287–294.
61 Fewtrell CMS, Foreman JC, Jordan CC, Oehme P, Renner H, Stewart JM. J Physiol 1982;330:393–411.
62 Odum L, Petersen LJ, Skov PS, Ebskov LB. Inflamm Res 1998;47: 488–492.
63 Toyoda M, Makino T, Kagoura M, Morohashi M. Arch Dermatol Res 2000;292:418–421.
64 Xiang Z, Nilsson G. Clin Exp Allergy 2000;30:1379–1386.
65 Carraway RE, Cochrane DE, Boucher W, Mitra SP. J Immunol 1989;143:1680–1684.
66 Cochrane DE, Carraway RE, Feldberg RS, Boucher W, Gelfand JM. Peptides 1993;14:117–123.
67 Schmidlin F, Bunnett NW. Curr Opin Pharmacol 2001;1: 575–582.

68 Molino M, Barnathan ES, Numerof R, et al. J Biol Chem 1997;272:4043–4049.
69 Katsarou-Katsari A, Filippou A, Theoharides TC. Int J Immunopathol Pharmacol 1999;12:7–11.
70 Graham DT, Wolf S. J Lab Clin Med 1953;42:238–254.
71 Murphy PM. N Engl J Med 2001;345:833–835.
72 Kimata H. Brain Behav Immun 2003;17:134–138.
73 Kimata H. Eur J Clin Invest 2003;33:513–517.
74 Choi IS, Koh YI, Chung SW, Lim H. J Korean Med Sci 2004;19:724–728.
75 Slominski A, Wortsman J. Endocrinol Rev 2000;21:457–487.
76 Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Physiol Rev 2000;80:979–1020.
77 Slominski A, Wortsman J, Pisarchik A, et al. FASEB J 15:1678–1693 2001.
78 Lytinas M, Kempuraj D, Huang M, Boucher W, Esposito P, Theoharides TC. Int Arch Allergy Immunol 2003;130:224–231.
79 Dhabhar FS, McEwen BS. Proc Natl Acad Sci USA 1999;96:1059–1064.
80 Kaneko K, Kawana S, Arai K, Shibasaki T. Exp Dermatol 2003;12:47–52.
81 Dhabhar F, McEwen BS. J Immunol 1996;156:2608–2615.
82 Singh LK, Pang X, Alexacos N, Letourneau R, Theoharides TC. Brain Behav Immunity 1999;13:225–239.
83 Theoharides TC, Singh LK, Boucher W, et al. Endocrinology 1998;139:403–413.
84 Singh LK, Boucher W, Pang X, et al. J Pharmacol Exp Ther 1999;288:1349–1356.
85 Clifton VL, Crompton R, Smith R, Wright IM. J Clin Endocrinol Metab 2002;87:267–270.
86 Katsarou-Katsari A, Singh LK, Theoharides TC. Dermatology 2001;203:157–161.
87 PFOadopoulou N, Kalogeromitros D, Staurianeas NG, Tiblalexi D, Theoharides TC. J Invest Dermatol 2005;125:952–955.
88 Buske-Kirschbaum A, Hellhammer DH. Ann NY Acad Sci 2003;992:231–240.
89 Paus R, Theoharides TCAP. Trends Immunol 2006;27:32–39.
90 Weiss ST. Ann Allergy Asthma Immunol 2001;87:5–8.
91 PFOiris S, Kotanidou A, Malagari K, Roussos C. Crit Care 2002;6:30–44.
92 Kips JC, Anderson GP, Fredberg JJ, et al. Eur Respir J 2003;22:374–382.
93 Lloyd CM, Gutierrez-Ramos JC. Methods Mol Biol 2004;239:199–210.
94 Wilson J. Clin Exp Allergy 2000;30:51–53.
95 Olivenstein R, Du T, Xu LJ, Martin JG. Pulm Pharmacol Ther 1997;10:223–230.
96 Van Rensen EL, Hiemstra PS, Rabe KF, Sterk PJ. Am J Respir Crit Care Med 2002;165:1275–1279.
97 Johnson JR, Wiley RE, Fattouh R, et al. Am J Respir Crit Care Med 2004;169:378–385.
98 Naureckas ET, Ndukwu IM, Halayko AJ, Maxwell C, Hershenson MB, Solway J. Am J Respir Crit Care Med 1999;160:2062–2066.
99 Sadakane K, Ichinose T, Takano H, et al. Int Arch Allergy Immunol 2002;128:220–228.
100 Cho SH, Anderson AJ, Oh CK. Clin Rev Allergy Immunol 2002;22:161–174.
101 Bradding P. Curr Opin Allergy Clin Immunol 2003;3:45–50.
102 Brightling CE, Bradding P, Pavord ID, Wardlaw, AJ. Clin Exp Allergy 2003;33:550–556.
103 O'Sullivan, SM. Proc Am Thorac Soc 2005;2:162–165.
104 Dakhama A, Lee YM, Gelfand EW. Pediatr Infect Dis J 2005;24:S159–S169 discussion.
105 Van Rijt LS, van Kessel CH, Boogaard I, Lambrecht BN. J Clin Virol 2005;34:161–169.
106 Sackesen C, Pinar A, Sekerel BE, Akyon Y, Saraclar Y. Turk J Pediatr 2005;47:227–231.
107 Pelaia G, Vatrella A, Gallelli L, et al. Respir Med 2005.
108 Matsuse H, Kondo Y, Saeki S, et al. Int Arch Allergy Immunol 2005;138:267–272.
109 Williams JV, Crowe JE Jr, Enriquez R, et al. J Infect Dis 2005;192:1149–1153.
110 Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. J Allergy Clin Immunol 2005;116:571–577.
111 Kristjansson S, Bjarnarson SP, Wennergren G, et al. J Allergy Clin Immunol 2005;116:805–811.
112 Gualano RC, Vlahos R, Anderson, GP. Pulm Pharmacol Ther 2006;19:18–23.
113 Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. Proc Natl Acad Sci USA 1998;95:588–593.
114 Aderem A, Ulevitch RJ. Nature 2000;406:782–787.
115 Cristofaro P, Opal, SM. Drugs 2006;66:15–29.
116 Akira S, Takeda K, Kaisho T. Nat Immunol 2001;2:675–680.
117 Heine H, Lien E. Int Arch Allergy Immunol 2003;130:180–192.
118 Varadaradjalou S, Feger F, Thieblemont N, et al. Eur J Immunol 2003;33:899–906.
119 McCurdy JD, Olynych TJ, Maher LH, Marshall JS. J Immunol 2003;170:1625–1629.
120 Ikeda RK, Miller M, Nayar J, et al. J Immunol 2003;171:4860–4867.
121 Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. J Allergy Clin Immunol 2004;114:174–182.
122 Anthony M, Lance JW. Proc Aust assoc Neurol 1971;8:43–46.
123 Cairns JA, Walls AF. J Immunol 1996;156:275–283.
124 Masuda A, Yoshikai Y, Aiba K, Matsuguchi T. J Immunol 2002;169:3801–3810.
125 Supajatura V, Ushio H, Nakao A, et al. J Clin Invest 2002;109:1351–1359.
126 Laube BL, Curbow BA, Costello RW. Respir Med 2002;96:823–828.
127 Kilpelainen M, Koskenvuo M, Helenius H, Terho EO. Clin Exp Allergy 2002;32:256–263.
128 Lawrence DA. Environ Health Perspect 2002;110:A230–A231.
129 Joachim RA, Quarcoo D, Arck PC, Herz U, Renz H, Klapp BF. Psychosom Med 2003;65:811–815.
130 Liu LY, Coe CL, Swenson CA, Kelly EA, Kita H, Busse WW. Am J Respir Crit Care Med 2002;165:1062–1067.
131 Gordon DJ, Rifkind BM. N Engl J Med 1989;321:1311–1316.
132 Schmaling KB, McKnight PE, Afari N. J Asthma 2002;39:501–510.
133 Bienenstock J. Am J Respir Crit Care Med 2002;165:1034–1035.
134 von Hertzen LC. J Allergy Clin Immunol 2002;109:923–928.
135 Dendorfer U, Oettgen P, Libermann TA. Mol Cell Biol 1994;14:4443–4454.
136 Chen E, Fisher EB, Bacharier LB, Strunk RC. Psychosom Med 2003;65:984–992.
137 Wright RJ, Mitchell H, Visness CM, et al. Am J Public Health 2004;94:625–632.
138 Fagan J, Galea S, Ahern J, Bonner S, Vlahov, D. Psychosom Med 2003;65:993–996.
139 Centers for Disease Control and Prevention (CDC) Self-reported increase in asthma severity after the September 11 attacks on the World Trade Center—Manhattaan, New York, 2001. MMWR Morb Mortal Wkly Rep 2002;51:781–784.
140 Smith A, Nicholson K. Psychoneuroendocrinology 2001;26:411–420.
141 Hasler G, Gergen PJ, Kleinbaum DG, et al. Am J Respir Crit Care Med 2005;171:1224–1230.
142 Wright RJ, Cohen S, Carey V, Weiss ST, Gold DR. Am J Respir Crit Care Med 2002;165:358–365.
143 Kapoor U, Tayal G, Mittal SK, Sharma VK, Tekur U. Indian J Pediatr 2003;70:965–968.
144 Buske-Kirschbaum A, von Auer K, Krieger S, Weis S, Rauh W, Hellhammer D. Psychosom Med 2003;65:806–810.
145 Forsythe P, Ebeling C, Gordon JR, Befus AD, Vliagoftis H. Am J Respir Crit Care Med 2004;169:220–226.
146 Crisp AJ, Champan CM, Kirkham SE, Schiller AL, Keane SM. Arthritis Rheum 1984;27:845–851.
147 Koldewijn EL, Hommes OR, Lemmens WAJG, Debruyne FMJ, Van Kerrebroeck PEV. J Urol 1995;154:169–173.

148 Woolley DE. Ann Rheum Dis 1995;54:533–534.
149 Tetlow LC, Woolley DE. Ann Rheum Dis 1995;54:549–555.
150 de Paulis A, Ciccarelli A, Marinò I, de Crescenzo G, Marinò D, Marone G. Arthritis Rheum 1997;40:469–478.
151 de Paulis A, Marino I, Ciccarelli A, et al. Arthritis Rheum 1996;39:1222–1233.
152 Gotis-Graham I, Smith MD, Parker A, McNeil HP. Ann Rheum Dis 1998;57:664–671.
153 Theoharides, T. C. Synovial mast cells in inflammatory arthritis. in E. D. Meyers (ed.), Encyclopedia of Molecular Cell Biology and Molecular Medicine, Larkspur, CA: Ramtech Ltd., 2005.
154 Conti P, Reale M, Barbacane RC, Castellani ML, Orso C. Immunol Lett 2002;80:105–111.
155 Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner MB. Science 2002;297:1689–1692.
156 Mattheos S, Christodoulou S, Kempuraj D, Kempuraj B, Karalis K, Theoharides TC. FASEB J 2003;17:C44.
157 Webster EL, Barrientos RM, Contoreggi C, et al. J Rheumatol 2002;29:1252–1261.
158 McEvoy AN, Bresnihan B, FitzGerald O, Murphy EP. Arthritis Rheum 2001;44:1761–1767.
159 Lowry PJ, Woods RJ, Baigent S. Pharmacol Biochem Behav 1996;54:305–308.
160 Uzuki M, Sasano H, Muramatsu Y, et al. Clin Sci 2001;100:577–589.
161 Kohno M, Kawahito Y, Tsubouchi Y, et al. J Clin Endocrinol Metab 2001;86:4344–4352.
162 Thomason BT, Brantley PJ, Jones GN, Dyer HR, Morris JL. J Behav Med 1992;15:215–220.
163 Herrmann M, Scholmerich J, Straub RH. Rheum Dis Clin North Am 2000;26:737–763.
164 Forman MB, Oates JA, Robertson D, Robertson RM, Roberts LJ II, Virmani R. N Engl J Med 1985;313:1138–1141.
165 Kaartinen M, Penttilä A, Kovanen PT. Circulation 1994;90:1669–1678.
166 Constantinides P. Circulation 1995;92:1083–1088.
167 Laine P, Kaartinen M, Penttilä A, Panula P, Paavonen T, Kovanen PT. Circulation 1999;99:361–369.
168 Patella V, de Crescenzo G, Ciccarelli A, Marino I, Adt M, Marone, G. Int Arch Allergy Immunol 1995;106:386–393.
169 Jenne DE, Tschopp J. Biochem J 1991;276:567.
170 Lee M, Kovanen PT, Tedeschi G, Oungre E, Franceschini G, Calabresi L. J Lipid Res 2003;44:539–546.
171 Lee M, Calabresi L, Chiesa G, Franceschini G, Kovanen PT. Arterioscler Thromb Vasc Biol 2002;22:1475–1481.
172 Kovanen PT. Curr Opin Lipidol 1996;7:281–286.
173 Lindstedt L, Lee M, Castro GR, Fruchart JC, Kovanen PT. J Clin Invest 1996;97:2174–2182.
174 Gristwood RW, Lincoln JC, Owen DA, Smith IR. Br J Pharmacol 1981;74:7–9.
175 Genovese A, Spadaro G. Allergy 1997;52:67–78.
176 Christian EP, Undem BJ, Weinreich D. J Physiol 1989;409:297–312.
177 Laine P, Naukkarinen A, Heikkila L, Penttilä A, Kovanen PT. Circulation 2000;101:1665–1669.
178 Deliargyris EN, Raymond RJ, Theoharides TC, Boucher WS, Tate DA, Dehmer GJ. Am J Cardiol 2000;86:913–918.
179 Clejan S, Japa S, Clemetson C, Hasabnis SS, David O, Talano JV. J Cell Mol Med 2002;6:583–592.
180 Suzuki M, Inaba S, Nagai T, Tatsuno H, Kazatani Y. Am J Cardiol 2003;91:331–333.
181 Kounis NG, Zavras GM. Circulation 1996;94:1789.
182 Kounis NG, Grapsas ND, Goudevenos JA. Circulation 1999;100:E156.
183 Deedwania PC. JACC 1995;25:1504–1506.
184 Freeman LJ, Nixon PGF, Sallabank P, Reaveley D. Am Heart J 1987;114:477–482.
185 Deanfield JE, Shea M, Kensett M, et al. Lancet 1984;2: 1001–1005.
186 Rozanski A, Bairey CN, Krantz DS, et al. N Engl J Med 1988;318:1005–1012.
187 Pang X, Alexacos N, Letourneau R. J Pharm Exp Therap 1998;287:307–314.
188 Huang M, Pang X, Letourneau L, Boucher W, Theoharides TC. Cardiovasc Res 2002;55:150–160.
189 Huang M, Pang X, Karalis K, Theoharides TC. Cardiovasc Res 2003;59:241–249.
190 Diener HC, Weimar C, Katsarava Z. Curr Opin Neurol 2005;18:299–304.
191 Theoharides TC, Donelan J, Kandere-Grzybowska K, Konstantinidou A. Brain Res Rev 2005;49:65–76.
192 Spierings EL. Clin J Pain 2003;19:255–262.
193 Theoharides TC. Perspect Biol Med 1983;26:672–675.
194 Dimitriadou V, Aubineau P, Taxi J, Seylaz J. Neuroscience 1987;22:621–630.
195 Rozniecki JJ, Letourneau R, Sugiultzoglu M, Spanos C, Gorbach J, Theoharides TC. J Pharmacol Exp Ther 1999;290:1427–1435.
196 Dimitriadou V, Buzzi MG, Theoharides TC, Moskowitz, MA. Neuroscience 1992;48:187–203.
197 Esposito P, Chandler N, Kandere-Grzybowska K, et al. J Pharmacol Exp Ther 2002;303:1061–1066.
198 Olness K, Hall H, Rozniecki JJ, Schmidt W, Boucher W, Theoharides TC. Headache 1999;39:101–107.
199 Wilhelms O-H, Kreusser W, Ritz E. Miner Electrolyte Metab 1981;6:294–301.
200 Kandere-Grzybowska K, Gheorghe D, Priller J, et al. Brain Res 2003;980:213–220.
201 Esposito P, Basu S, Letourneau R, Jacobson S, Theoharides, TC. Brain Res 2003;968:192–198.
202 Reuter U, Bolay H, Jansen-Olesen I, et al. Brain 2001;124:2490–2502.
203 Theoharides TC, Spanos CP, Pang X, et al. Endocrinology 1995;136:5745–5750.
204 Cao J, PFOadopoulou N, Kempuraj D, et al. J Immunol 2005;174:7665–7675.
205 Pollard H, Bischoff S, Llorens-Cortes C, Schwartz JC. Brain Res 1976;118:509–513.
206 Panula P, Yang H-YT, Costa E. Proc Natl Acad Sci USA 1984;81:2572–2576.
207 Yamatodani A, Maeyama K, Watanabe T, Wada H, Kitamura Y. Biochem Pharmacol 1982;31:305–309.
208 Bugajski AJ, Chlap Z, Gadek-Michalska A, Borycz J, Bugajski J. Inflamm Res 1995;44(Supp.1):S50–S51.
209 Gadek-Michalska A, Chlap Z, Turon M, Bugajski J, Fogel WA. Agents Actions 1991;32:203–208.
210 Matsumoto I, Inoue Y, Shimada T, Aikawa T. J Exp Med 2001;194:71–78.
211 Roberts F, Calcutt CR. Neuroscience 1983;9:721–739.
212 Kjaer A, Larsen PJ, Knigge U, Jorgensen H, Warberg J. Eur J Endocrinol 1998;139:238–243.
213 Lytinas M, Kempuraj D, Kandere K, et al. Endocrinology 2003, in press.
214 Bethin KE, Vogt SK, Muglia LJ. Proc Natl Acad Sci USA 2000;97:9317–9322.
215 Theoharides TC, Donelan JM, PFOadopoulou N, Cao J, Kempuraj D, Conti P. Trends Pharmacol Sci 2004;25:563–568.
216 Hogaboam CM, Befus AD, Wallace JL. J Immunol 1993; 151:3767–3774.
217 Leal-Berumen I, O'Byrne P, Gupta A, Richards CD, Marshall JS. J Immunol 1995;154:4759–4767.
218 Gupta AA, Leal-Berumen I, Croitoru K, Marshall JS. J Immunol 1996;157:2123–2128.
219 Lin TJ, Hirji N, Nohara O, Stenton GR, Gilchrist M, Befus AD. J Immunol 1998;161:6265–6272.
220 Gordon JR, Zhang X, Stevenson K, Cosford K. Cell Immunol 2000;205:128–135.
221 Lin TJ, Issekutz TB, Marshall JS. J Immunol 2000;165:211–220.
222 Kalesnikoff J, Huber M, Lam V, et al. Immunity 2001;801–811.

223. Coulombe M, Battistini B, Stankova J, et al. J Leukoc Biol 2002;71:829–836.
224 Mellor EA, Austen KF, Boyce JA. J Exp Med 2002;195:583–592.
225 PFOadopoulou NG, Oleson L, Kempuraj D, Donelan J, Cetrulo CL, Theoharides TC. J Mol Endocrinol 2005;35:R1–R8.
226. Zhu F, Gomi K, Marshall JS. J Immunol 1998;161:2541–2551.
227. Leal-Berumen I, Snider DP, Barajas-Lopez C, Marshall JS. J Immunol 1996;156:316–321.
228. Calderon GM, Torres-Lopez J, Lin TJ, et al. Infect Immun 1998;66:2755–2761.
229. Gonzalez-Espinosa C, Odom S, Olivera A, et al. J Exp Med 2003;197:1453–1465.