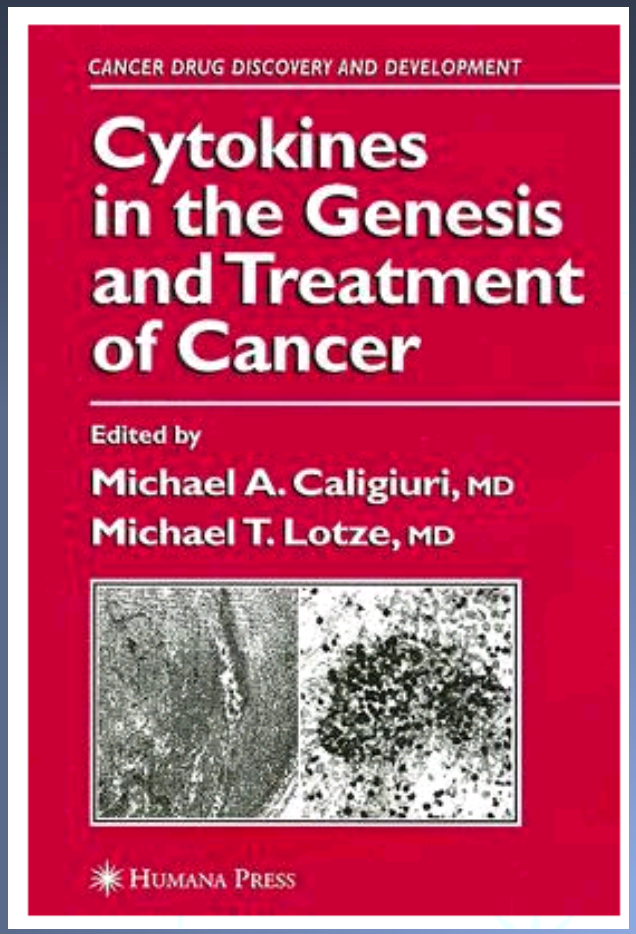

Rx Cancer

Concurrent Session I: Cytokines

Co-chairs: Michael T. Lotze, MD

Crystal Mackall, MD

FivePrime™

IL-34

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ABOUT US | DISCOVERY | PIPELINE | COLLABORATIONS | NEWS | CAREERS

Pipeline

- Overview
- Oncology
- Metabolic Disease
- Regenerative Medicine
- Immunology

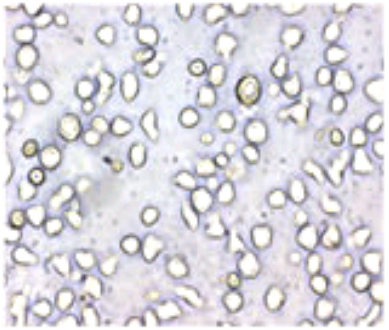
Immunology

FPT025: ***Infectious Disease and Cancer Immunotherapy***

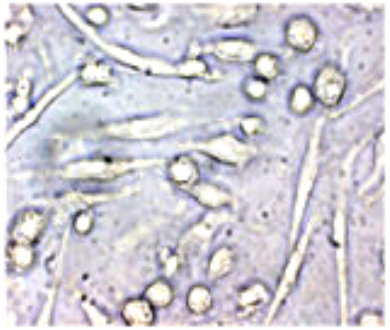
FPT025, recently designated as IL-34, is a novel cytokine unrelated to any known class of cytokines. It stimulates proliferation of monocytes and is involved in early differentiation of dendritic cells. Using our ligand-receptor matching technology, we recently discovered the receptor for FPT025. FPT025 is currently being evaluated as an immuno-stimulant in

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 vis 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.**



FTP025



FTP025 is a novel cytokine that differentiates hematopoietic stem cells. The figure above shows the accompanying morphological changes.

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

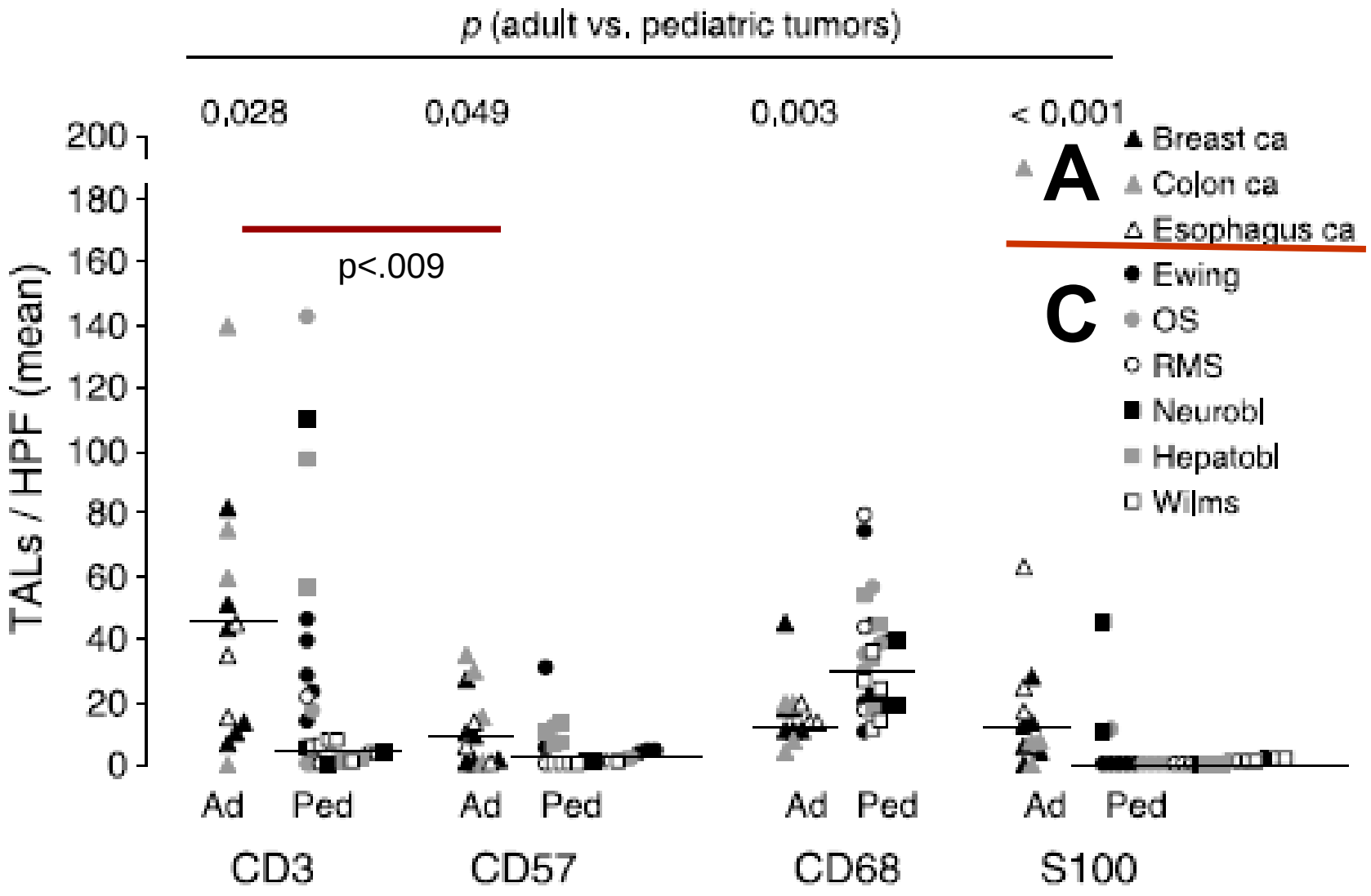
Chronic Inflammatory Conditions Associated with Cancer

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

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

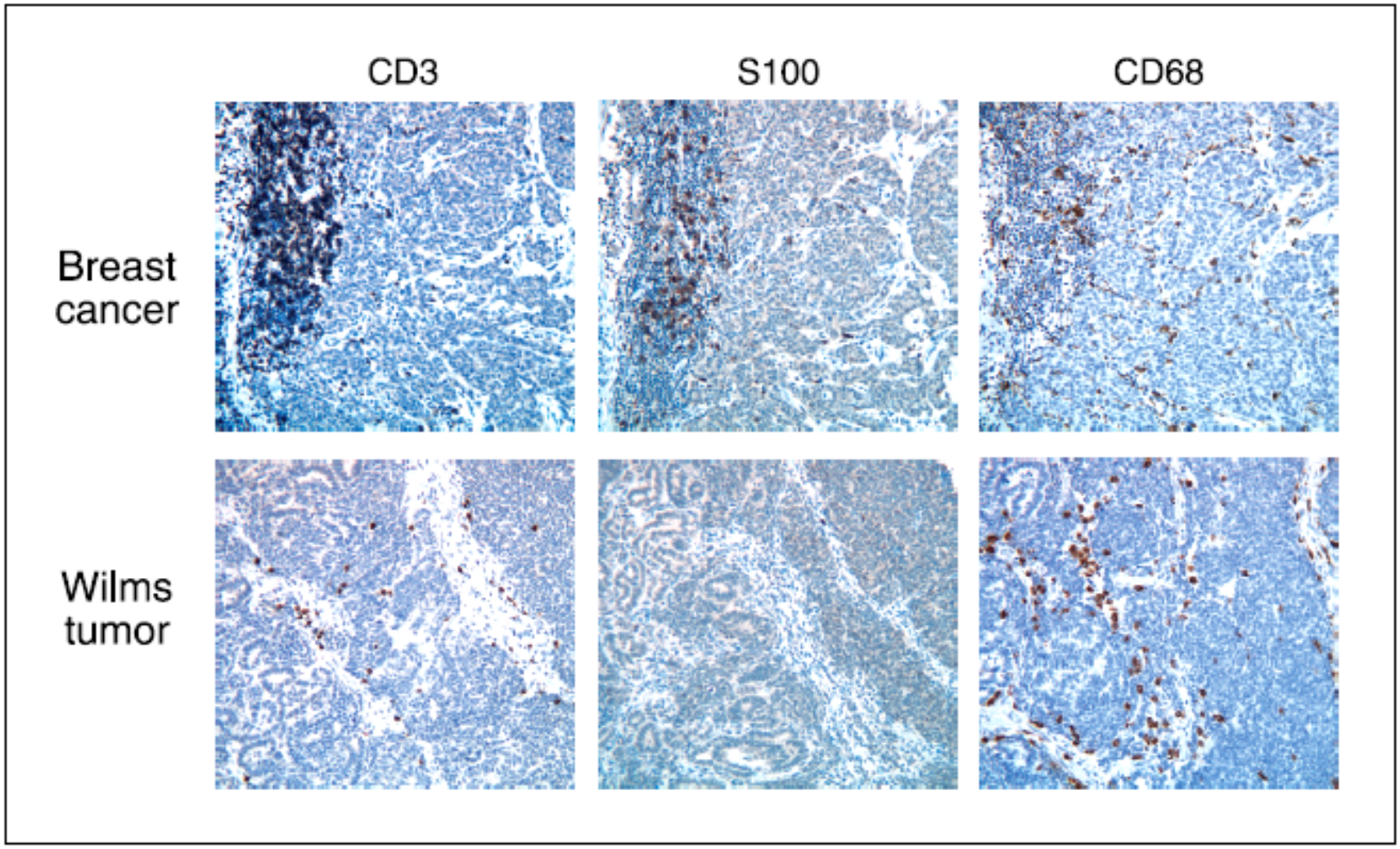
Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

Marked Decrease in Dendritic Cells in Pediatric Cancers



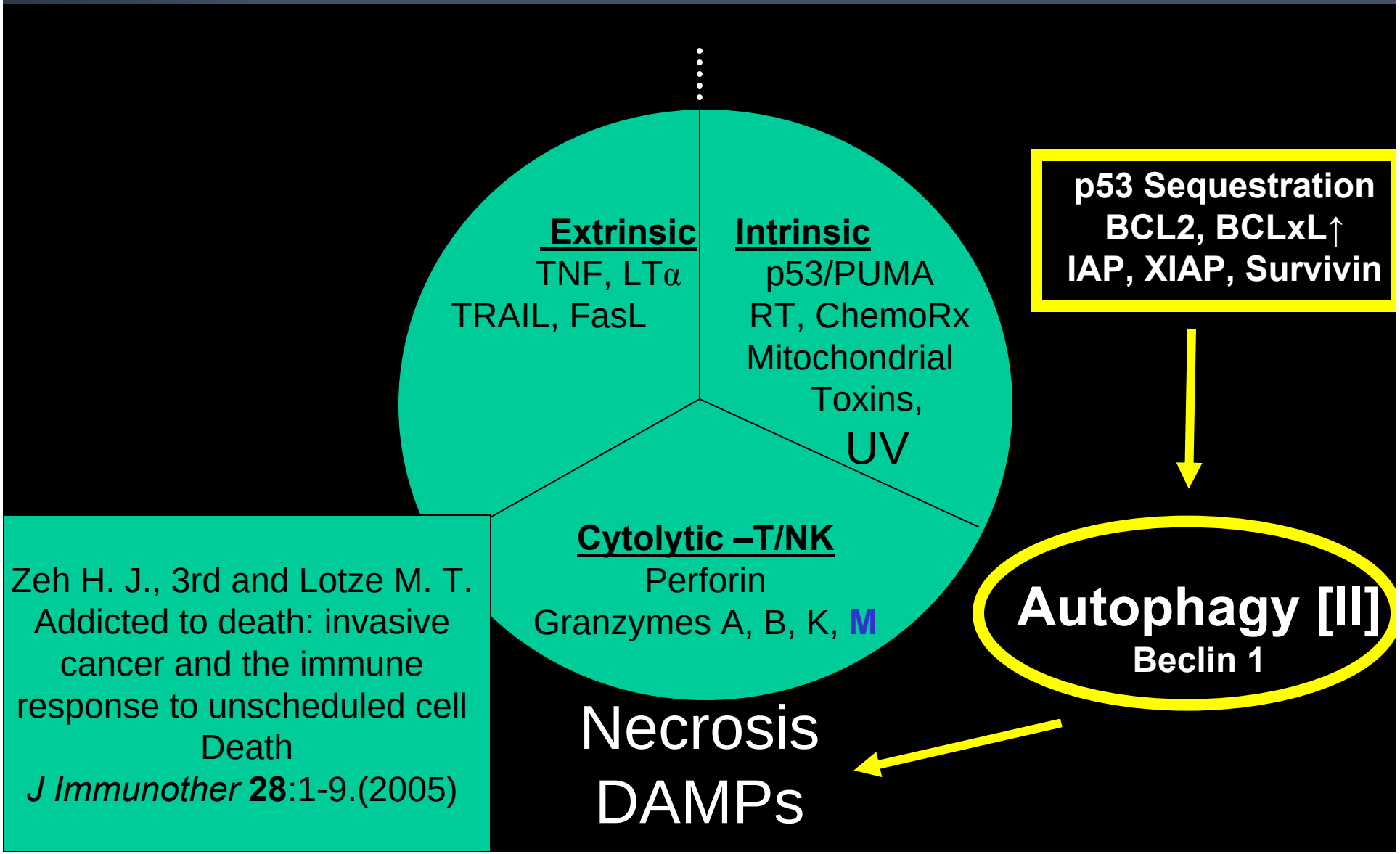
Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

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.

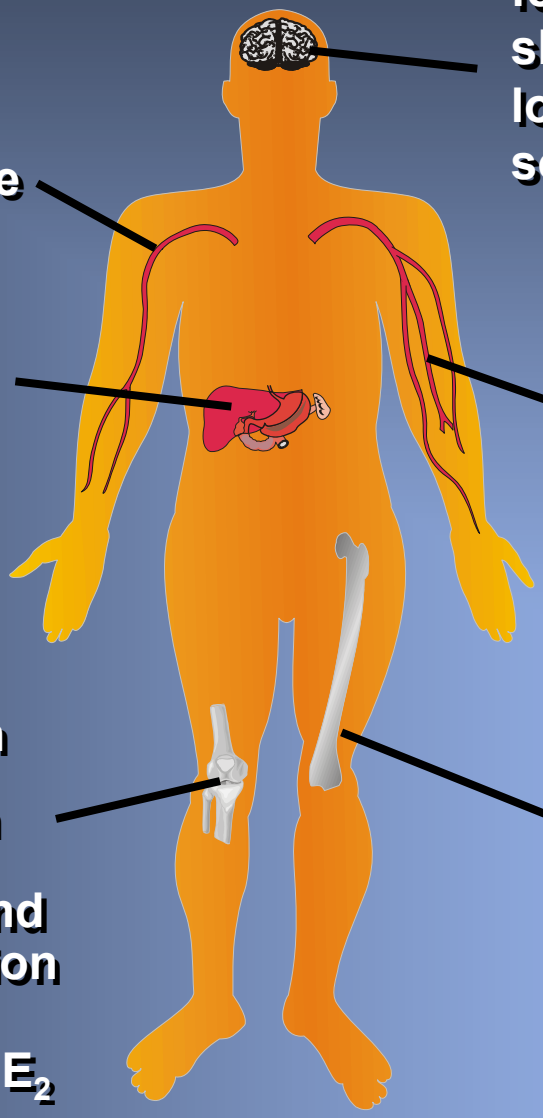
Death Used to be Simpler
Apoptosis [I], Autophagy [II] and Necrosis [III]



Zeh H. J., 3rd and Lotze M. T. Addicted to death: invasive cancer and the immune response to unscheduled cell Death
J Immunother **28**:1-9.(2005)

DAMP Lab 2007

IL-1 : A Pleiotropic Cytokine



fever
sleep
loss of appetite
social withdrawal

procoagulant state

acute phase
protein production

vasodilation

adhesion molecules

chemotaxis

increased permeability

collagenase
and stromelysin

bone resorption

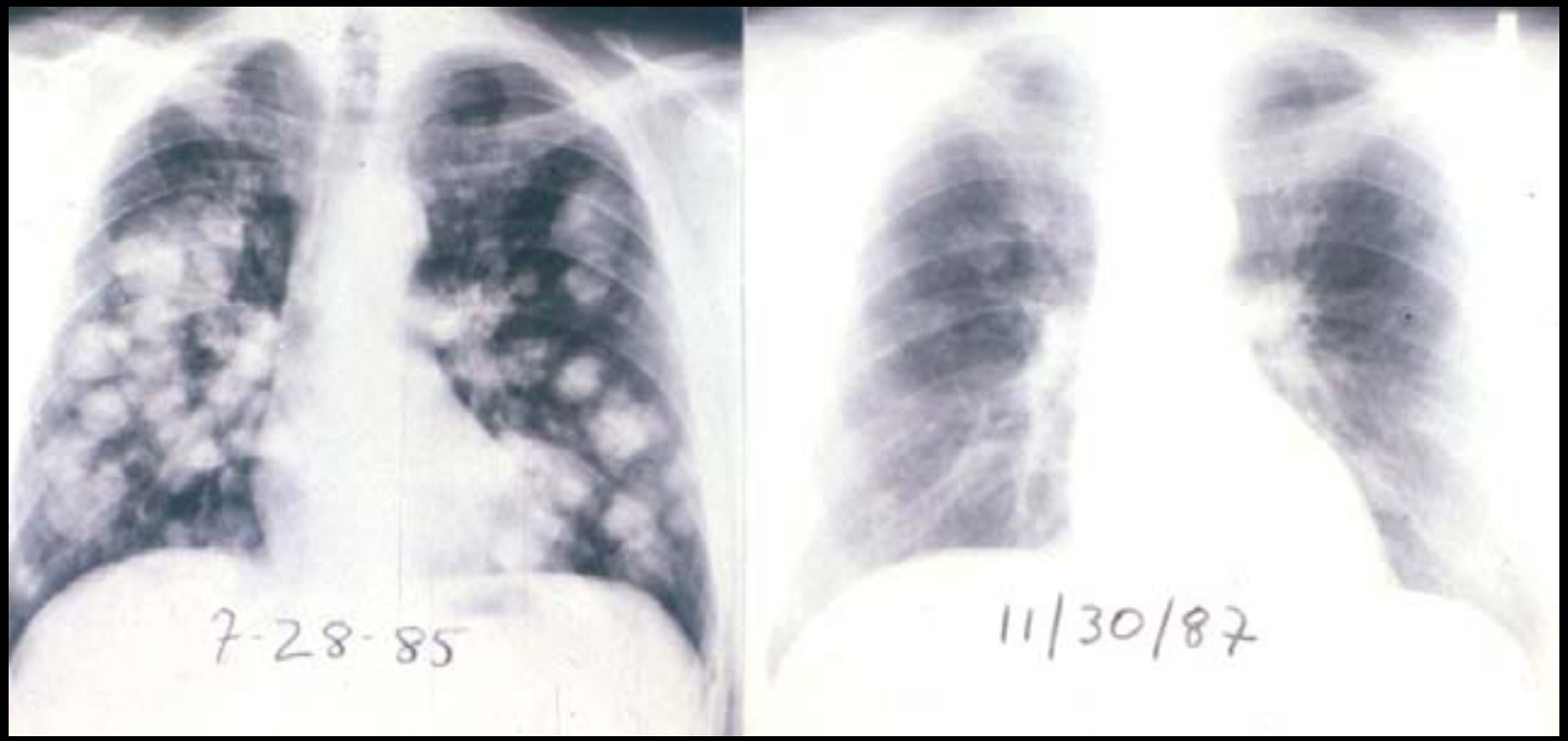
fibroblast and
epithelial cell proliferation

Prostaglandin E_2

immune activation

stimulation of
hematopoiesis

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.

IL-4

IL-7

IL-10

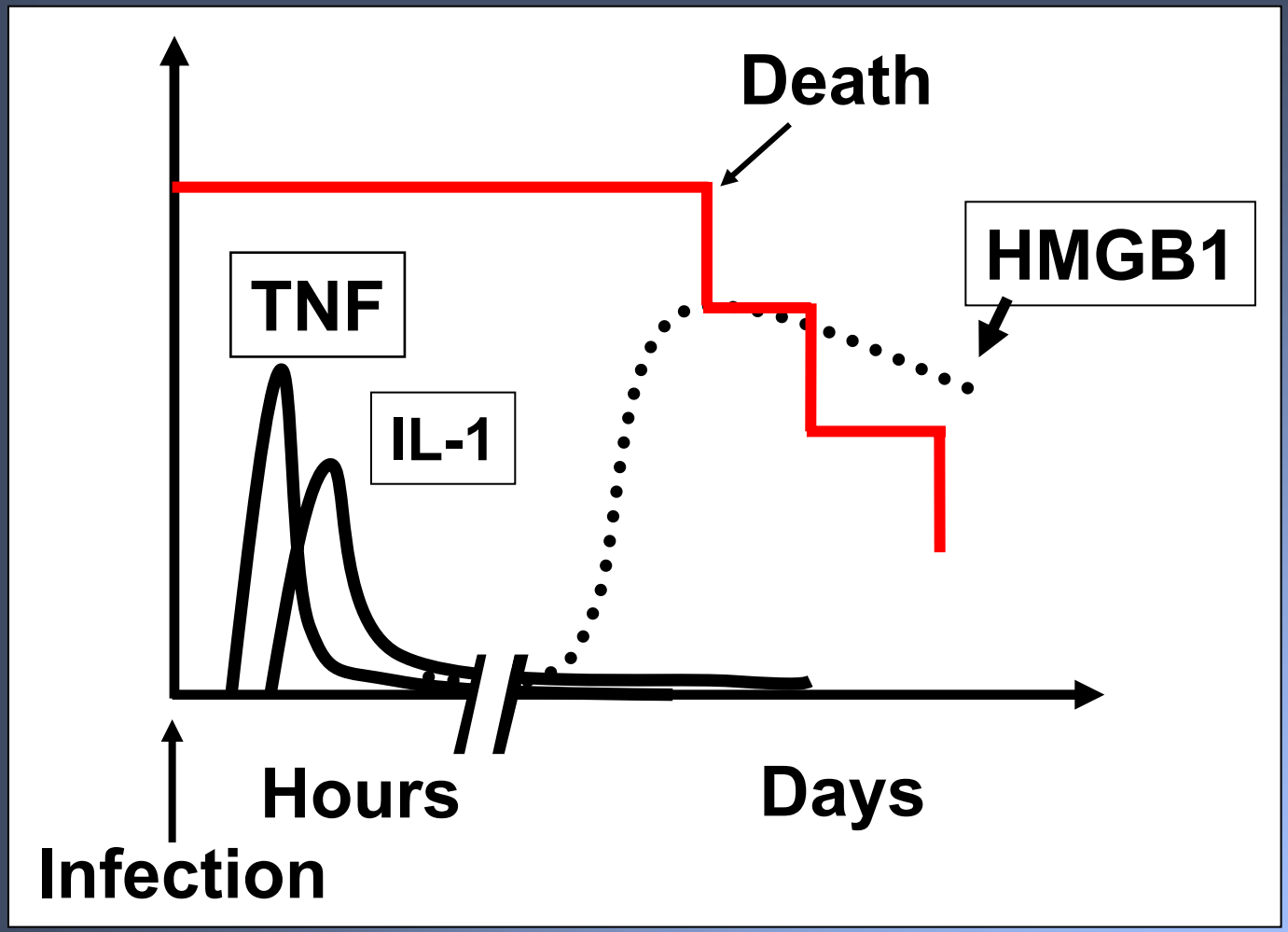
IL-12

IL-17

IL-18

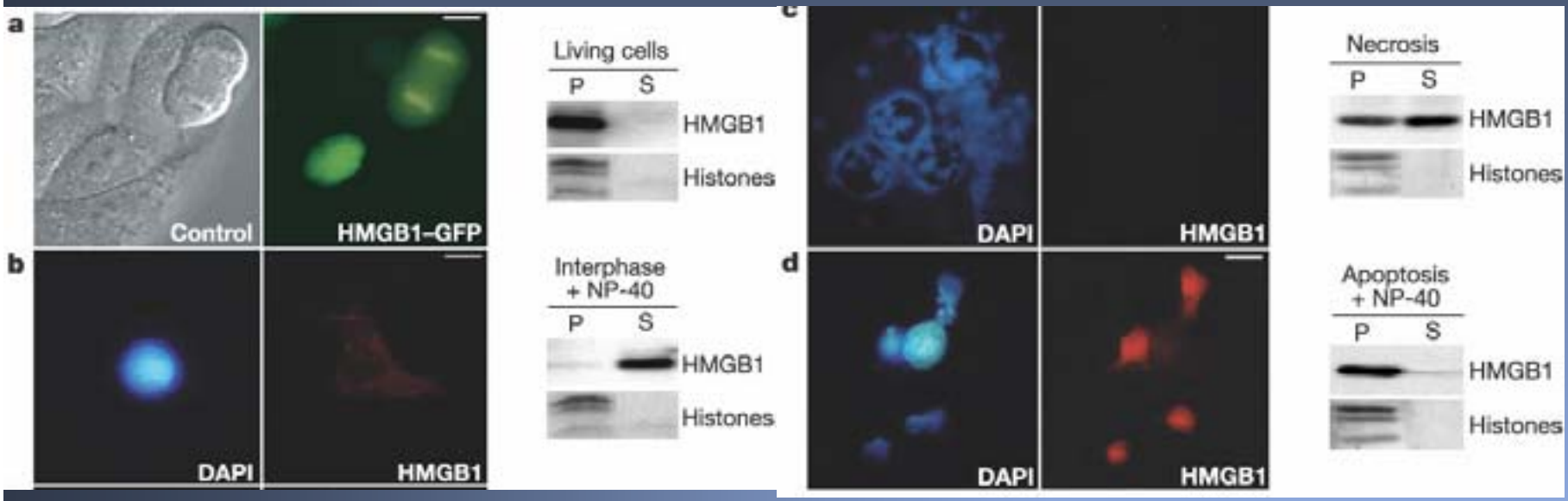
IL1-F7B

Identification of a Putative Mediator of Sepsis



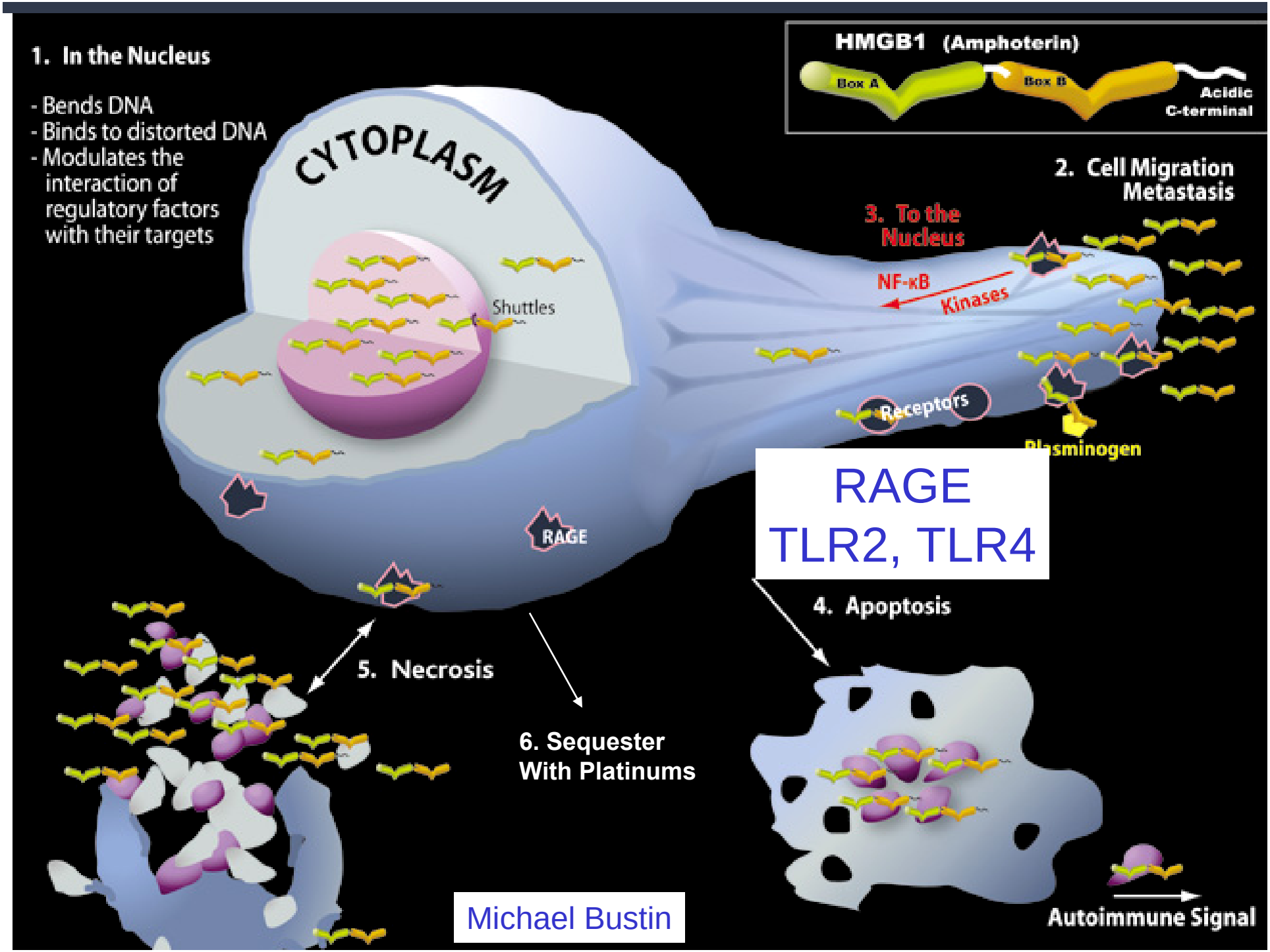
Wang and Tracey, ***Science*** 285:248, 1999

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

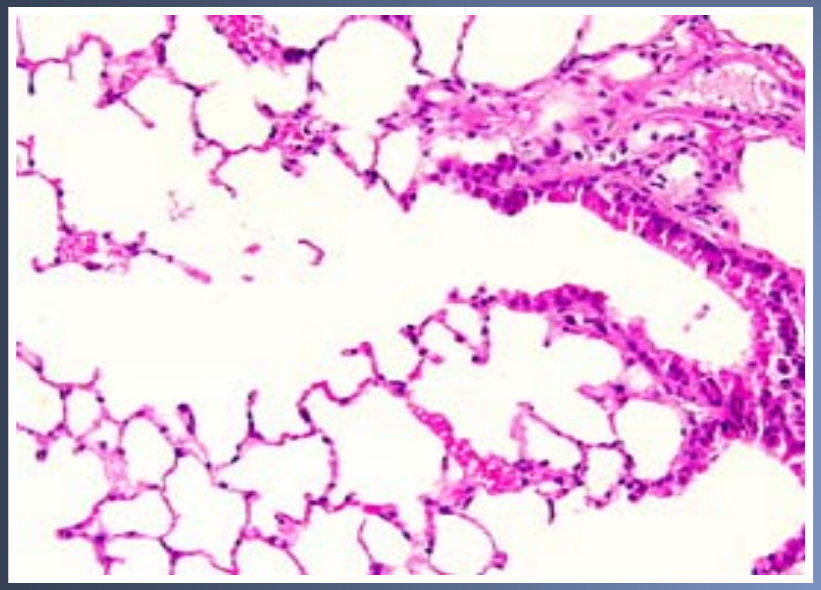


P=cells; S=medium

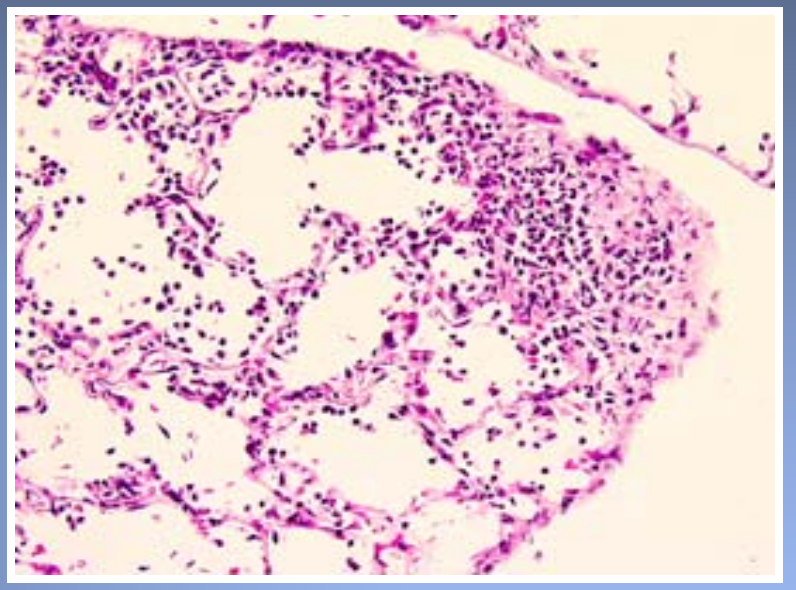
Nature July 11, 2002


1. In the Nucleus
- Bends DNA
- Binds to distorted DNA
- Modulates the interaction of regulatory factors with their targets
HMGB1 (Amphoterin)
Box A
Box B
Acidic C-terminal
CYTOPLASM
Shuttles
2. Cell Migration Metastasis
3. To the Nucleus
NF-κB
Kinases
Receptors
Plasminogen
RAGE
RAGE
TLR2, TLR4
4. Apoptosis
5. Necrosis
6. Sequester With Platinums
Autoimmune Signal
Michael Bustin

rHMGB1 Induces Acute Local Inflammation



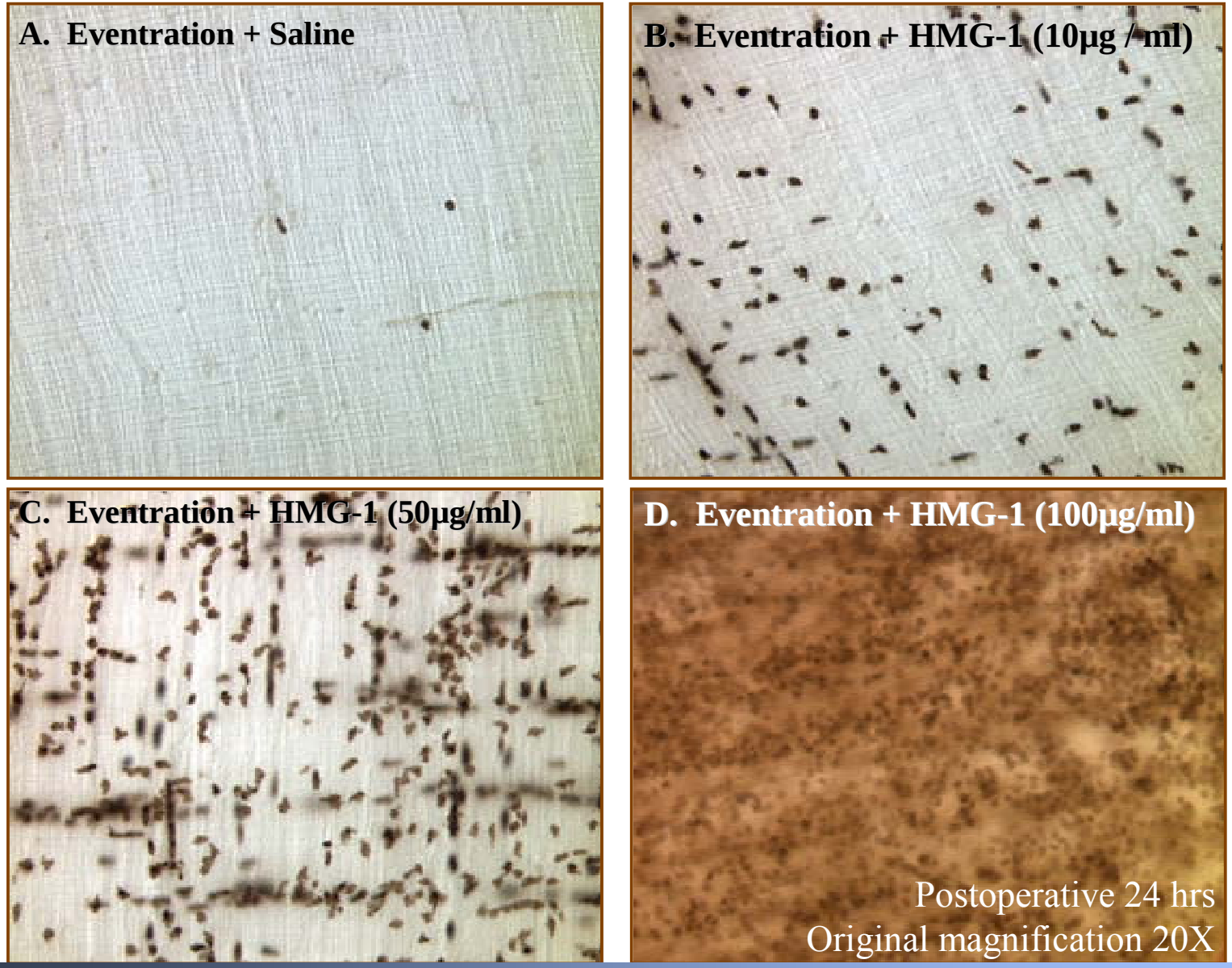
Vehicle



rHMGB-1

Abraham et al., *J. Immunology* 2000, 165:2950-2954.

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

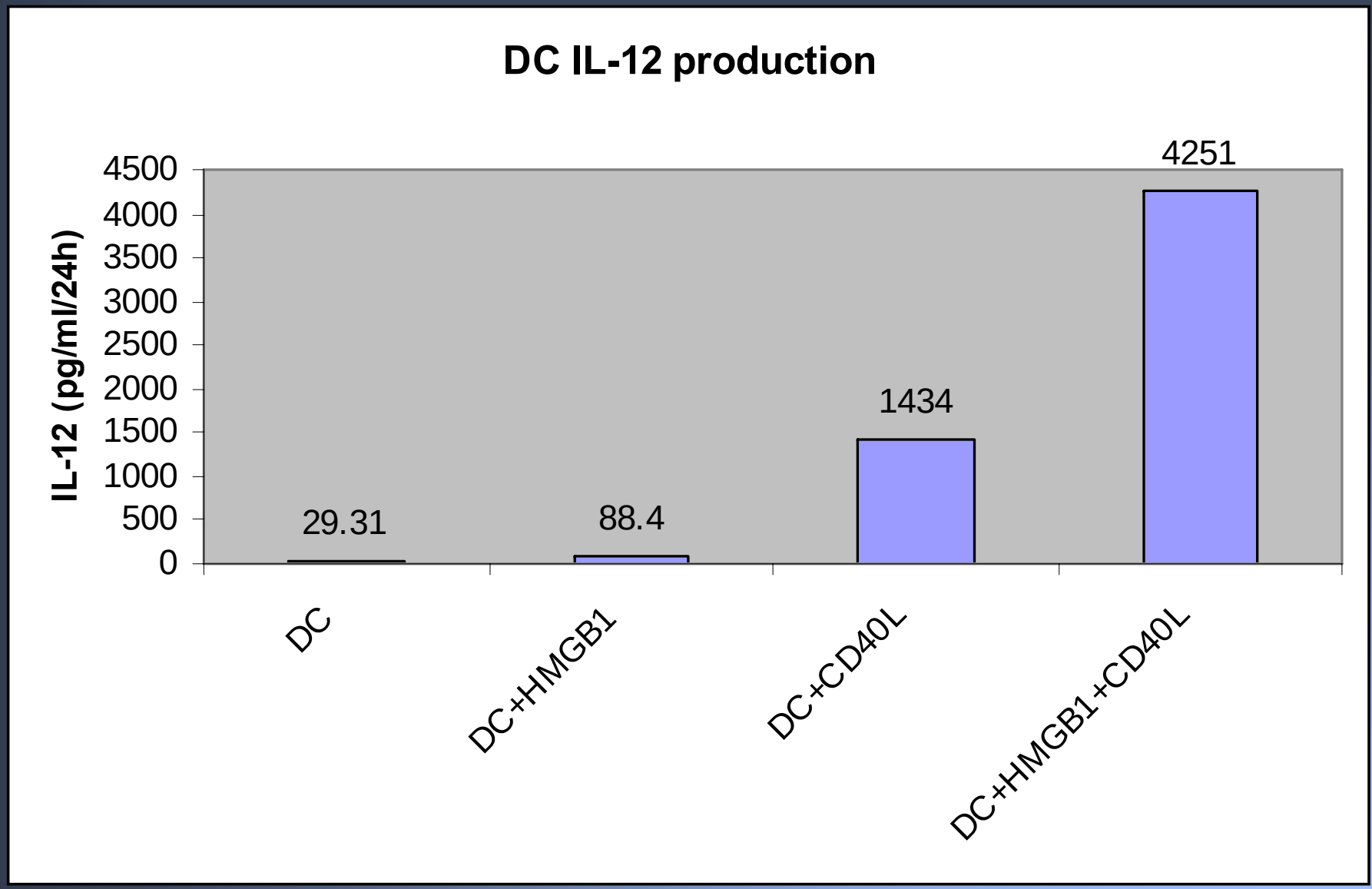


Bauer And Lotze

Anti-HMGB1 Antibodies

- Endotoxemia
- Arthritis
- Acute lung injury
- **Ischemia reperfusion injury**
- Hemorrhagic shock
- Colitis
- Pancreatitis
- Cerebral Ischemia
- Cancer

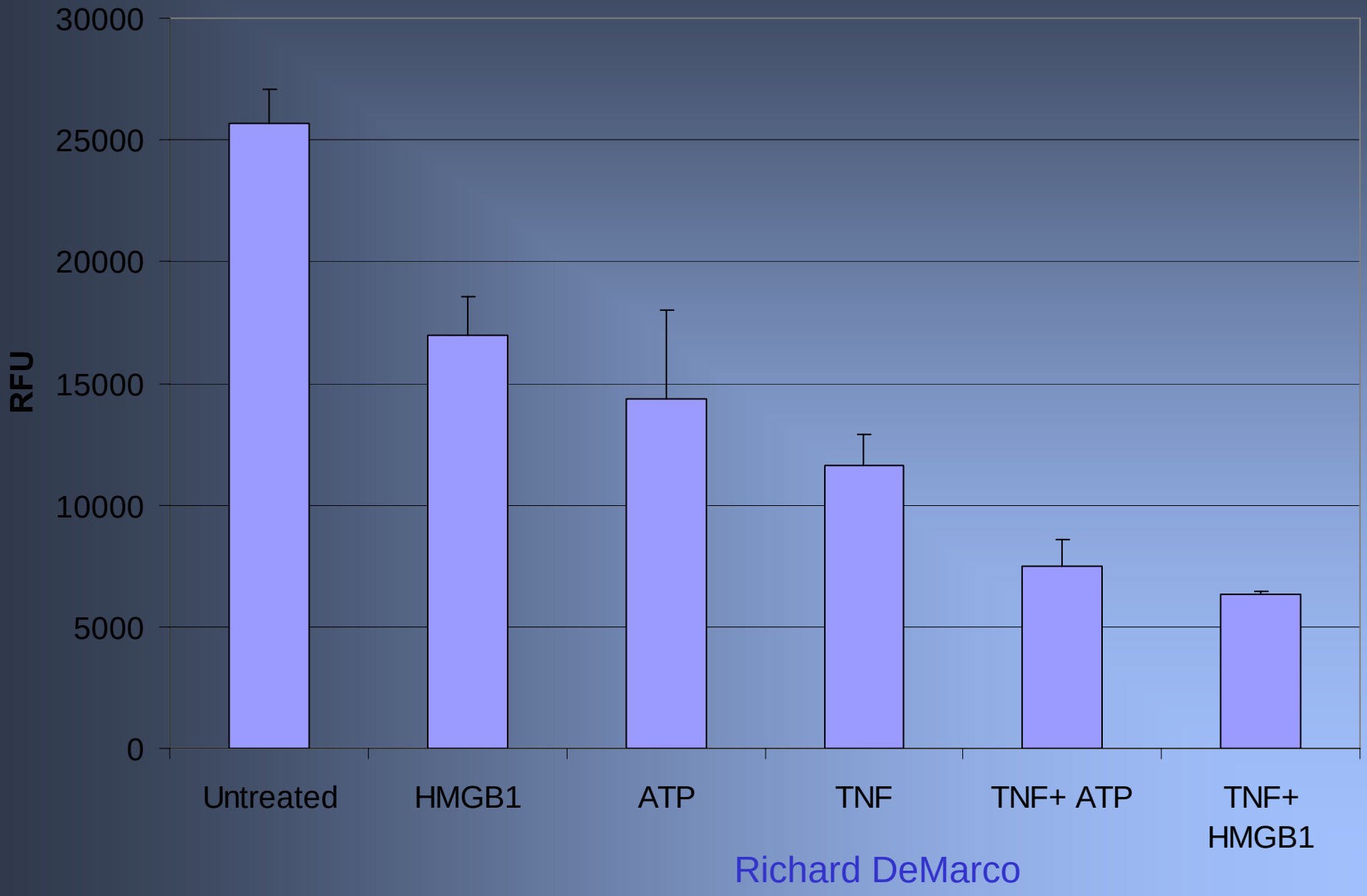
Maturation of Human iDC



Michael DeVera

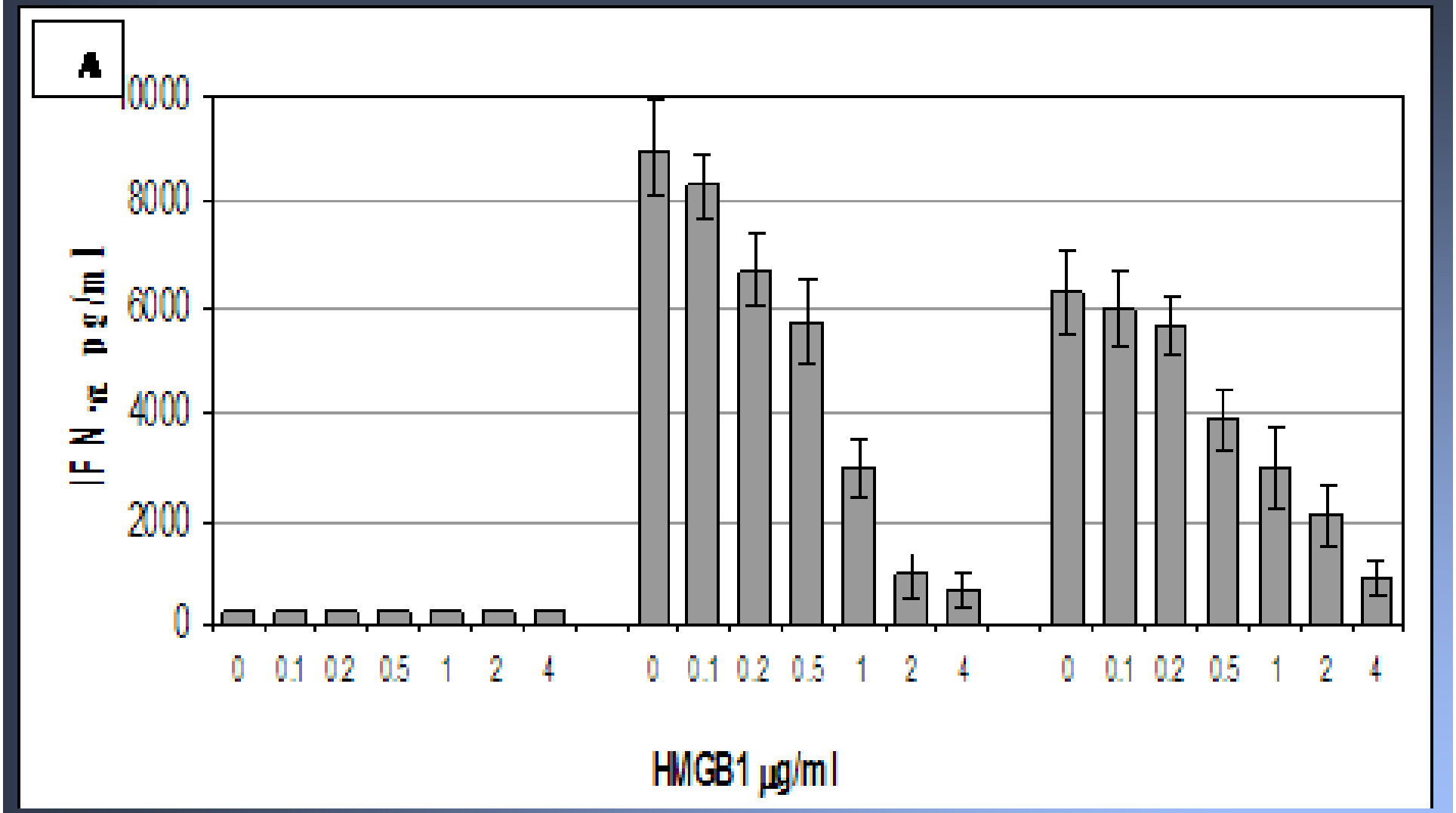
Maturation of Human MDC

FITC Dextran Uptake



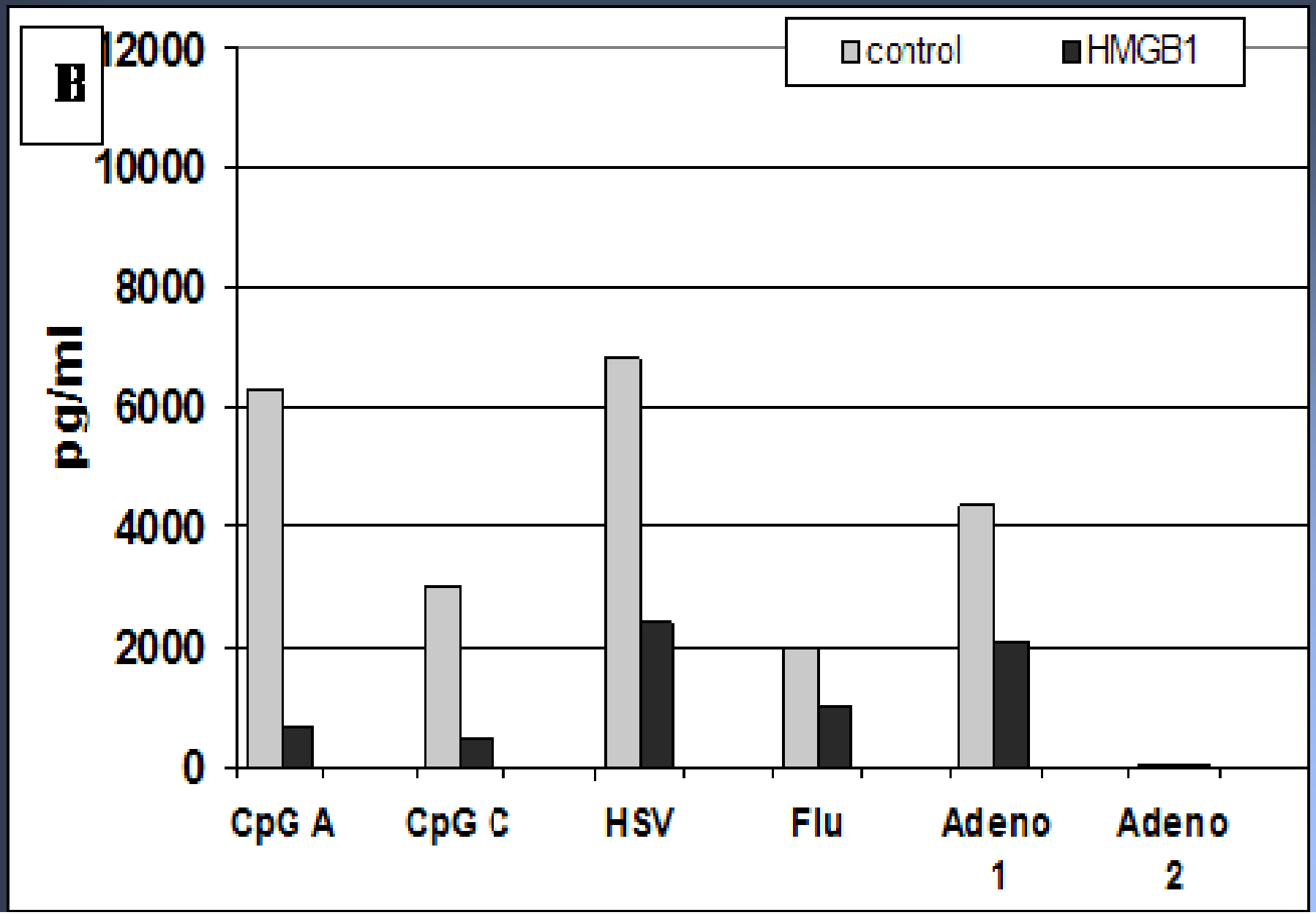
Richard DeMarco

HMGB1 Inhibits PDC Maturation



Popovic PJ, DeMarco R, Lotze MT, Winikoff SE, Bartlett DL, Krieg AM, Guo ZS, Brown CK, Tracey KJ, Zeh HJ 3rd. High mobility group B1 protein suppresses the human plasmacytoid dendritic cell response to TLR9 agonists. J Immunol. 2006 Dec 15;177(12):8701-7.

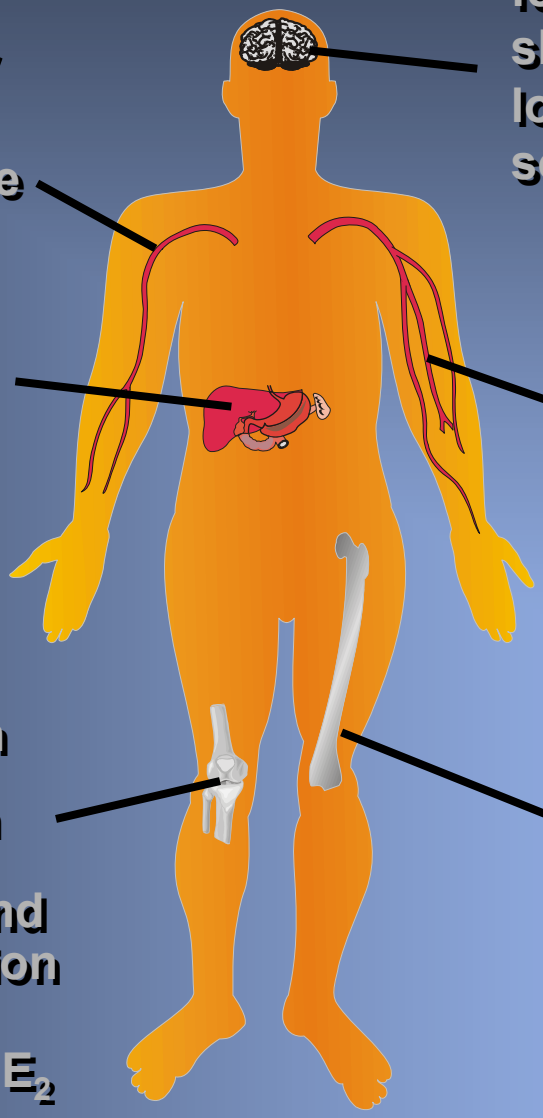
HMGB1 Inhibits PDC Maturation



IL-1 : A Pleiotropic Cytokine

Signaling Pathways:

p38 IKK
AP-1 other



fever
sleep
loss of appetite
social withdrawal

procoagulant state

acute phase
protein production

vasodilation

adhesion molecules

chemotaxis

increased permeability

collagenase
and stromelysin

bone resorption

fibroblast and
epithelial cell proliferation

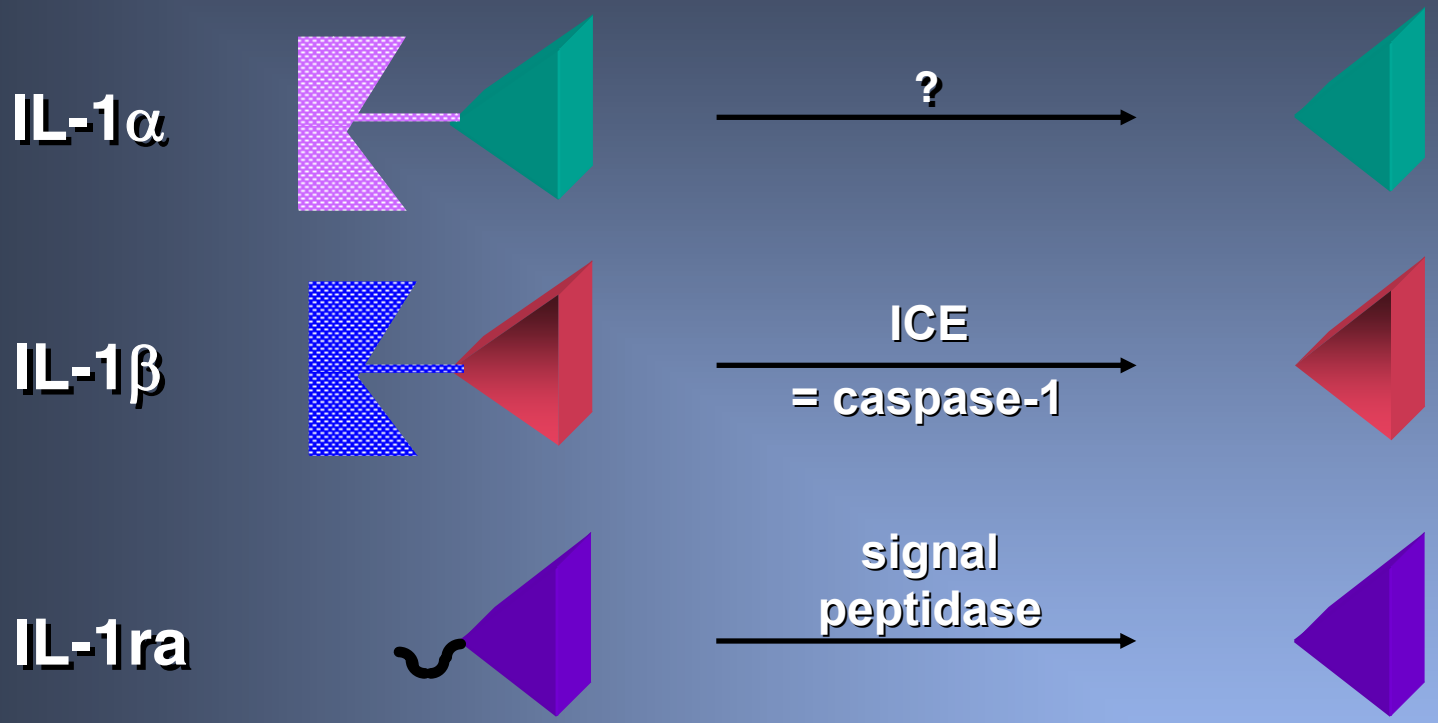
Prostaglandin E_2

immune activation

stimulation of
hematopoiesis

John Sims

The IL-1 Family

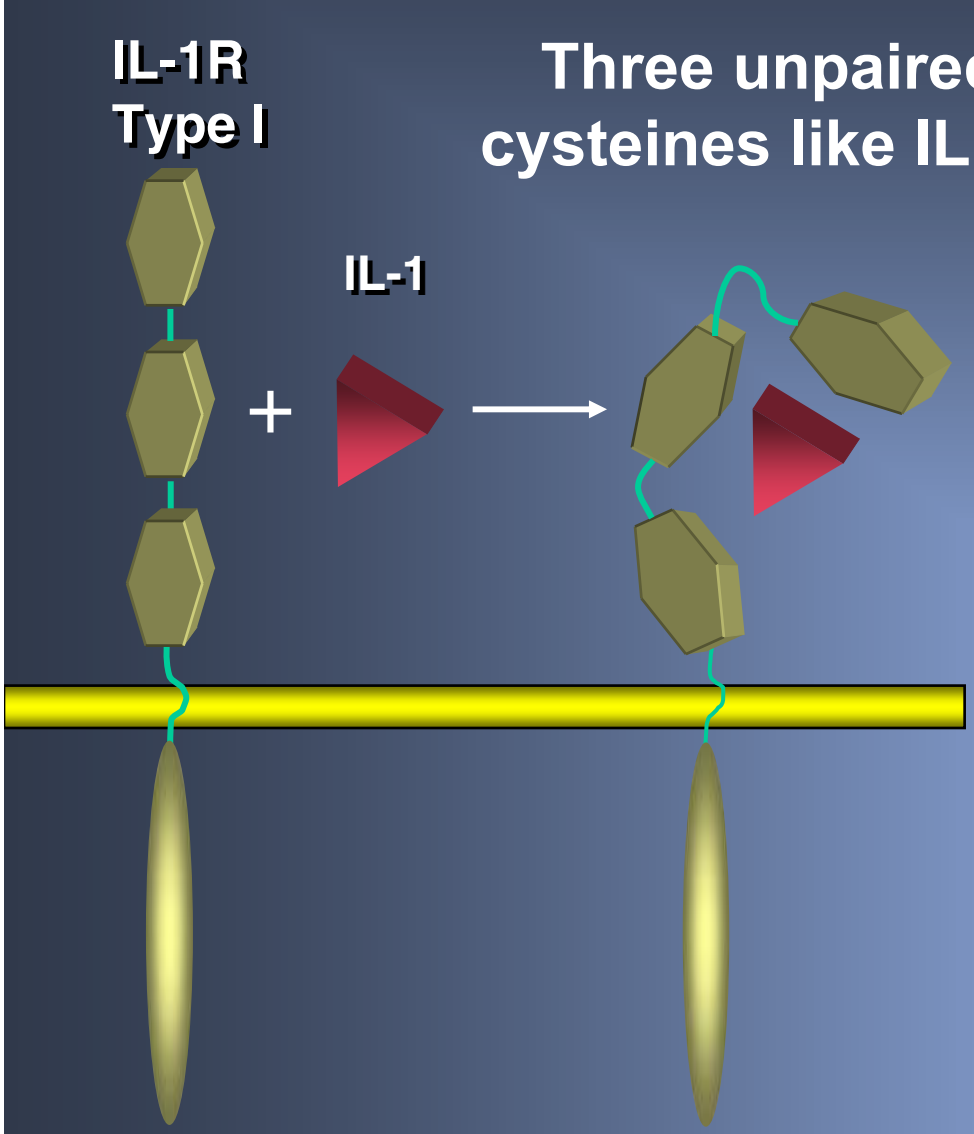


IL-1α
IL-1β — agonists

IL-1ra — antagonist

John Sims

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



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

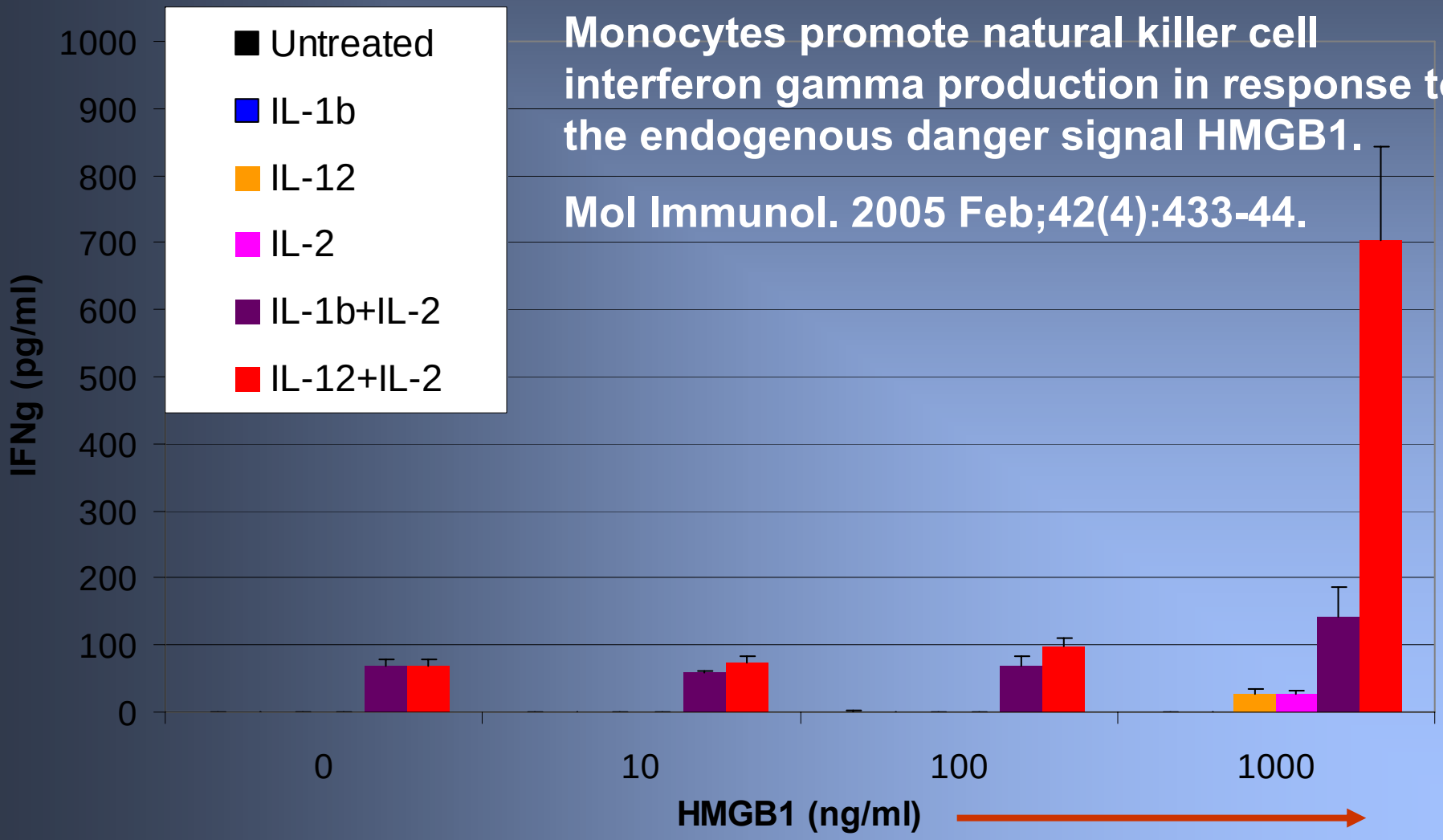
John Sims

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.

Mol Immunol. 2005 Feb;42(4):433-44.



The Expanded IL-1 Family

Unknown

all chromosome 2q

+ Hideaki Tahara

chromosome 11q

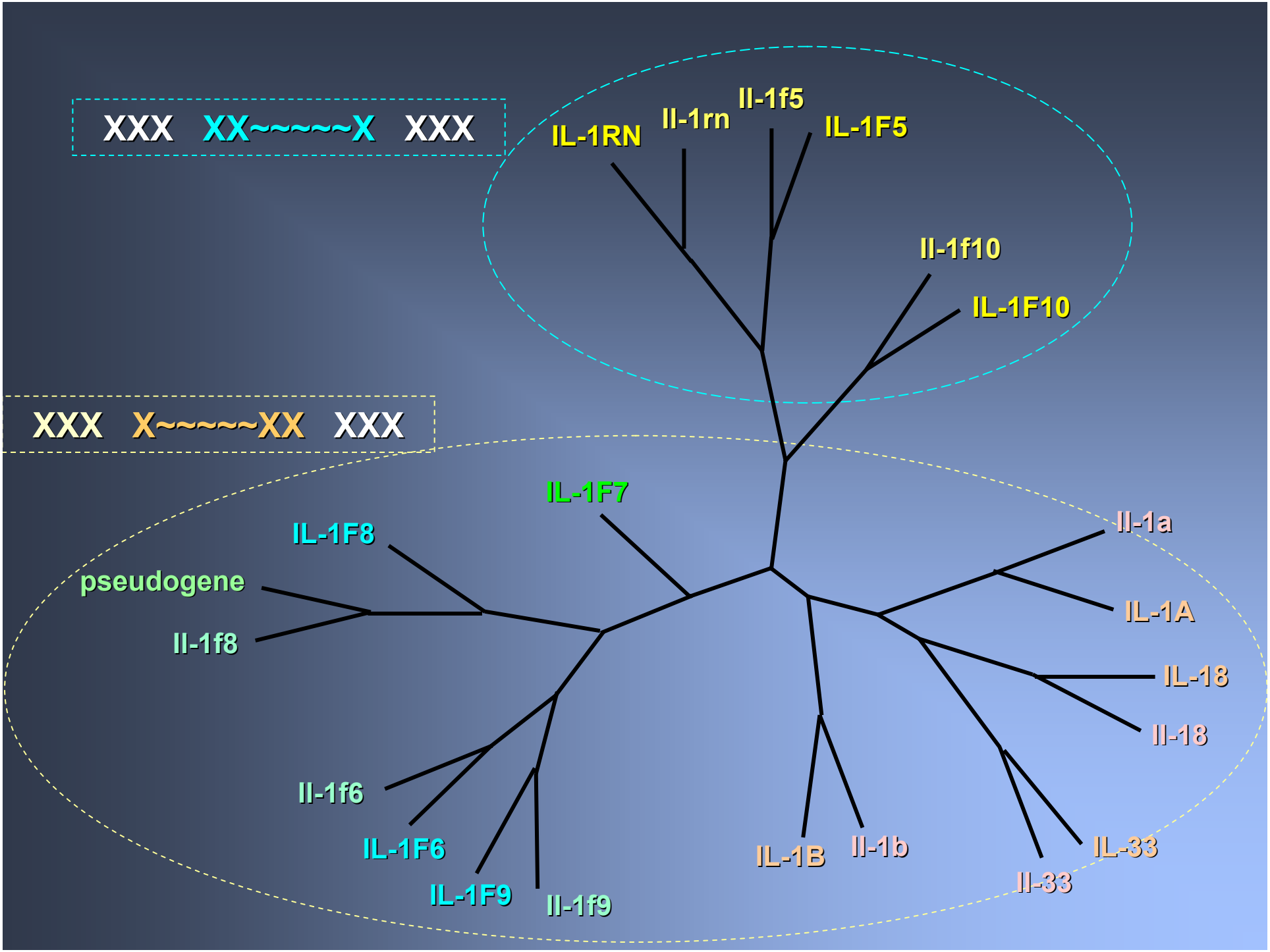
NK-4

chromosome 9p

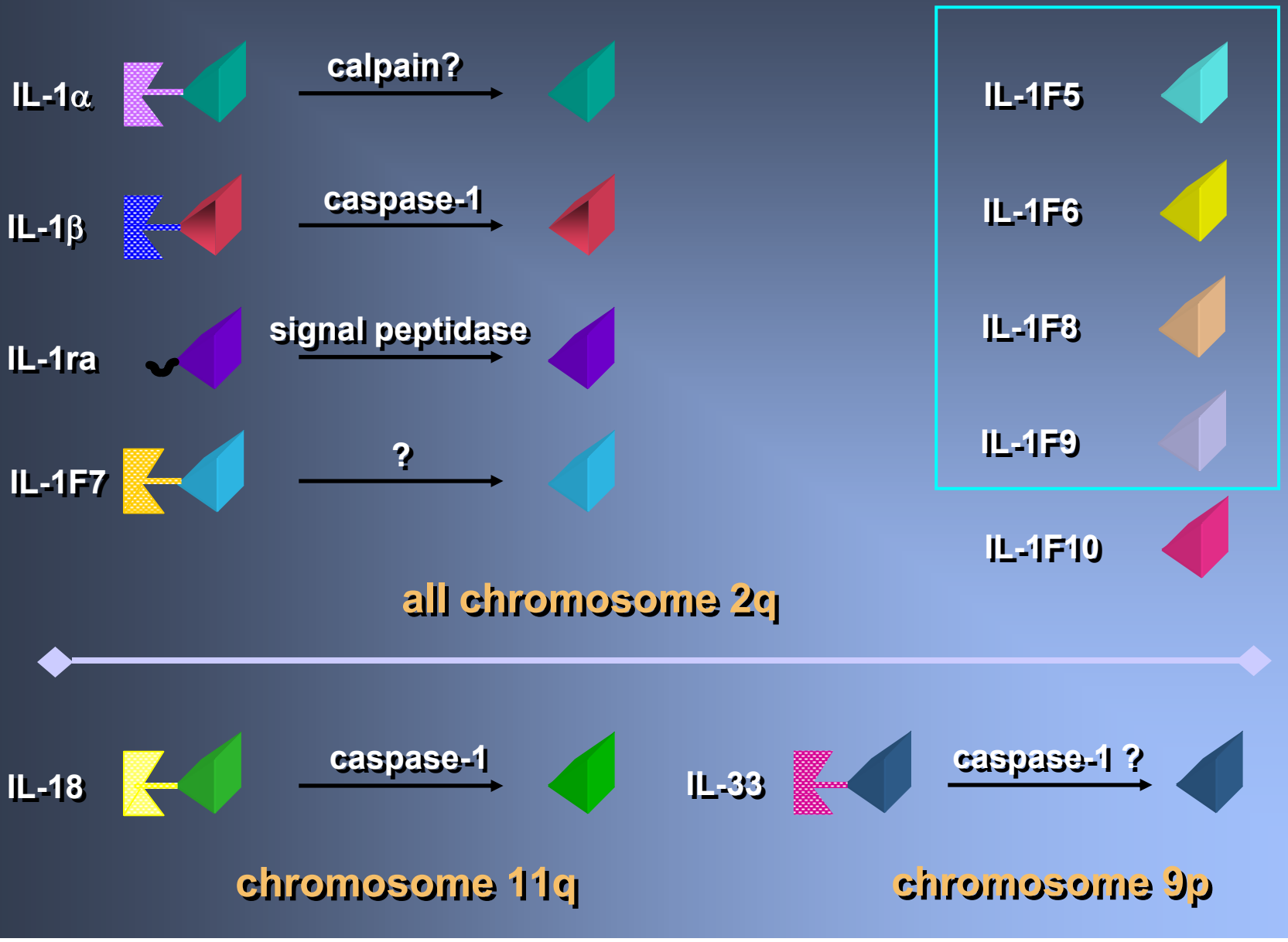
John Sims

Innate immunity mediated by the cytokine IL-1 homologue 4 (IL-1H4/IL-1F7) induces IL-12-dependent adaptive and profound antitumor immunity. Gao W, Kumar S, Lotze MT, Hanning C, Robbins PD, Gambotto A. J Immunol. 2003 Jan 1;170(1):107-13. Department of Molecular Genetics, University of Pittsburgh, PA 15219, USA.

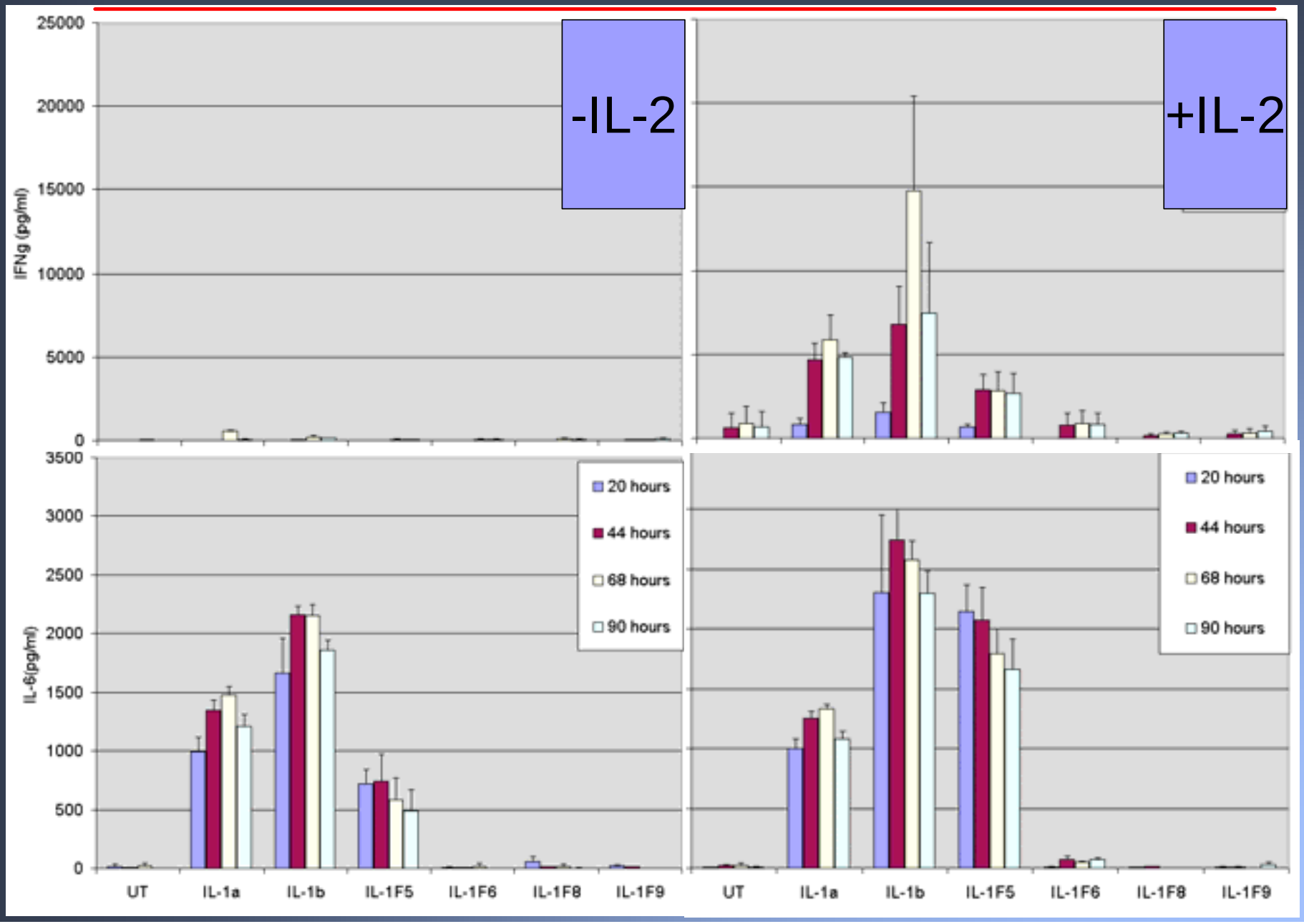
- IL-F7 by adenovirus-mediated gene transfer (AdIL-1H4) directly into murine tumors.
- Treatment of an established MCA205 mouse fibrosarcoma by single intratumoral injection of AdIL-1H4 resulted in significant growth suppression.
- The anti-tumor activity of IL-1H4 was abrogated in nude and SCID mice 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.


XXX XX~~~~~X XXX
XXX X~~~~~XX XXX
IL-1RN
Il-1rn
Il-1f5
IL-1F5
Il-1f10
IL-1F10
IL-1F7
IL-1F8
pseudogene
Il-1f8
Il-1a
IL-1A
IL-18
Il-18
Il-1f6
IL-1F6
IL-1F9
Il-1f9
IL-1B
Il-1b
IL-33
Il-33

Can we find a biological function for any of the novel IL-1 family members?

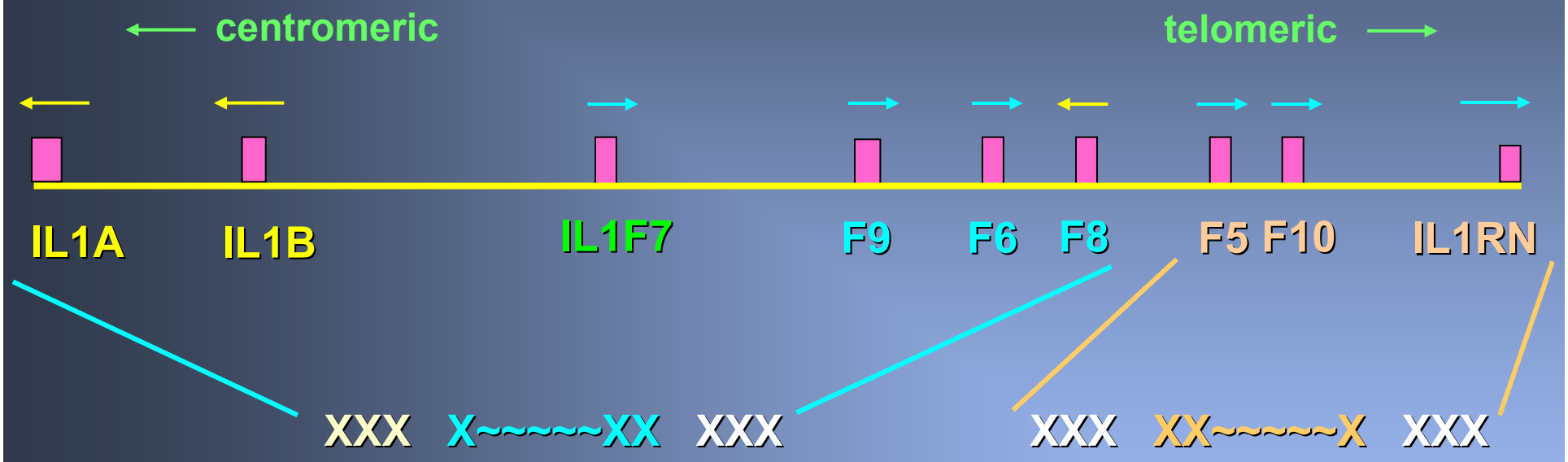


IL1F1, F2, F4 and F5 But Not F6-F10 Synergize with IL-2/HMGB1



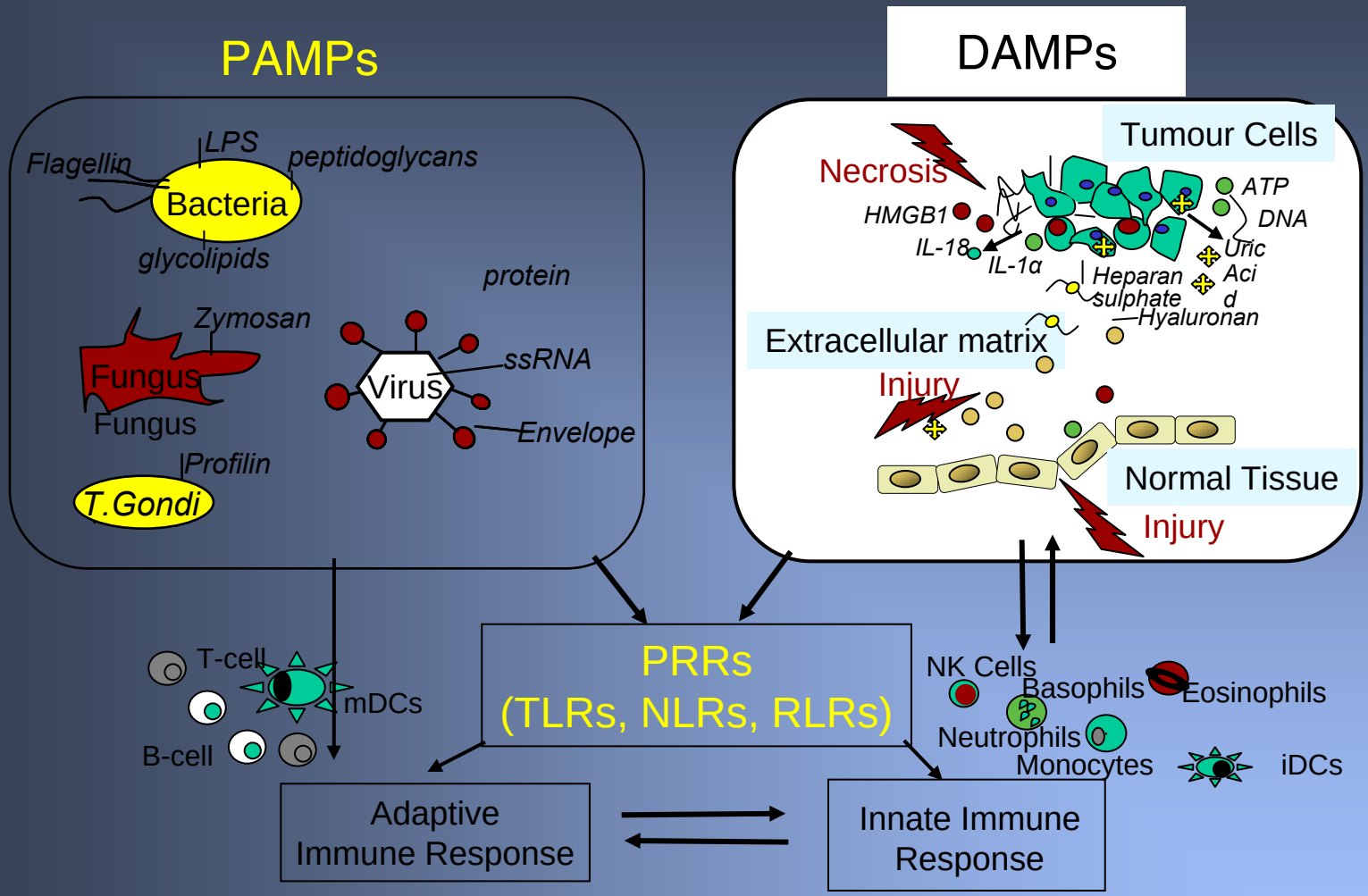
IL-1 locus

human chromosome 2q



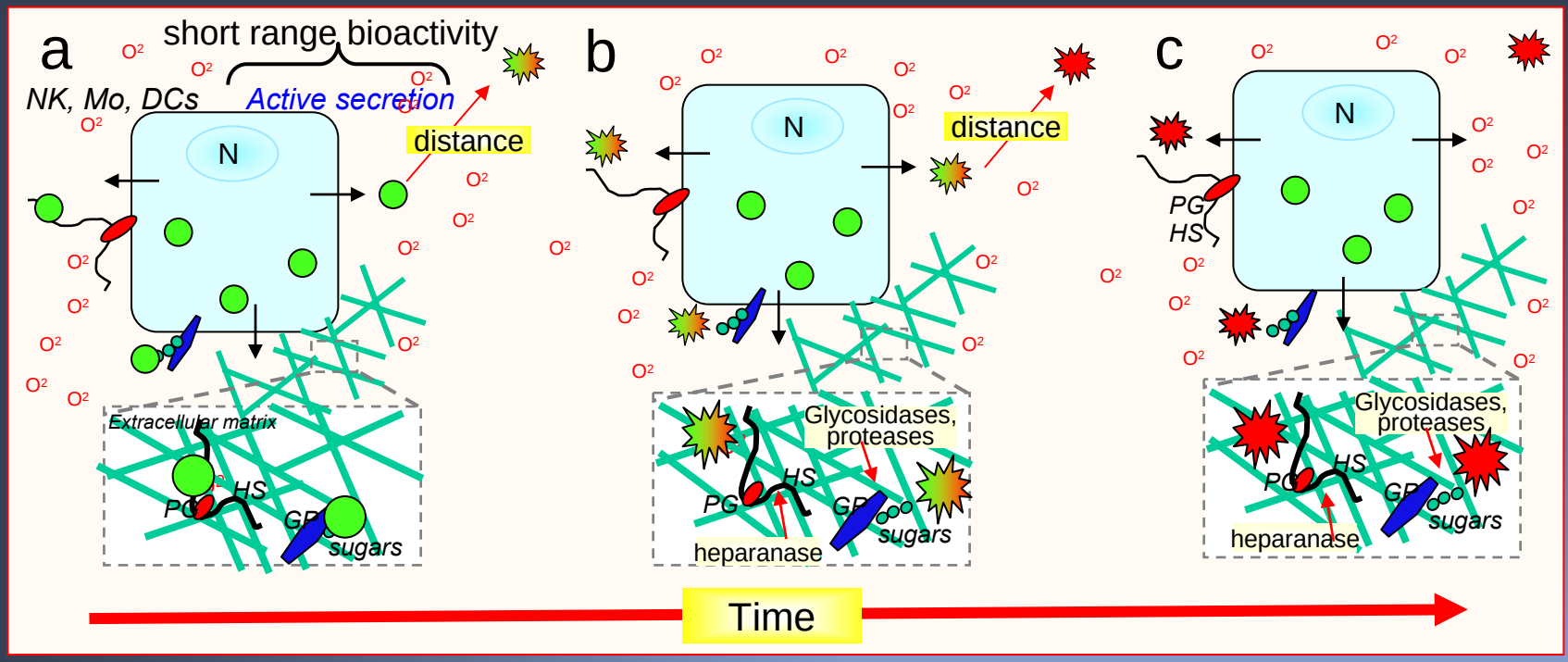
Blumberg H, Dinh H, Trueblood ES, Pretorius J, Kugler D, Weng N, Kanaly ST, Towne JE, Willis CR, Kuechle MK, Sims JE, Peschon JJ. Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. J Exp Med. 2007 Oct 29;204(11):2603-14.

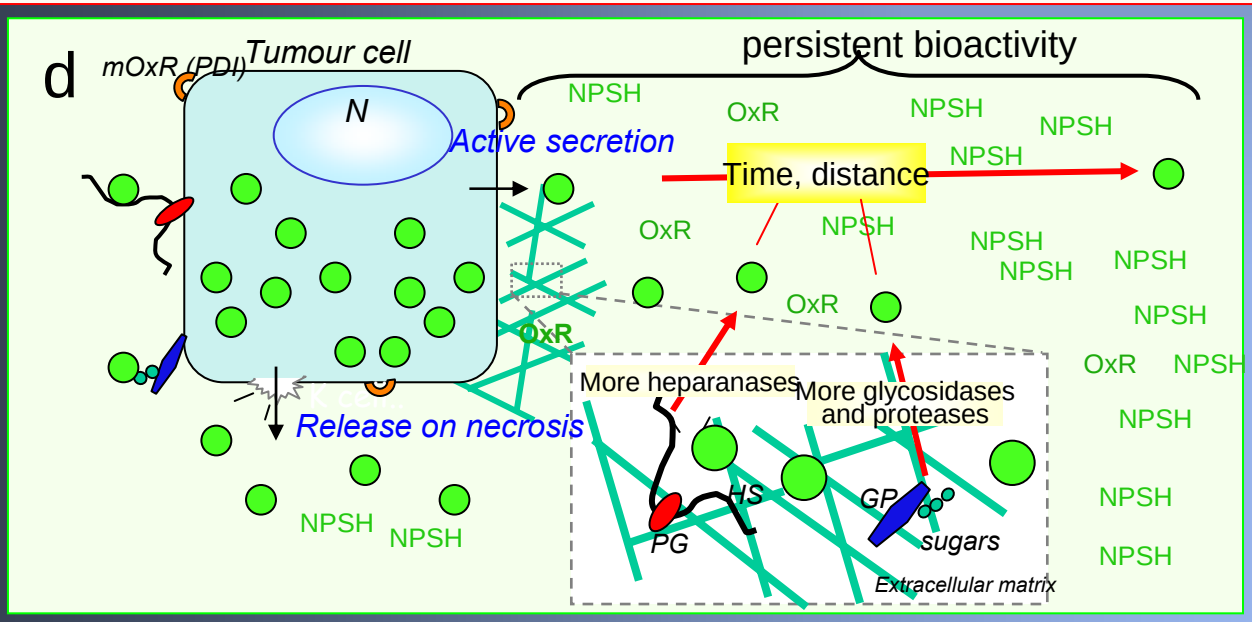
PAMPS and DAMPS and Redox-Signal 0



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]





Cytosolic or nuclear proteins, reduced, functional

Cytosolic or nuclear proteins, Partially oxidized

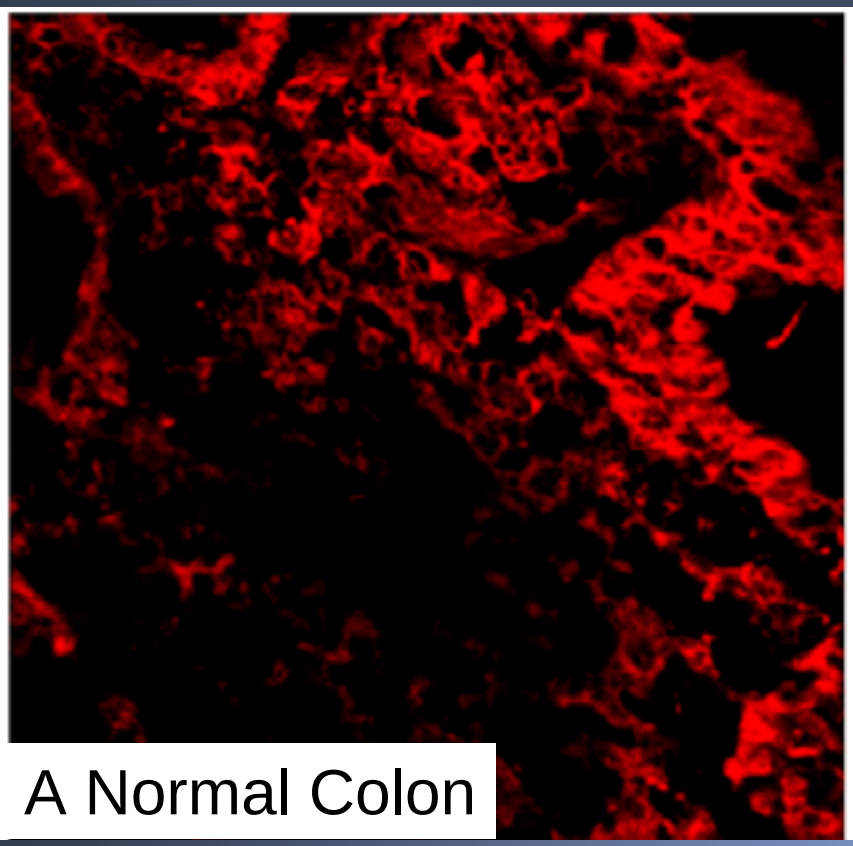
Cytosolic or nuclear proteins, Oxidized, non functional

PG: proteoglycan
HS: heparansulfates

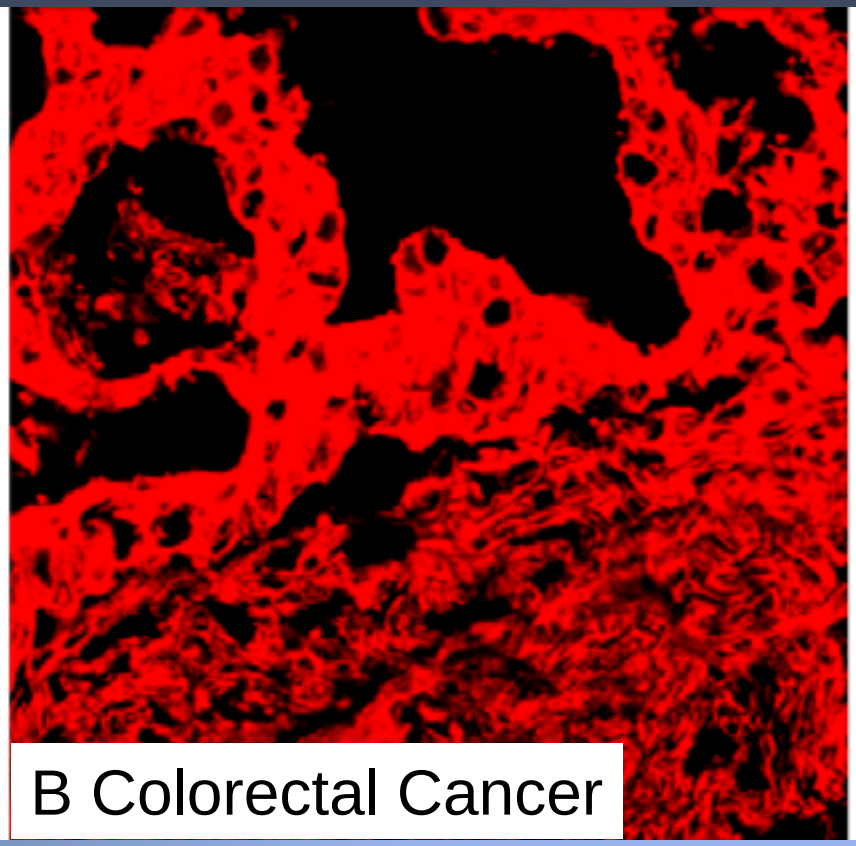
GP: glycoprotein

Membrane Oxido-Reductase

Neoplastic Tissues Contain Abundant Free Thiols



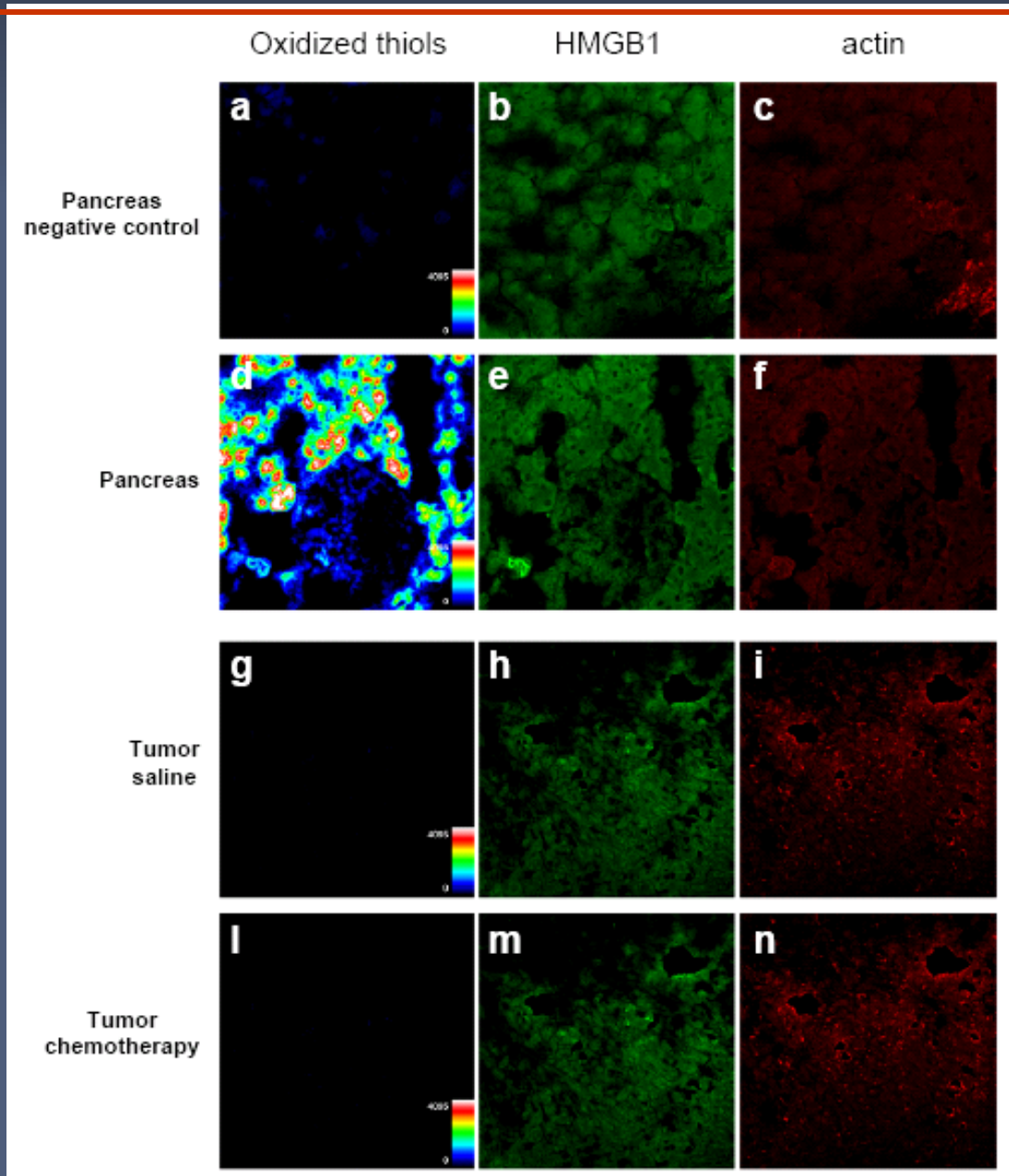
A Normal Colon



B Colorectal Cancer

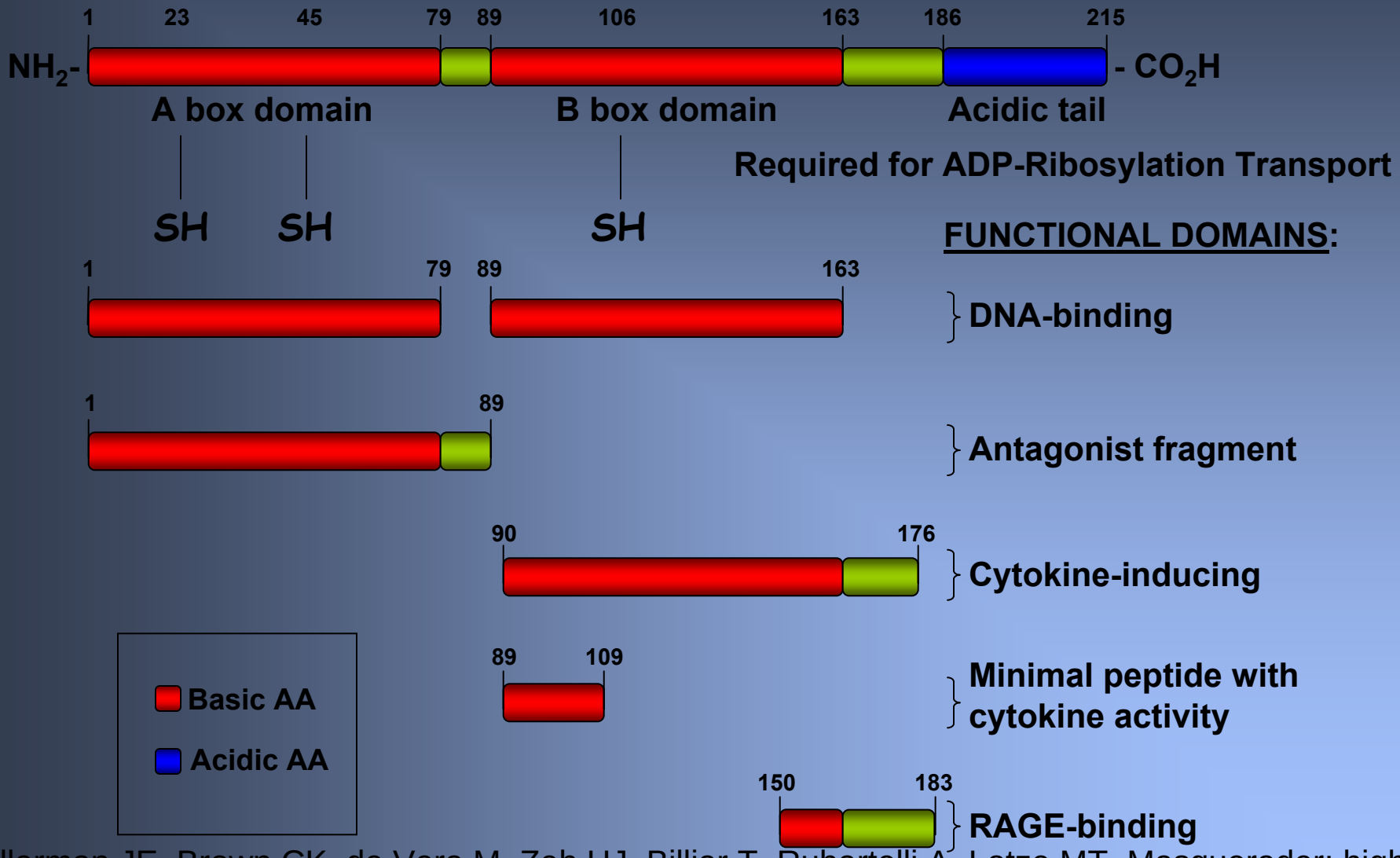
Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol. 2007 Oct;28(10):429-36.

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



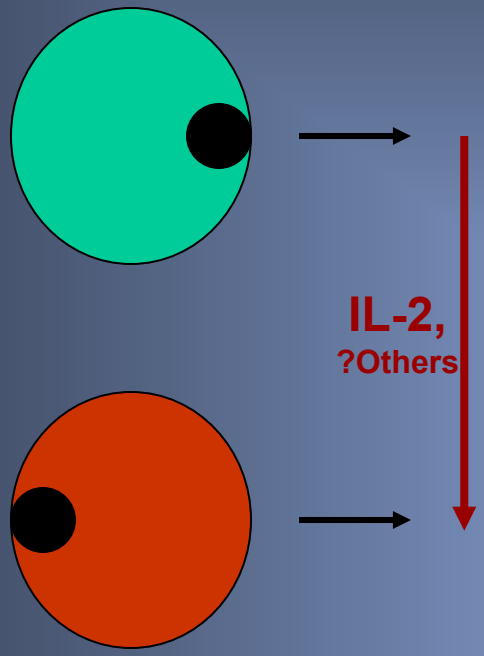
Nicole Schapiro
Pier Mastoberardino

Protein Structure of HMGB1 Revealing Oxidation Sensitive Unpaired Cysteines



Ellerman JE, Brown CK, de Vera M, Zeh HJ, Billiar T, Rubartelli A, Lotze MT. Masquerader: high mobility group box-1 and cancer. Clin Cancer Res. 2007 May 15;13(10):2836-48.

HMGB1 And Tumor Life/Death [Addicted to Death]



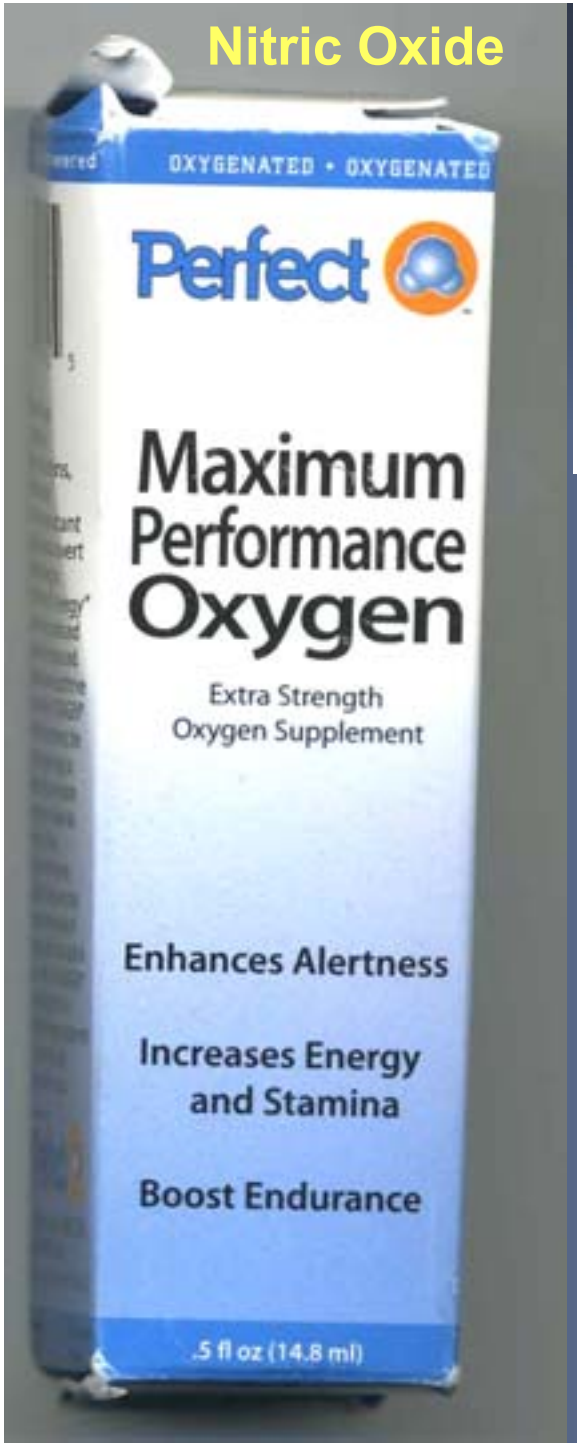
Release from necrotic cells
No inflammatory mediators
Stimulate PDC to limit immune effectors
Promotes tumor proliferation/survival

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

Nitric Oxide



Eosinophil Peroxidase

Arsenic Trioxide

Hyperbaric Oxygen

Lotfi R, Lotze M.T. Eosinophilic Granulocytes and Damage Associated Molecular Pattern Molecules [DAMPs]: Role in the Inflammatory Response Within Tumors. J Immunotherapy 2007 Jan;30(1):16-28.

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

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