Mast cells & basophils II: Roles in health & disease

Feb. 25, 2004
Advanced Immunology Course

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• The challenge: How to investigate definitively the potential roles of mast cells/basophils in health & disease?
• The “mast cell knock-in mouse”
• Roles of mast cells in “acute” & “chronic” models of asthma
• Mast cells: sentinels of innate immunity
• New approaches; “new” mast cell functions
• Promising questions for future investigation
Diverse potential mast cell activation mechanisms in host defense/pathology *

- Products of pathogens (LPS/CD14-TLR4†, E. coli FimH†/CD48, C. difficile toxin†, H. pylori, etc., etc.)
- Products of complement activation† (C3a/C3aR, C5a/C5aR, ? C3bR/CD35, etc.)
- IgE† (FcεRI†, CD23, galectin-3), IgG1† (FcγRIII†, mouse), LC† via antigens† or superAgs (e.g., S. aureus Protein A, P. magnus Protein L, HIV gp120, protein Fv in HBV & HCV)
- T cell-derived products (?)
- Other: neuropeptides (e.g., VIP, Neurotensin, Substance P†), ET-1†, SCF† & other cytokines, leukocyte products, defensins/LL-37, insect/reptile venom components, etc., etc.

* Responsiveness (and responses) of different MC populations can vary: “Mast cell (basophil) heterogeneity”
† MC-dependent response demonstrated in “MC-knock-in” KitW/KitW−v mice

Proposed mast cell (basophil ?) roles (a very incomplete list-I)

- Effector (& immunoregulatory) cell in: IgE- &/or (in mice) IgG1-associated immune responses, e.g., anaphylaxis, “asthma” & parasite immunity; some mouse models of “autoimmunity” (e.g., MS, RA)
- Regulate “immunologically non-specific” acute and chronic inflammation & “natural immunity” (e.g., IBD)
- Regulate wound healing, angiogenesis & tissue-remodeling
- Regulate T cell-dependent, “Ig-independent” responses (e.g., CS)
Proposed mast cell (basophil?) roles (a very incomplete list-II)

- Promote &/or retard tumor development, progression or metastasis
- Promote protective responses to diverse endogenous or exogenous noxious (non-microbial) agents
- Bi-directional interactions with peripheral nerves & promotion of “neurogenic inflammation” & neurite growth
- Regulate epithelial development, proliferation & function (e.g., “barrier function”, hair growth)

WBB6F₁-Kit<sup>W</sup>/Kit<sup>W-v</sup> Mice

- Markedly reduced KIT signaling:
  - Macrocytic anemia
  - Virtually lack germ cells (they are sterile), skin melanocytes, interstitial cells of Cajal, MAST CELLS *
  - Major T cell, B cell, granulocyte, monocyte & hemostatic functions essentially normal
  - Accept transplantation of hematopoietic cells that express wild type KIT

Kit<sup>W</sup>/Kit<sup>W-v</sup> mice & “mast cell knock-in”

Kit<sup>W</sup>/Kit<sup>W-v</sup> mice (important “details”)

- “Baseline” levels of mature mast cells (MCs) in Kit<sup>W</sup>/Kit<sup>W-v</sup> mice are very low (skin < 1.0 % of +/+ levels; airways, lungs, gut, peritoneal cavity and most other sites: undetectable) [but Kit<sup>W</sup>/Kit<sup>W-v</sup> mice have ~ normal levels of basophils].
- Kit<sup>W</sup>/Kit<sup>W-v</sup> mice have MC progenitors, which can give rise to MCs in vitro or, in certain (unusual) circumstances, in vivo * [always examine for this].
- “Mast cell knock-in” Kit<sup>W</sup>/Kit<sup>W-v</sup> mice: have undergone selective (local and/or systemic) “repair” of their MC deficiency with +/+ or genetically-altered MCs (of hematopoietic † or ES cell origin ‡) [but numbers, distribution &/or phenotype of these MCs may differ from those of MCs in wild type mice].

1. Mast cells *essential* for the response
2. Mast cells *contribute to* (or, possibly, *essential for*) the response
3. Response is *KIT-dependent but (probably) mast cell-independent*

**The importance of mast cells in “asthma models” in mice depends on the model analyzed**
“Asthma Models”

• **“OVA”** (without artificial adjuvant)*

  **Sensitization:** 10 mcg OVA i.p. (days 1, 3, 5, 7, 9, 11 & 13)

  **Challenge:** OVA (2, 20 or 200 mcg) or saline, intra-nasally x 3 over 6 days, beginning on day 40

• **“OVA/alum”** *(can induce “asthma” [?] without B cells or mast cells!)*†

  **Sensitization:** 20 mcg OVA in alum i.p. (days 1 & 14)

  **Challenge:** 1% OVA or saline by aerosol, 20 min x 3 (on days 28, 29 & 30)


Assessment of “asthma” features 24 h after last antigen (OVA) or saline challenge

• AHR to aerosolized methacholine (PenH in non-anesthetized, spontaneously breathing mice [BUXCO system])

• Eosinophil numbers (total lung and perivascular)

• Numbers of proliferating (BrdU+) cells in tracheal and bronchiolar epithelium
OVA-induced pulmonary changes (OVA sens. without alum)

*Saline* vs. *OVA*

W/W^v OVA vs. BMCMC→W/W^v OVA

arrows = eosinophils

Eosinophils (perivascular) 24 h after last OVA or saline challenge in the two models

<table>
<thead>
<tr>
<th>Challenge OVA (µg)</th>
<th>W/W^v</th>
<th>BMCMC</th>
<th>W/W^v</th>
<th>+/+</th>
<th>W/W^v</th>
<th>+/+</th>
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<tbody>
<tr>
<td>W/W^v</td>
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<td>20</td>
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<tr>
<td>200</td>
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<tr>
<td>0%</td>
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<tr>
<td>0%</td>
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</table>

All mice sensitized with OVA without alum (BrdU+ cells [arrows])

BrdU+ cells in tracheal epithelium 24 h after last OVA or saline challenge in the two models
Summary

**OVA/alum sensitization & OVA challenge**

- Mast cells are *not required* for antigen-specific IgE/IgG1 responses or for chronic inflammation of the airways.

- Mast cells are *not required* for antigen-induced immunologically non-specific airway hyper-responsiveness (AHR) to methacholine *

- Mast cells *may be required* for airway epithelial cell proliferation [*requires studies in mast cell knock-in mice to confirm*].

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Summary (continued)

**OVA *(without alum)* sensitization & OVA challenge**

- Mast cells are *not required* for antigen-specific IgE/IgG1 responses.

- Mast cells *significantly enhance* antigen-induced eosinophil infiltration of the airways/lungs (by ~ 2-3 fold).

- Mast cells *significantly enhance* antigen-induced AHR (by ~ 100 %).

- Mast cells *significantly enhance* antigen-induced increases in BrdU+ cells in airway epithelium (by ~ 4 fold in trachea).
Choose “models” carefully

- Because most studies of “asthma” (or autoimmunity) in mice employ protocols that induce very strong immune responses, it may be difficult to identify the contributions of mast cells (or other effectors) whose role is to initiate, amplify &/or perpetuate the inflammation or tissue remodeling induced by weak signals.

- **Patients with atopic asthma can be extremely sensitive to challenge with small amounts of antigen.**

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Mouse model of “chronic asthma” *

**Model:** OVA i.p. sensitization x 3 (without adjuvant) + OVA i.n. challenge (weekly x 9, starting on day 12)

**Control:** PBS i.p. + PBS i.n., schedule as for OVA

* M. Yu, M. Tsai & S. J. Galli, unpub. data
Histologic features of “chronic asthma” model *

OVA/OVA PBS/PBS

BSM (red) Collagen (yellow) Mucus (purple)

Intra-epithelial mast cells

* M. Yu, M. Tsai & S. J. Galli, unpub. data

Features of Chronic Asthma Model
(Generally, measured 24 h after last OVA challenge)

<table>
<thead>
<tr>
<th>Features</th>
<th>Expression in Various Mice</th>
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<tbody>
<tr>
<td></td>
<td>Kit^{W/Kit^{W+v}}</td>
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<tr>
<td>Acute Bronchoconstriction (OVA)</td>
<td>−/+</td>
</tr>
<tr>
<td>AHR to Methacholine (24 h after OVA)</td>
<td>+</td>
</tr>
<tr>
<td>Increased BAL: pmn, eos, mono, lymph</td>
<td>−/+</td>
</tr>
<tr>
<td>Eosinophil infiltration</td>
<td>+</td>
</tr>
<tr>
<td>Increased lung mast cells</td>
<td>ND</td>
</tr>
<tr>
<td>Intra-epithelial mast cells</td>
<td>ND</td>
</tr>
<tr>
<td>Increased goblet cells</td>
<td>−/+</td>
</tr>
<tr>
<td>Increased collagen deposition</td>
<td>−/+</td>
</tr>
<tr>
<td>Increased bronchial smooth muscle</td>
<td>−/+</td>
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</table>
Clustering of 503 genes in the lungs of various mice sensitized/challenged with OVA (or PBS) *
(Tissues sampled 24 h after the last OVA or PBS challenge)

“Chronic asthma” model

“Reference” mRNA
(C57BL/6 : WBB6F1-+/+ = 1:1):
90% naive lungs
10% from lungs after treatment with OVA/OVA, H₂O₂ or LPS

~ 6,000 genes of ~ 34,000 tested exhibited 2-100 fold changes in expression compared to reference mRNA

* M. Yu, M. Tsai & S. J. Galli, unpub. data

Critical protective role of mast cells in a model of acute septic peritonitis

Bernd Echtenacher, Daniela N. Männel & Lothar Hültmann *

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* GSF-Institut für Experimentelle Hämatologie, Max-Planckstrasse 25, D-81377 Munich, Germany

Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α

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* Department of Pathology, Barnes-Jewish Hospital, and † Departments of Pathology and Molecular Microbiology, Washington University School of Medicine, St Louis, Missouri 63110, USA
† Department of Veterinary Microbiology, Azabu University, Kanagawa, Japan

Use of “mast cell knock-in mice” to demonstrate that mast cells and TNF-α can be essential for optimal innate immunity to bacteria

NATURE • VOL 381 • 2 MAY 1996
Survival in CLP can be KIT-dependent

Survival in CLP can be mast cell- & TNF-α-dependent


Features of CLP in wild type (+/+), C3 -/-, or human C3-treated C3 -/- mice


<table>
<thead>
<tr>
<th></th>
<th>+/+</th>
<th>C3-/-</th>
<th>C3 -/- + HuC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLP mortality (24h)</td>
<td>20%</td>
<td>100%</td>
<td>40%</td>
</tr>
<tr>
<td>PMC degranulation (3h)</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Peritoneal TNF-α (3h,% +/+ )</td>
<td>100</td>
<td>33</td>
<td>79</td>
</tr>
<tr>
<td>Neutrophil influx (3h,% +/+ )</td>
<td>100</td>
<td>52</td>
<td>102</td>
</tr>
<tr>
<td>Bacterial clearance (E.coli, CFU x10⁴ in peritoneal fluid)</td>
<td>1h 1.6</td>
<td>35</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>3h 5.7</td>
<td>116</td>
<td>34</td>
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</table>
Can the effectiveness of innate immunity be enhanced by agents that modulate mast cells numbers or function?

The c-kit ligand, Stem Cell Factor (SCF), can enhance innate immunity through effects on mast cells

J. Exp. Med. 188:2343-2348, 1998

Marcus Maurer, Bernd Echtenacher, Lothar Hültner, George Kollias, Daniela N. Männel, Keith E. Langley & Stephen J. Galli

SCF treatment increases mast cell numbers and CLP survival in C57BL/6 mice

A-C: rrSCF, rrSCF-peg or vehicle s.c. daily, beginning 21 d before CLP and until death or 14 d after CLP

CLP: 50% ligation, 0.7 mm needle puncture X 1; Mast cell counts at end of 21 d SCF or vehicle treatment period
The ability of SCF treatment to enhance survival in CLP is (at least in part) mast cell-dependent.

rrSCF-peg (30 μg/kg) or vehicle s.c. daily, beginning 21 d before CLP (and 4 weeks after transfer of WBB6F1-+/+ BMCMCs [MCs] to some WBB6F1- KitW/KitW−/ KitW− mice) and until death or 14 d after CLP.

CLP: 50% ligation, 0.7 mm needle puncture X 1

SCF can enhance CLP survival independently of TNF-α or increases in peritoneal mast cells.

CLP: (A) 50% ligation, 0.9 mm needle puncture X 1; (B-D) 80% ligation, 0.9 mm needle X 2

B-D: rrSCF-peg (30 μg/kg) or vehicle s.c. daily, beginning 21 d before CLP & until death or 14 d after CLP.
**Mast cells (MCs) in innate immunity**

- *"Sentinel/effectector" role*: Detect pathogens (via C’Rs, CD14-TLR4, CD48); initiate, amplify & modulate inflammatory/immune responses (TNF-α is one key mediator)
- **Other roles** (direct effects on pathogens, other effects on tissues, etc.)
- The protective role of MCs in one model of innate immunity (CLP in mice) can be enhanced therapeutically (with SCF)


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**In vitro differentiation of mast cells from mouse embryonic stem (ES) cells**

<table>
<thead>
<tr>
<th>Wild-type ES cells</th>
<th>Genetically-altered ES cells</th>
<th>ES cells from nuclear transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 ES cells</td>
<td></td>
<td>Undifferentiated ES cells</td>
</tr>
<tr>
<td>50,000-fold expansion</td>
<td></td>
<td>Primary plating</td>
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<tr>
<td></td>
<td></td>
<td>Single-cell suspension in methylcellulose-based media containing SCF and IL-11 to form embryoid bodies (EBs)</td>
</tr>
<tr>
<td>~10⁸ ESMCs</td>
<td></td>
<td>Mast cell differentiation</td>
</tr>
<tr>
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<td></td>
<td>Transfer EBs to liquid media containing SCF and IL-3. EBs attach to the bottom of tissue culture plates and give rise to embryonic stem cell-derived mast cells (ESMCs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expansion of mast cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Remove adherent cells and expand ESMCs in SCF- and IL-3- containing media</td>
</tr>
</tbody>
</table>

**In vitro studies**

**In vivo studies**


Mast cells can protect mice against hypothermia induced by the ligand of “receptor X” (RX)


Mast cell expression of “receptor X” (RX) can promote optimal survival after CLP

C57BL/6-Kit<sup>W-sh</sup>/Kit<sup>W-sh</sup> Mice

- Markedly reduced KIT signaling (mutation not in KIT coding region)**:
  - Virtually lack skin melanocytes & MAST CELLS ***
- Not anemic; fertile
- Accept transplantation of bone marrow-derived cultured mast cells (BMCMCs) that express wild type KIT (M. Grimbaldeston S. J. Galli, et al., unpub. data)

*A “better” mast cell-deficient mouse ?*


Synergistic effect of IgE-dependent mast cell activation & anti-CD3-dependent T cell activation on skin swelling in vivo (“passive contact sensitivity” or “PCS”)

Susumu Nakae, H. Suto, M. Tsai & S. J. Galli, unpub. data.
Proposed/proven mast cell functions (a very incomplete list-I)

- **Effector (and immunoregulatory) cell in:** IgE- &/or (in mice) IgG_{1}-associated immune responses, e.g., anaphylaxis, "asthma" & parasite immunity; some mouse models of "autoimmunity" (e.g., MS, RA)
- **Regulate** "immunologically non-specific" acute and chronic inflammation & "natural immunity"
- **Regulate wound healing, angiogenesis & tissue-remodeling**
- **Regulate T cell-dependent, "Ig-independent (?)" responses** (CS; LC migration)* * = unpub. data

Proposed/proven mast cell functions (a very incomplete list-II)

- **Promote &/or retard tumor development, progression, or metastasis**
- **Bi-directional interactions with peripheral nerves & promotion of "neurogenic inflammation" & neurite growth** *
- **Promote protective responses to diverse endogenous (ET-1)* or exogenous noxious (non-microbial) agents**
- **Regulate epithelial development, proliferation* & function** (e.g., "barrier function", hair growth)

* = unpub. data, Galli lab
Viruses?

Some of MANY


Effects of monomeric IgE on:
- Reduce toxicity of endogenous products
- Promote homeostasis

Mast cell functions/products

Uncertainties/Opportunities:
- FcεRI surface expression
- Mast cell survival?
- Mast cell mediator/cytokine secretion?

Therapeutic manipulation

Mice vs. humans

Natural immunity, (non-immunological tissue damage)

Acquired immunity to parasites (allergic diseases) (autoimmune diseases)