Concurrent Session I: Cytokines
Co-chairs: Michael T. Lotze, MD
Crystal Mackall, MD
IL-34

A novel cytokine, FPT025, was identified in a high-throughput functional screen with human monocytes;

- No sequence homology with known cytokine families;
- Stimulates monocyte proliferation/survival at 5 ng/ml (0.2 nM);
- Specifically binds to human monocytes and activates ERK1/2 phosphorylation via c-fms [M-CSF Receptor];
- Promotes CFU-GM and CFU-M colony formation from human bone marrow cells;
- Stimulates expression of myeloid cell surface markers.
Interleukin-35: A Novel Cytokine that Mediates Regulatory T Cell Function  Dario Vignali, Ph.D., Associate Member, Department of Immunology, St. Jude Children’s Research Hospital  Regulatory T (TR) cells are a critical sub-population of CD4+ T cells that are essential for maintaining self tolerance and preventing autoimmunity, limiting chronic inflammatory diseases, such as asthma and inflammatory bowel disease (IBD), and regulating homeostatic lymphocyte expansion. However, they also suppress natural immune responses to parasites and viruses as well as anti-tumor immunity induced by therapeutic vaccines. While the manipulation of TR function is an important goal of immunotherapy, the molecules that mediate their suppressive activity remain largely unknown. Here we demonstrate that Epstein-Barr virus-induced gene 3 (Ebi3; IL27β) and interleukin-12 alpha (Il12a; IL12α/p35) are highly expressed by Foxp3+ (forkhead box P3) TR cells but not by resting or activated effector CD4+ T (TE) cells, and that an Ebi3/Il12a heterodimer is constitutively secreted by TR but not TE cells. Both Ebi3 and Il12a mRNA are dramatically upregulated in TR cells co-cultured with TE cells, thereby boosting Ebi3/Il12a production in trans. TR cell-restriction of this cytokine is due to Ebi3 being a downstream target of Foxp3, a transcription factor that is required for TR cell development and function. Ebi3−/− and Il12a−/− TR cells have significantly reduced regulatory activity in vitro and fail to control homeostatic proliferation and cure IBD in vivo. As these phenotypic characteristics are distinct from those of other IL-12-family members, this novel Ebi3/Il12a heterodimeric cytokine has been designated interleukin-35 (IL-35). Ectopic expression of IL-35 confers regulatory activity on naïve T cells, while recombinant IL-35 suppresses T cell proliferation. Taken together, these data identify IL-35 as a novel inhibitory cytokine that is specifically produced by TR cells and is crucial for maintaining self tolerance.
### Chronic Inflammatory Conditions Associated with Cancer

<table>
<thead>
<tr>
<th>Chronic inflammation</th>
<th>Associated cancer</th>
<th>Aetiological agent</th>
<th>Percent predisposed that progress to cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchitis</td>
<td>Lung cancer</td>
<td>Tobacco smoke</td>
<td>11–24</td>
</tr>
<tr>
<td>Gastritis</td>
<td>Gastric cancer</td>
<td><em>Helicobacter pylori</em></td>
<td>1–3</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>Cervical cancer</td>
<td>Human papillomavirus</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Warts</td>
<td>Non-melanoma skin cancer</td>
<td>Ultraviolet light, human papillomavirus</td>
<td>Varies with skin pigment and solar intensity</td>
</tr>
<tr>
<td>Asbestosis</td>
<td>Mesotheloma</td>
<td>Asbestos fibres</td>
<td>10–15</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Colorectal cancer</td>
<td>Gut pathogens, altered gut permeability</td>
<td>1*</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Pancreatic cancer</td>
<td>Tobacco, genetic factors</td>
<td>≤10%*</td>
</tr>
<tr>
<td>Oesophagitis</td>
<td>Oesophageal cancer</td>
<td>Gastric acid, alcohol, tobacco</td>
<td>15</td>
</tr>
<tr>
<td>Sunburned skin</td>
<td>Melanoma, basal-cell carcinoma, squamous-cell carcinoma</td>
<td>Ultraviolet light</td>
<td>Varies with skin pigment and solar intensity, ≤9% of Caucasians</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Hepatocellular carcinoma</td>
<td>Hepatitis B virus, hepatitis C virus</td>
<td>10</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Burkitt’s lymphoma, Hodgkin’s disease</td>
<td>Epstein–Barr virus</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>Gall bladder cancer</td>
<td>Bacteria, gall bladder stones</td>
<td>1–2*</td>
</tr>
<tr>
<td>Cystitis</td>
<td>Bladder cancer</td>
<td>Gram-negative uropathogens, pelvic irradiation, carcinogens</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Per year. *In susceptible populations. *At cholecystectomy.

Marked Decrease in Dendritic Cells in Pediatric Cancers

Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.
Comparable Number of Macrophages and Decreased DCs in Pediatric Cancer

Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.
Death Used to be Simpler
Apoptosis [I], Autophagy [II] and Necrosis [III]

Extrinsic
- TNF, LTα
- TRAIL, FasL

Intrinsic
- p53/PUMA
- RT, ChemoRx
- Mitochondrial Toxins, UV

Cytolytic – T/NK
- Perforin
- Granzymes A, B, K, M

Necrosis
- DAMPs

p53 Sequestration
- BCL2, BCLxL↑
- IAP, XIAP, Survivin

Zeh H. J., 3rd and Lotze M. T.
Addicted to death: invasive cancer and the immune response to unscheduled cell Death
IL-1: A Pleiotropic Cytokine

- Fever
- Sleep
- Loss of appetite
- Social withdrawal
- Vasodilation
- Adhesion molecules
- Chemotaxis
- Increased permeability
- Immune activation
- Stimulation of hematopoiesis

- Procoagulant state
- Acute phase protein production
- Collagenase and stromelysin
- Bone resorption
- Fibroblast and epithelial cell proliferation
- Prostaglandin E₂
Melanoma Patient Response Before and After High Dose IL-2

Bethesda 1987
The Cytokine Handbook, 4th Edition Two-Volume Set

Edited By

Angus Thomson, Starzl Transplantation Institute of the University of Pittsburgh, Pennsylvania, U.S.A.
Michael Lotze, Molecular Medicine Institute of the University of Pittsburgh, Pennsylvania, U.S.A.

Description
The fourth edition of The Cytokine Handbook provides an encyclopedic coverage of the molecules that induce and regulate immune responses. Now expanded to two volumes, co-edited by Michael T Lotze, and written by over 120 international experts, the scope of the book has been broadened to include a major emphasis on the clinical applications of cytokines. The early chapters discuss individual cytokines, chemokines and receptors. Additional chapters discuss the clinical implications and applications of cytokines, including cytokine gene transfer, antisense therapy and assay systems. This book is essential for researchers and clinicians interested in cytokines, including anyone working in cancer biology, transplantation, infectious diseases, autoimmunity or bioinformatics.
Identification of a Putative Mediator of Sepsis

HMGB1 Is A Link with Induction of Inflammation - Necrotic Cell Death: Marco Bianchi

P=cells; S=medium

Nature July 11, 2002
Michael Bustin

1. In the Nucleus
- Bends DNA
- Binds to distorted DNA
- Modulates the interaction of regulatory factors with their targets

2. Cell Migration Metastasis

3. To the Nucleus

4. Apoptosis

5. Necrosis

6. Sequester With Platinums

RAGE

TLR2, TLR4
rHMGB1 Induces Acute Local Inflammation

Chemokine Activity of rhHMG-1
In the Rat Jejunal Muscularis Externa

A. Eventration + Saline

B. Eventration + HMG-1 (10µg/ml)

C. Eventration + HMG-1 (50µg/ml)

D. Eventration + HMG-1 (100µg/ml)

Postoperative 24 hrs
Original magnification 20X

Bauer And Lotze
Anti-HMGB1 Antibodies

- Endotoxemia
- Arthritis
- Acute lung injury
- Ischemia reperfusion injury
- Hemorrhagic shock
- Colitis
- Pancreatitis
- Cerebral Ischemia
- Cancer
Maturation of Human iDC

DC IL-12 production

- DC: 29.31 pg/ml/24h
- DC+HMGB1: 88.4 pg/ml/24h
- DC+CD40L: 1434 pg/ml/24h
- DC+HMGB1+CD40L: 4251 pg/ml/24h

Michael DeVera
Maturation of Human MDC
FITC Dextran Uptake

- Untreated
- HMGB1
- ATP
- TNF
- TNF + ATP
- TNF + HMGB1

Richard DeMarco
HMGB1 Inhibits PDC Maturation

HMGB1 Inhibits PDC Maturation
IL-1: A Pleiotropic Cytokine

Signaling Pathways:
- p38
- IKK
- AP-1
- other

- fever
- sleep
- loss of appetite
- social withdrawal
- vasodilation
- adhesion molecules
- chemotaxis
- increased permeability
- immune activation
- stimulation of hematopoiesis

- procoagulant state
- acute phase protein production
- collagenase and stromelysin
- bone resorption
- fibroblast and epithelial cell proliferation
- Prostaglandin E₂

John Sims
The IL-1 Family

IL-1α

IL-1β

IL-1ra

? → ICE → caspase-1

signal peptidase

IL-1α agonists

IL-1β

IL-1ra antagonist

John Sims
IL-1 binds to the Type I IL-1 receptor

IL-1R
Type I

Three unpaired cysteines like IL-18

IL-1
IL-1 and Disease

• IL-1ra is approved for treatment of rheumatoid arthritis

• IL-1 inhibition is effective in other arthritides, including ankylosing spondylitis, systemic onset juvenile idiopathic arthritis, and adult onset Still’s disease

• Particularly notable is the success of IL-1ra therapy in hereditary periodic fever syndromes (Muckle-Wells Syndrome, FCAS, NOMIDS/CINCA)

• Suggestive evidence (over-expression in human disease, genetics, mouse models) for IL-1 as a disease driver in
  – chronic inflammatory diseases (IBD, MS, fibrosis)
  – acute inflammation (stroke)
  – osteoarthritis
  – diabetes
  – cardiovascular disease
  – pain
HMGB-1 Enhances IL-1/12 + IL-2 dependent IFNγ Production from PBMC [24hr]

DeMarco RA, Fink MP, Lotze MT.

Monocytes promote natural killer cell interferon gamma production in response to the endogenous danger signal HMGB1.

The Expanded IL-1 Family

- **IL-1α**: calpain?
- **IL-1β**: caspase-1
- **IL-1ra**: signal peptidase
- **IL-1F7**: ?
- **IL-1F5**, **IL-1F6**, **IL-1F8**, **IL-1F9**, **IL-1F10**: chromosome 2q

- **IL-18**: caspase-1 → **IL-33**, **NF-HEV**, **NK-4**, chromosome 11q
- **IL-33**, **NF-HEV**, **NK-4**, chromosome 9p

Unknown

John Sims

Hideaki Tahara

- IL-F7 by adenovirus-mediated gene transfer (AdIL-1H4) directly into murine tumors.
- Treatment of an established MCA205 mouse fibrosarcoma by single intratumoral injection of AdIL-1H4 resulted in significant growth suppression.
- The anti-tumor activity of IL-1H4 was abrogated in nude and SCID mice and in IL-12-, IFN-gamma-, or Fas ligand-deficient mice. In contrast, IL-1H4 was able to confer substantial anti-tumor effects in NKT-deficient mice.
Can we find a biological function for any of the novel IL-1 family members?

**IL-1α** → **calpain?**

**IL-1β** → **caspase-1**

**IL-1ra** → **signal peptidase**

**IL-1F7** → **?**

All chromosome 2q

**IL-1F5**

**IL-1F6**

**IL-1F8**

**IL-1F9**

**IL-1F10**

**IL-18** → **caspase-1**

**IL-33** → **caspase-1?**

Chromosome 11q

Chromosome 9p
IL1F1, F2, F4 and F5 But Not F6-F10 Synergize with IL-2/HMGB1
Rubartelli A, Lotze MT. Inside, outside, upside down: Damage associated molecular pattern molecules and Redox. Trends in Immunology, in press [2007].
Normal Leaderless Secretory Proteins [IL-1Fx, IL-18, FGF, HMGB1]

**a** short range bioactivity

NK, Mo, DCs

**b** Active secretion

**c** persistent bioactivity

**d** Tumour cell

mOxR (PDI)

Normal Leaderless Secretory Proteins: IL-1Fx, IL-18, FGF, HMGB1

- **a** short range bioactivity: NK, Mo, DCs
- **b** Active secretion: **c** persistent bioactivity
- **d** Tumour cell: mOxR (PDI)

Glycosidases, proteases

Extracellular matrix

Release on necrosis

OxR

NPSH

Membrane Oxido-Reductase
Neoplastic Tissues Contain Abundant Free Thiols

Pancreatic Cancer is a Good Target for Oxidants-No Free Thiols!!

Nicole Schapiro
Pier Mastoberardino
Protein Structure of HMGB1 Revealing Oxidation Sensitive Unpaired Cysteines

FUNCTIONAL DOMAINS:

- Minimal peptide with cytokine activity
- Cytokine-inducing DNA-binding
- Antagonist fragment
- RAGE-binding

HMGB1 and Tumor Life/Death [Addicted to Death]

Release from necrotic cells
- No inflammatory mediators
- Stimulate PDC to limit immune effectors
- Promotes tumor proliferation/survival

Goal: Oxidize HMGB1/DAMPs

Limit Release

Release from macrophages, NK/DC
- With inflammatory mediators, IL-2
- Promotes enhanced inflammation with NK cells
- Diminishes tumor growth

Goal: Promote Immunity, IL-2

 IL-2, ?Others
Conclusions

- HMGB1 is indeed a pleiotrophic cytokine – an endogenous danger signal promoting DC maturation and found in the serum of acute and chronic inflammatory states.
- HMGB1 synergizes with other cytokines in the mouse and man to promote acute immune reactivity.
- HMGB1 in chronic inflammation may promote PDC suppression, promoting healing.
- Targeting HMGB1 with antibodies or soluble receptors OR pro-oxidant therapies may represent important strategies for cancer treatment.
Contributors

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