Inflammation

Chapter 2

Feb 16, 2005

Chemotaxis and chemoinvasion assays

- Migration and invasion was assayed in 24-well cell-culture chambers using inserts with 8-μm pore membranes as described.
- Membranes were pre-coated with fibronectin (2.5–7.5 μg ml-1) for chemotaxis or Matrigel (28 μg per insert) and fibronectin for invasion studies. Breast cancer cells were resuspended in chemotaxis buffer (DMEM/ 0.1% BSA/ 12 mM HEPES) at 2 or 4 10^4 cells per ml.
- After incubation for 6 or 24 h for chemotaxis or chemoinvasion assays, respectively, cells on the lower surface of the membrane were stained and counted under a light microscope in at least five different fields (original magnification, 200).
- Assays were performed in triplicates. Chemokinesis was tested in checkerboard assays and was uniformly negative for both CXCL12 and CCL21.

Boyden chamber

Figures removed for copyright reasons.

Checkerboard assay

nM Top / Bottom	0	1	10	100
0	0.2	0.2	0.3	0.5
1	0.8	0.3	0.4	0.4
10	2.7	2.3	0.6	0.6
100	3.4	3.5	0.8	0.7

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Cells Behave Better on BD Matrigel™ Matrix

- BD Matrigel™ Matrix is a solubulized basement membrane preparation extracted from EHS mouse sarcoma, a tumor rich in ECM proteins.
- Its major component is laminin, followed by collagen IV, heparan sulfate proteoglycans, and entactin.
- At room temperature, BD Matrigel™ Matrix polymerizes to produce biologically active matrix material resembling the mammalian cellular basement membrane. Cells behave as they do *in vivo* when they are cultured on BD Matrigel™ Matrix.
- It provides a physiologically relevant environment for studies of cell morphology, biochemical function, migration or invasion, and gene expression.

Aulus Cornelius Celsus

- *De medicina* (Florence: Nicolaus Laurentii, 1478)
- Compilation of knowledge of diet, pharmacy, and surgery from the time of Imperial Rome, circa 30 A.D.
- Printed four times during the fifteenth century.
- *De medicina* contains the first history of medicine, and it was Celsus who originally translated Greek medical terms into Latin.

Vascular changes

- Redness
- Swelling
- Heat
- Pain

- Loss of function

- Vasodilation and increased blood flow
  - Histamine, NO
- Increased vascular permeability
  - Endothelial gaps
    - Histamine, leukotrienes
    - Kinins, complement, etc.
  - Injury (direct, leukocyte)
- Stasis, margination, rolling, sticking

Leukocyte extravasation

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Source: Figure 2-6 in [RC]
Kumar, V., A. K. Abbas, and N. Fausto. *Robbins and Cotran Pathologic Basis of Disease*, 7th ed. Philadelphia PA: Elsevier, 2005. ISBN: 0721601871.

Rolling: selectins

- L-selectin (CD62L) on leukocytes
  - Homing receptor for lymphocytes to enter lymph nodes via high endothelial venules
  - For PMNs to cytokine activated endothelium
  - Binds to GlyCAM-1, MadCAM-1, CD34
- E-selectin (CD62E) only on activated endothelial cells
  - Recognizes Lewis X or Lewis A
  - Homing receptor for effector and memory T cells, especially skin
- P-selectin (CD62P) secretory granules of platelets and Weibel-Palade bodies of endothelial cells

Sticking: ICAM-1, VCAM-1

- Ig superfamily members are endothelial adhesion molecules that bind integrins on leukocytes
- Integrins are heterodimeric glycoproteins
  - b_2 integrins LFA-1 and Mac-1 bind to ICAM-1
  - b_1 integrins such as VLA-4 bind to VCAM-1
- Once stuck, leukocytes transmigrate (diapedesis) between endothelial cells in venules to leave the circulatory system (extravasate)

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Source: Figure 2-10 in [RC]

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Source: Figure 2-11A in [RC]

Oxygen-dependent killing

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Source: Figure 2-11B in [RC]

- Phagocytosis stimulates respiratory burst
- NADPH or phagocyte oxidase (Phox)
- PMNs produce myeloperoxidase that converts H_2O_2 to HOCl
- Efficient killing

Chronic granulomatous disease

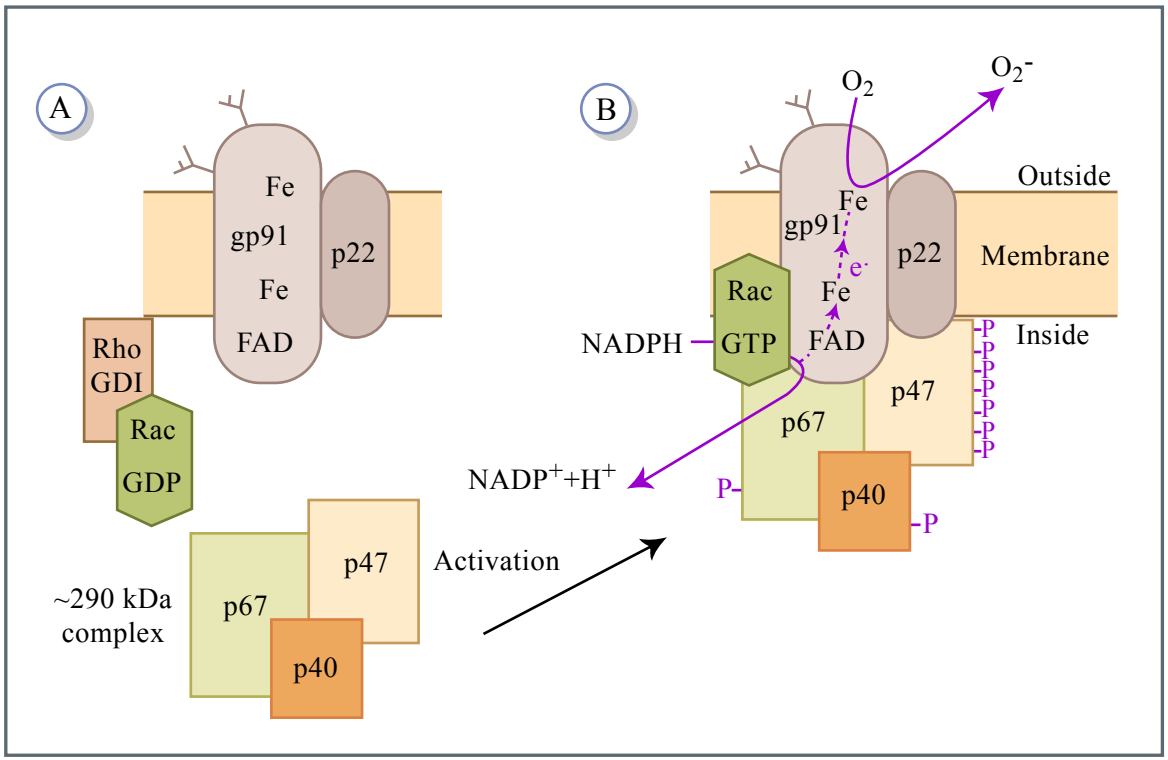

Figure by MIT OCW.

- NADPH oxidase made up of 7 proteins
- X-linked $gp91^{Phox}$
- Autosomal $p47^{Phox}$ and $p67^{Phox}$
- Recurrent opportunistic infections (catalase-producing organisms)

Cytokines and chemokines

- TNF and IL-1 produced by activated macrophages
  - Endothelial activation, priming of PMNs
  - Acute phase response, septic shock
- Chemokines are potent chemoattractants
  - CXC (alpha) act mostly on PMNs (IL-8)
  - CC (beta) act on other phagocytes (MCP-1, MIP-1a)
  - C (lymphotactin) and CX_3C (fractalkine)

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Source: Figure 2-21 in [RC]

Morphologic patterns of acute inflammation

- Serous inflammation
  - Transudate (s.g. < 1.012) or effusion
- Fibrinous inflammation
  - Exudate (s.g. > 1.012) with fibrin
  - Meninges, pericardium, pleura
- Suppurative or purulent inflammation
  - Pus or purulent exudate
  - PMNs, necrotic debris, and pyogenic bacteria

Chronic inflammation

- Prolonged duration (weeks or months)
- Simultaneous active inflammation, tissue destruction, and attempts at repair
  - Persistent infections
  - Prolonged exposure to toxic agents
  - Autoimmunity

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Source: Figure 2-28 in [RC]

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Source: Figure 2-31 in [RC]

Granulomatous inflammation

- Characterized by activated macrophages that take on an epithelioid appearance
- Pale pink granular cytoplasm and indistinct cell boundaries
- Multinucleate giant cells