Mast Cells
in the brain
**MAST CELLS - 1. Basic information**

- Produced in bone marrow - myeloid lineage
- Develop from CD34/CD117 pluripotent progenitor
- Immature committed progenitors released in the blood. Require ligation of CD117 / the KIT receptor by stem cell factor SCF (in humans) to mature. SCF also acts as a MC homing signal. Neuron and glia secrete SCF.
- Immature MCs travel to various tissues
- Tissue specific maturation and differentiation - Triggered by cytokines in the cell’s environment
MAST CELLS - 2. Familiar functions

✦ Express the high-affinity FcεRI and bind the constant region of IgE

✦ Constitutively bound to IgE before the antibody variable region binds antigen.

✦ Activated by IgE cross-linked by antigen binding >> Rapid response effector cell. Other things can activate MCs. More than 50 different MC receptors have been identified.

✦ Release manyyyyy substances (vasoactive and neuroactive, cytokines, chemokines, growth factors, proteases) - prestored granules and de novo synthesized
Mast cell activators

Receptor-binding agonists
- IgE + antigen or IgE alone
- Ig light chain
- Complement
- Neuropeptides
- Microbial products
- Cytokines
- Chemokines

Physical activators
- Temperature
- Pressure

Cell–cell contact
- OX40/OX40L
- CD40/CD40L
- TCR/MHCII

Mast cell molecules

Preformed mediators
- Histamine
- Proteases
- Serotonin
- Heparin
- IL-4, TNF, GM-CSF

T and B cell ligands
- PD-L1, OX40L, CD30L, CD40L, CCL19, 4-1BB

Newly synthesized mediators
- Lipid derived: prostaglandins
- Leukotrienes
- PAF
- Cytokines
- Growth factors
- Chemokines
- Free radicals
- Others: substance P

TRENDS in Neurosciences
**Fig. 14.11 Molecules released by mast cells on activation.** Mast cells produce a wide variety of biologically active proteins and other chemical mediators. The enzymes and toxic mediators listed in the first two rows are released from the preformed granules. The cytokines, chemokines, and lipid mediators are synthesized after activation.

<table>
<thead>
<tr>
<th>Class of product</th>
<th>Examples</th>
<th>Biological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Tryptase, chymase, cathepsin G, carboxypeptidase</td>
<td>Remodel connective tissue matrix</td>
</tr>
<tr>
<td>Toxic mediator</td>
<td>Histamine, heparin</td>
<td>Toxic to parasites, increase vascular permeability, cause smooth muscle contraction, anticoagulation</td>
</tr>
<tr>
<td>Cytokine</td>
<td>IL-4, IL-13</td>
<td>Stimulate and amplify T&lt;sub&gt;H&lt;/sub&gt;2-cell response</td>
</tr>
<tr>
<td></td>
<td>IL-3, IL-5, GM-CSF</td>
<td>Promote eosinophil production and activation</td>
</tr>
<tr>
<td></td>
<td>TNF-α (some stored preformed in granules)</td>
<td>Promotes inflammation, stimulates cytokine production by many cell types, activates endothelium</td>
</tr>
<tr>
<td>Chemokine</td>
<td>CCL3</td>
<td>Attracts monocytes, macrophages, and neutrophils</td>
</tr>
<tr>
<td>Lipid mediator</td>
<td>Prostaglandins D&lt;sub&gt;2&lt;/sub&gt;, E&lt;sub&gt;2&lt;/sub&gt;, Leukotrienes C4, D&lt;sub&gt;4&lt;/sub&gt;, E4</td>
<td>Smooth muscle contraction, chemotaxis of eosinophils, basophils, and T&lt;sub&gt;H&lt;/sub&gt;2 cells, increase vascular permeability, stimulate mucus secretion, bronchoconstriction</td>
</tr>
<tr>
<td></td>
<td>Platelet-activating factor</td>
<td>Attracts leukocytes, amplifies production of lipid mediators, activates neutrophils, eosinophils, and platelets</td>
</tr>
</tbody>
</table>
MAST CELLS - *The nitty gritty*

- Found in mucosal tissue of eyes, nose, airways & in connective tissue around blood vessels - and brains of mammals and others.
- Mediate allergic reactions, anaphylaxis - rapid release, degranulation, of preformed histamine, prostaglandins, etc.
- Recruit eosinophils, basophils, TH2, B cell, lymphocytes initiating an “inflammatory cascade”
- Evolutionary goals - defend from parasitic invasion, deliver antigen to immune tissues, expel invaders by muscle contractions in gut, lung
Background:

- MCs have been found in the brains of multiple animal species
- Brain distribution is heterogenous but sorts by species
- Some species have more brain MCs than others
- MC numbers vary widely by individual
- In rats high numbers of MCs have been found in the thalamus
- PN: the BBB matures, glial cells proliferate and brain’s vascular network grows

Setup:

- Male Long- Evans rats - PN8, PN11, PN15, PN21, PN30, adults

- 3-5 per age group

- Brains removed from anesthetized rats

- Serial coronal slices through thalamus

- Alternate sections stained and viewed with fluorescent or scanning confocal

- MCs counted in thalamus and pia, analyzed stereologically
Fig. 1 - Developmental changes in mast cell number. (A) Pial mast cell population was 3558 ± 404 at PN0, increased to 4958 ± 844 at PN8, peaked at 5550 ± 764 at PN11, then decreased by 74% to 1466 ± 220 at PN15, and reached adult levels of 46 ± 14 at PN30. (B) Thalamic mast cells first appear at PN8 (138 ± 38), increase rapidly between PN15 and 21, and reach adult levels by PN30 (1424 ± 267 at P30; 1566 ± 267 at P112). (C, D) Distribution of mast cells in the pia and thalamus respectively, along the anterior–posterior axis with reference to bregma (n=5–8 animals/age).
Fig. 2 – DAPI stained tissue to test for nuclear fragmentation in pial mast cells. (A–C) Pial mast cell with its characteristic pattern of eu- and heterochromatin. (D–F) Apoptotic pial mast cell with fragmented chromatin (white arrows). Optical slices (z axis, 1 μm). Scale bar: 5 μm.

Fig. 3 – Localization of mast cells in relation to the blood vessel wall. Mast cells are labeled with avidin (red) and endothelia are labeled with RECA-1 (white). (A–C) This is an example of a mast cell localized on the brain side of the endothelial cell. (D–F) This mast cell lies in the lumen of the blood vessel. (D–F) Composite image of seven 1-μm serial optical slices. Scale bars: 10 μm.
Fig. 4 – To determine the cellular localization of mast cells relative to the smooth muscle of the blood vessel and its basal lamina, triple-label immunocytochemistry was used. Mast cells are located between the smooth muscle actin-positive cell (red) and the laminin-delineated basal lamina (white). (A–D) At PN11 thalamic mast cells (A) are associated with large blood vessels with smooth muscle containing α-SMA (B). Laminin (C) is present in the basal lamina of the smooth muscle and surrounds the mast cell. (D) Overlay of panels A–C. (E–G) A similar arrangement is shown for PN30 as in PN11. Additionally, at this age, laminin is present in neuronal cell bodies (G). Images are composites of 4 (A–D) and 6 (E–H) optical slices (z axis, 1 μm). Scale bar, 20 μm.
Fig. 5 – Mast cells in the thalamus seen express the integrin $\alpha_4$. (A–C) Mast cells in the PN11 pia are labeled with avidin (A) and contain $\alpha_4$ integrin immunoreactivity (B). (C) Overlay. Image is a composite of ten 1-μm optical sections acquired using confocal microscopy. A similar observation was made in the PN30 thalamus (D–F). Scale bar 20 μm.
Fig. 6 - Mast cells are associated with blood vessels that have acquired astrocytic endfeet. Triple label immunocytochemistry is used on tissue from PN8, PN15 and PN30 to examine the physical interaction of mast cells and astrocytes. Panels A1-A4
Fig. 7 – The figure shows the frequency with which mast cells contact GFAP-positive processes from PN8-30. The percent mast cells contacted by GFAP-positive processes (circles) remains at about 80% at all ages. The OD of GFAP-positive processes (diamonds) increases with age.
Fig. 8 – Bar graphs reveal properties of vasculature in relation to localization of mast cells in development. (A) The small blood vessels significantly increase in density from PN8 to PN15 and PN30. (B) The percent of blood vessels in each size category is shown over development. (C) Mast cells are preferentially located on large blood vessels at all ages.
Fig. 9 – Perivascular mast cells are preferentially located at blood vessel branch points. (A–C) (A) Mast cells at P15 are located at (B) endothelial (RECA-1, white) branch points (arrows). (C) Overlay of panels A and B. Images are composites of 13 optical slices (z axis, 1 μm) acquired using confocal microscopy. Scale bar, 20 μm. (D) Mast cells are preferentially located at blood vessel branch points at PN15 and PN30. Blood vessel density increases significantly from PN8 to PN15 and P30 (Tukey Test, P = 0.008 for PN8 vs. P15, and P = 0.001 for P8 vs. P30; n = 3 per age group). Branch points compose between 2.4% and 6.9% of the blood vessel volume from PN8 to PN30, whereas the proportion of mast cells located at branch points increases from 6.4% at PN8 to 36.2% at PN30. (E) The proportion of mast cells at branch points (MCBP) vs. the proportion of branch points of all blood vessels indicates that mast cells are localized at branch points at P15 and P30. n = 3 animals/group. BP, branch point; MC, mast cells. Scale bar = 30 μm.

**Background:**

- There is anatomical evidence of communication between MCs and neurons and MCs and glial cells - neuronal/glial signals effecting MC behavior and MC releasing signals that effect neuronal/glial signaling
- MCs express neuropeptide receptors and neurons contacting MCs secrete neuropeptides
- A mast cell can release NGF on a neuron after being stimulated by NGF released by that same neuron
- Histamine secreting MCs associate closely with histamine receptor bearing Schwann cells
- MC degranulation is stimulated by MC binding to a glial cell monolayer
- MCs may have a role in demyelination - an integral glial function, or BBB hyperpermeability
- Authors previously demonstrated myelin activation of MCs via scavenger receptor and MC adherence to rat oligodendrytes in culture. They hypothesize heterotypc binding of MCs/ODC may trigger bidirectional signaling and alter function in both cells.
Peritoneal mast cells (RPMC) and macrophages (RPMAC) harvested from CO2 killed male and femal Wister rats

Enriched oligodendryte culture prepared from mixed primary culture of neonatal rat brain

RPMC were added to ODC monolayers and incubated 30/60 min

Non-adherent cells pelleted and resuspended in the supernatant. Chymase activity measured in supernatant, adherent /non-adherent cells. Adhesion and secretion/free chymase in supernatant calculated from calibration curve of enzymatic activity.

Setup:

ODC samples incubated with/without RPMCs examined with EM (1)

Western blot analysis of actin/tubulin protein expression in cytoskeleton of ODCs with/without RPMCs (3)

ODCs with/without RPMCs incubated with anti-alpha-tubulin III and F-actin marker palloidin, fluoresced and imaged for cytoskeleton morphology (2)

Western blot analysis of citrullinated protein expression in ODCs incubated with 30,000 cells worth of RPMC, RPMAC, RPMC sonicate, RPMC conditioned medium, RPMAC sonicate - IHC stained with a citrullinated protein anti-body (8) (9)

Fluorescent videoimaging of glass plated ODC cell body or cell process Ca2+ response to samples of - resting MCs, histamine, RPMC cell conditioned medim, whole RPMC garnules, RPMC sonicte, macrophage sonicate or 48/80 (MC avtivator) (5) (6)

ODCs incubated with 30,000 RPMCs, labeled with Annexin V-FITC, treated with anti-cleaved Capsase 3 and fluoresced (7)

Analysis:

Fig. 1. Appearance of ODC after 30 min (A and B) or 60 min incubation either in the absence (A and C) or in the presence (B and D) of 30,000 RPMC/well. Some adherent RPMC are evident in B and D (white arrows). A contraction of the cell body is evident after RPMC addition. Diff-Quik stained smears; original magnification = ×400.
Fig. 2. Detection of actin (A and B) and tubulin (C and D) assembly in ODC after 60 min incubation either in the absence (A and C) or following the addition of 30,000 RPMC/well, by immunofluorescence. Actin was revealed using RITC-phalloidin binding. Tubulin was revealed by using YOYO monoclonal antibody and FITC-conjugated anti-mouse IgG. The presence of RPMC is associated with an evident reduction of actin microfilaments in the ODC processes (B) and of the microtubule assembly (D). Original magnification = ×200.
Fig. 3. Western blotting analysis of the TX-100 cytoskeletal extract of ODC incubated for 2 h either alone or with 30,000 inflammatory cells/well. Lane 1 = RPMAC alone; Lane 2 = RPMC alone; Lane 3 = ODC + RPMAC; Lane 4 = ODC + RPMC; Lane 5 = ODC alone; 5 μg of proteins was loaded in each lane. Actin and tubulin were revealed in the extract obtained from ODC alone (lane 5) or ODC + RPMAC (lane 3), while in the presence of RPMC (lane 4) the signals for these proteins were strongly diminished.
Fig. 4. Evidence that following interaction with ODC, RPMC are activated to degranulate. A and B) Diff-Quik stained smears. ODC after 2 h of incubation alone (A) or following RPMC (30,000/well) addition (B); some degranulating RPMC interacting with ODC are seen in B, with many granules sticking mainly on ODC processes (original magnification = ×400). C) Chymase secretion from RPMC interacting with the ODC monolayer. The enzyme activity is absent in the supernatant of the ODC monolayer incubated 60 min alone, but is increased if RPMC (30,000/well) were added. The spontaneous release of chymase from RPMC incubated under the same experimental setting but in the absence of ODC (polyornithine (PO) coated slides) was significantly lower. In the inset the extent of adhesion of RPMC on ODC is reported. The addition of anti-SR-A/II blocking antibody (α-SR) inhibited, but did not block the secretory process and the adhesion (Fig. 4C and inset in Fig. 4C), suggesting other mechanisms of interaction. Values are the mean ± SD of at least three experiments. D) Scanning electron microscope appearance of one RPMC interacting with the ODC surface. Note that some granules are secreting and other, already secreted, are attached to the ODC surface (Original magnification = 9000×).
Fig. 5. Spatial and temporal properties of $[Ca^{2+}]$ response induced by RPMC in ODC. (A) The numbers indicate seconds after RPMC adhesion (time 0). Red asterisks in the drawing indicate the position of adherent RPMC. The intensities of fluorescence ratio values are represented according to the pseudocolor calibration bar, where blue corresponds to the $[Ca^{2+}]$ at rest. Cell response was initially confined to the cell processes to which RPMC adhered. After a delay $[Ca^{2+}]$ raised in the nearby processes and in the cell body. (B) The corresponding time course of $[Ca^{2+}]$ variation recorded in two of the cell processes (1 and 2) and in the soma (3). The recording windows were positioned as illustrated in the drawing.
Fig. 6. Proportion of ODC eliciting Ca\(^{2+}\) responses in different experimental conditions (see text for further details). RPMCson or wg = sonicate of RPMC or RPMC whole granules; RPMACson = sonicate of RPMAC; RPMCcond med = supernatant of RPMC activated with 48/80; 48/80 ctrl = the same amount of 48/80 used to activate RPMC is added at the same dilution as negative control; HIST = histamine. Values are the mean ± SD of four different experiments.
Fig. 7. Evaluation of the apoptotic programme in ODC incubated 2 h either alone or in the presence of 30,000 RPMC/well. A and C Annexin V-FITC binding; B and D immunofluorescence revealing active Caspase 3. Original magnification = 200×. The red fluorescence in B and D reveals the actin assembly through labelled phalloidin binding.
Fig. 8. Evaluation of the presence of citrullinated proteins in ODC by immunofluorescence following 2 h incubation. 30,000 inflammatory cells or supernatant from 40,000 activated RPMC (RPMC cond. med.) were added. A = ODC alone; B = ODC + RPMC; C = ODC + RPMC; D = ODC + RPMC cond. med. Original magnification = 200×.
Fig. 9. Evaluation of the presence of citrullinated proteins in ODC by western blotting following 2 h incubation. 30,000 inflammatory cells or supernatant from 30,000 activated RPMC (RPMC cond med) were added/well. Lane 1 = ODC alone; Lane 2 = ODC + RPMC; Lane 3 = ODC + RPMAC; Lane 4 = ODC + RPMC cond med; Lane 5 = RPMC sonicate alone as negative control. 5 µg of proteins was loaded in each lane. Standard molecular weights (KDa) are shown on the right.
RESEARCH QUESTIONS

1. *Do activated or deficient mast cells alter the timing or spatial pattern of embryonic angiogenesis?* *Or, neonatal embryogenesis?*

2. *Is the protein expression in the neonatal NVU different in kind or quantity after an activated or deficient mast cell embryonic environment?*