Eosinophils Modulate Tumor Microenvironment By Oxidizing DAMPs From Necrotic Tumor

Ramin Lotfi, MD
University of Ulm / German Red Cross, Germany
Institute of Transfusion Medicine and Immunogenetics

University of Pittsburgh, Hillmann Cancer Center, USA
Mode of Cell Death is Important for Danger Recognition

- Release Contents
  - Modulate Immune Response,
  - Induce Angiogenesis & Tumor Proliferation

- Ingested by Phagocytes
  - Apoptotic Bodies

- Contents
  - Sequestered in Apoptotic Bodies

- Necrosis
- Apoptosis

- unexpected “Bloody Death”
- programmed “Silent Death”
Damage-associated Molecular Patterns (DAMPs):

Cell Constituents:
- HMGB1
- Heat shock proteins
- Uric Acid, ATP, Adenosine
- s100 proteins
- Hepatoma derived growth factor
- Cardiolipin

Secreted molecules:
- Fibrinogen domain A
- Surfactant protein A

Matrix elements:
- Heparan sulfate
- Soluble hyluranan
- Fibronectin
Eos are Attracted By Cell Debris And Found In Necrotic Tissues

Stenfeldt AL, Wenneras C. (Immunology 2004)

In vitro

Cormier SA, Lee NA (J Leuk Biol, 2006)

In vivo
**Eosinophils**
- Asthma/autoimmune/allergic diseases
- Helminth infections
- Cancer (colorectal cancer patients with eosinophilia have a better prognosis)  
  *Lotfi et al., J Immunother. 2007*
- Highly cytotoxic granules (MBP, EPO)
- Highest oxid. Burst compared to other leukocytes
Summary of background information and hypotheses

- Tumors undergo necrosis => release of DAMPs
- DAMPs influence tumor microenvironment
- Eos are attracted by DAMPs

Interaction between Eos & DAMPs ??
Experimental design:

Induction of necrosis by repeated freeze/thaw cycles to obtain DAMPs

Stimulated Eos with DAMPs

Read-Out:
Degranulation (Release of MBP&EPO)
Oxidative Burst (Generation of ROS)
Necrotic Material induce Eos Degranulation

A

HCT-116

CACO-2

Intracellular MBP (log 10 fluorescence)

Eos Count

w/o Lysate

+ Lysate

B

EPO Release x10^3 Eos Equivalent

EPO Release x10^3 Eos Equivalent

Untreated PMA CACO HCT

C

Intracellular MBP

D

Lysed HCT cells

EPO Release
Necrotic Material induce Eos Degranulation

Stimulation with HCT-Lysate

Eosinophil Count

Intracellular MBP (log scale)

No lysate
2 min
5 min
30 min
120 min
Necrotic Material Enhance Eos Oxid. Burst

A

- Stimulated with lysate
- Non-stimulated

Eosinophil Oxid. Burst

B

Eosinophils
Granulocytes

Oxid. Burst

D

Eosinophil Burst

- Untreated
- HCT-Lysate

Rel. Oxid. Burst

Lysed MSC Cells/ml

10^5 10^4 10^3 10^2
H2O2 Neutralizes The Effect Of Necrotic Material On Eosinophils.
Biology of HMGB1

a In the nucleus
- Binds DNA
- Binds to distorted DNA
- Modulates interaction of transcription factors with DNA

b At the cell surface
- Axonal sprouting and neurite outgrowth
- Cell migration and metastasis of tumour cells

c Extracellular
- Binds RAGE, TLR2 and TLR4
- Signals through NF-κB

d Necrosis
- Released from cell
- Drives inflammation and/or repair

HIGH-MOBILITY GROUP BOX 1 PROTEIN (HMGB1): NUCLEAR WEAPON IN THE IMMUNE ARSENAL

*Michael T. Lotze and *Kevin J. Tracey
The Receptor For Advanced Glycation End Products, RAGE on Eosinophils.
RAGE Participates in DAMPs-Induced Eosinophil Activation

C

Stimulation

No lysate

HCT-lysate + anti-RAGE

HCT-lysate + anti-HMGB1

HCT-lysate

No lysate

MSC-lysate + anti-RAGE

MSC-lysate + anti-HMGB1

MSC-lysate

Eosinophil Count

Intracellular MBP (log scale)

D

Eos Oxid. Burst

anti-RAGE

-  -  +

HCT-Lysate

-  +
Human Eosinophils Degranulate Following HMGB1 Treatment
HMGB1 Specifically Enhances the Oxid. Burst of Eos When Compared With Neutros

![Graph showing the oxidative burst of eosinophils and granulocytes with different concentrations of HMGB1 over time.](image)
HMGB1 Serves As Chemoattractants For Human Granulos And Eos
Inhibition of HMGB1 Induced Eos Migration

![Graph showing inhibition of HMGB1 induced Eos migration. The graph compares HMGB1 (10μg/ml), HMGB1 + goat IgG, Oxidized HMGB1, and HMGB1 + anti-RAGE in terms of Eos migration (Fold Increase).]
HMGB1
Enhance Survival Of Human Eosinophils
Oxidized DAMPs Lose Their Capacity to Stimulate DCs

Marker of differentiation  Aktivierungs-märker  Differenzierungs-märker
Eosinophils Promote Oxidation of DAMPs
Dr. Lotze's DAMP Lab

ramin.lotfi@uni-ulm.de

Lotfi et al., J Immunol., Oct 1, 2009