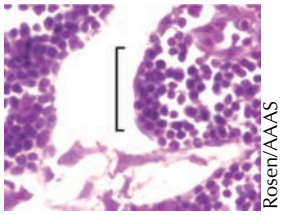
Research Roundup

Traffic jam suppresses immunity

Organ rejection in transplant patients can be prevented using the drug FTY720, but the drug's mechanism of action has been unknown. Now, Suzanne Mandala, Hugh Rosen, and colleagues (Merck Research Laboratories, Rahway, NJ) show that it acts by retaining lymphocytes in lymph nodes, preventing their circulation through the bloodstream.



Rosen/AAAS

FTY720 causes lymphocytes to get trapped in nodes (bracket).

B and T lymphocytes are either retained in lymph nodes in order to contact antigens and antigen-presenting cells or sent back out into the bloodstream for circulation. Circulating lymphocytes may enter tissues, where they mediate protective activities or cause tissue damage. Most immunosuppressive drugs work by inhibiting lymphocyte function: they either kill the cells directly, inhibit their production, or block signals downstream of antigen recognition. But FTY720 works in a novel manner.

Rosen's group found that a phosphate ester metabolite of FTY720 mimics the natural lipid sphingosine-1-phosphate (S1P) and binds several forms of S1P receptors. Activation of the receptors causes the sequestration of lymphocytes at nodes and a corresponding decrease in circulating lymphocytes. "This is the first indication that S1P and its receptors regulate lymphocyte trafficking and that, if manipulated pharmacologically, [they] can give rise to immunosuppression," says Rosen. Unlike S1P, the side effects of which include low blood pressure, FTY720 is better tolerated in clinical use. The reasons behind this distinction are yet unclear, but may involve the failure of FTY720 to bind all S1P receptors. ■

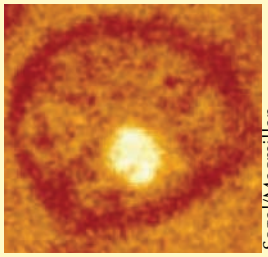
Reference: Mandala, S., et al. 2002. *Science.* 10.1126/science.1070238

Proteases commit microbicide

White blood cells use proteases to kill their targets, according to new results from Emer Reeves, Hiu Lu, Anthony Segal (University College, London, England), and colleagues. The findings contradict previously accepted notions that reactive oxygen species (ROSs) are the killing agents.

ROSs like superoxide are produced by NADPH oxidase activity when white blood cells, primarily neutrophils, phagocytose pathogens. For decades it has been thought that high concentrations of the toxic ROSs resulting from this respiratory burst are directly responsible for killing microorganisms. But the new results prove otherwise.

Segal's group showed that mice deficient in protease activity have increased sensitivity to microbes. Additionally, purified neutrophils from the protease-deficient mice are impaired in their antimicrobial activity in vitro. "Our results are conclusive," says Segal. "By taking out the proteases, we do not interfere with the respiratory burst, but do interfere with killing."



Segal/Macmillan

Neutrophils kill engulfed microbes (white) using proteases.

The proteases are activated through increases in both pH and hypertonicity in the phagocytotic vacuoles. These changes arise when NADPH oxidase activity drives electrons into the vacuole. To compensate for the charge transfer across the membrane, K^+ also enters. The resulting hypertonic conditions release the cationic proteases from their bound state on an anionic proteoglycan matrix. K^+ entry also results in alkaline conditions that stimulate protease activity. The proteases are then free to bind to and digest the target microbes. ■

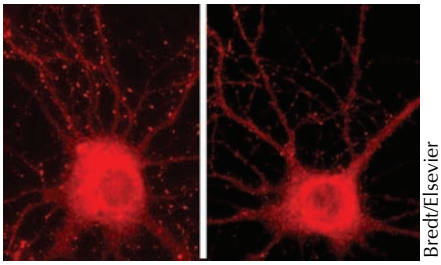
Reference: Reeves, E., et al. 2002. *Nature.* 416:291–297.

Learning by palmitoylation

Learning is probably driven by use-dependent changes in synaptic strength. Now, Alaa El-Husseini, David Bredt (University of California at San Francisco, San Francisco, CA), and colleagues have identified regulated protein palmitoylation as one means to control the strength of synapse responses.

Synaptic activity is controlled by AMPA channels, which respond to the neurotransmitter glutamate by letting in Na^+ until the neuron fires. Ca^{2+} influx through NMDA receptors (also activated by glutamate) alters synaptic responses by influencing the number of AMPA receptors clustered at the synapse.

It now appears that protein palmitoylation is key to this modulation. The localization of AMPA receptors is controlled by the PDZ domain protein PSD-95, which also clusters at synapses and binds to the AMPA-trafficking protein stargazin. The clustering of PSD-95 is known to require its palmitoylation. Now, Bredt and colleagues demonstrate that increased glutamate receptor activity accelerates depalmitoylation of PSD-95 and that depalmitoylation causes endocytosis and dispersion of both PSD-95 and AMPA receptors, leading to depression of synaptic strength.



Bredt/Elsevier

Clusters of AMPA receptors (red spots) disperse when PSD-95 palmitoylation is blocked (right).

"This defines a mechanism for activity-dependent changes in AMPA receptor numbers and defines protein palmitoylation as a new signaling mechanism at synapses," says Bredt. The enzymes that add and remove PSD-95 palmitoyl groups have not been identified, but Bredt speculates that they will be Ca^{2+} regulated, as Ca^{2+} entry into the cell (e.g., through NMDA receptors) is required for AMPA dispersion. ■

Reference: El-Husseini, A., et al. 2002. *Cell.* 108:849–863.

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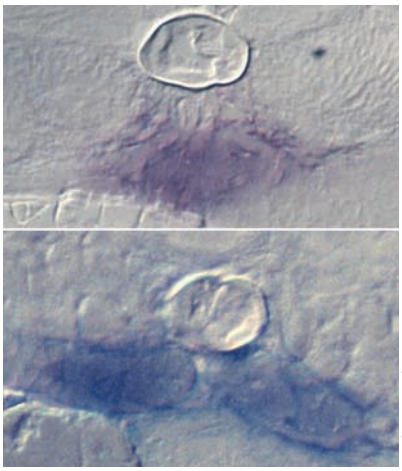
Pressured into making a kidney

Developmental biologists spend a great deal of time studying molecular signals that determine the when and where of organogenesis. But recent results indicate they may be overlooking something. Mechanical forces provided by the vasculature are also at work, according to a new study by Fabrizio Serluca, Ian Drummond, and Mark Fishman (Harvard Medical School, Boston, MA), who examine the development of the zebrafish kidney glomerulus.



Fishman/Elsevier

MMP-2 blockage (bottom) stops glomerulus formation by podocytes (blue).

The glomerulus is made up of unique layers of vessel endothelium that filter fluid from the blood without removing proteins or cells. Fluid flows between the endothelial cells, before moving sequentially through the basement membrane of the kidney and epithelial cells known as podocytes, and finally into the tubules of the kidney. During zebrafish embryogenesis, the podocytes form the glomerulus by migrating and coalescing to an area surrounding a vessel outgrowth of the aorta.

Now, it seems that mechanical forces from these vessel endothelium cells initiate podocyte cell migration. Fishman's group identified multiple mutants with disrupted glomerular assembly, each of which shares the common feature of impaired circulation. The group showed that hemodynamic forces from the aorta are essential for glomerular development by stimulating podocyte migration. "Cell fate in the mutants is normal; only organogenesis is affected," says Fishman. "The cells just don't get together as they should."

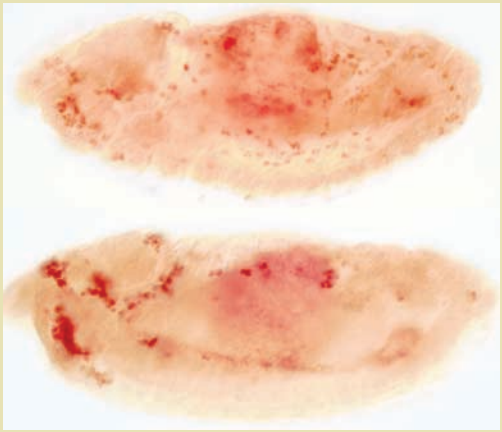
This force-initiated podocyte migration requires the collagen-degrading enzyme matrix metalloproteinase-2 (MMP-2). MMP-2 may be turned on by stretch-sensitive ion channels or by some other stretch-sensitive signal transduction pathway. In turn, MMP-2 may allow the vessels to migrate more easily into the epithelium by disrupting extracellular collagen. Alternatively, MMP-2 may activate matrix stores of inactive vessel-promoting signals. Homologues of the zebrafish signals may be involved in human disorders caused by hemodynamic forces, including atherosclerosis resulting from high blood pressure. ■

Reference: Serluca, F., et al. 2002. *Curr. Biol.* 12:492–497.

From migration to circulation

A pathway required for blood vessel development in vertebrates also functions in flies, according to new results from Nam Cho, Mark Krasnow (Stanford University, Stanford, CA), and colleagues. The results imply that cell migration, not angiogenesis, may have been the original function of the vascular endothelial growth factor receptor (VEGFR) tyrosine kinase family and its ligands.

The active migration of blood cells, or hemocytes, occurs during fly embryogenesis when the heart has not yet begun pumping. Fly blood does not travel through a closed circulatory system, but Cho found that flies have homologues of the VEGF pathway, which is required for vertebrate blood vessel development. This suggests that the proteins have a more ancient function.



Fishman/Elsevier

Blood cells (red) do not migrate when VEGFR is missing (bottom).

That function appears to be the migration of blood cells during embryogenesis, according to the new study. Cho and colleagues found that, in flies lacking VEGFR, blood cells differentiate, but do not migrate normally and aggregate in the anterior of the embryo. Inactivation of all three homologues of the VEGF ligand resulted in the same cell migration defect. Ectopic expression of a VEGF ligand caused misrouting of blood cells, suggesting that it may act as a chemoattractant to assure that the hemocytes travel along pathways where phagocytosis of apoptotic cells is required.

Blood cell chemoattractant activity has also been demonstrated in vitro for a vertebrate VEGF, but has largely been ignored. "While most of the press for VEGF involves its role in angiogenesis…it may have originally evolved for blood cell functions," says Cho. However, it is not known whether the vertebrate homologues still serve similar functions in blood cells. The simple genetics of the fly system should facilitate the identification of signaling molecules downstream of VEGF, which may aid in the discovery of therapeutic agents for blocking angiogenesis during tumor growth or increasing vascular growth after injuries such as heart attack. ■

Reference: Cho, N., et al. 2002. *Cell.* 108:865–876.