Research Roundup

Clonal diversity by asymmetry

When challenged by an intruder, the immune system generates a bewildering array of different T lymphocyte subsets. Cells in functionally distinct subsets somehow share specificity for an identical antigen. A mechanism for generating this clonal diversity is now explained by John Chang, Vikram Palanivel, Steven Reiner (University of Pennsylvania, Philadelphia, PA), and colleagues.

The subsets, say the researchers, arise from the first, asymmetric division that a naive T cell makes after being stimulated by antigen. The source of the asymmetry is the immune synapse—the connection of the naive T cell to the antigen-presenting cell (APC) that is stimulating it. As the T cell divides, the synapse-proximal T cell becomes an effector cell responsible for immediate fighting, whereas the synapse-distal cell becomes a memory T cell.

The model stands in contrast to two main theories that have been used to explain the generation of effector and memory T cells. Some researchers think that early visitors to APCs become effectors, whereas late visitors to the identical APC become memory cells. Other immunologists believe that effector cells develop first and then sometimes become memory cells later in life.

These models, says Reiner, "are noneconomical, nonparsimonious. We were very dissatisfied with the models."

There were also hints that something else was going on. T cell differentiation was known to require stimulation, a pause, and then more stimulation—a process termed "priming." Perhaps, thought Reiner, the pause arose because cells being stimulated did not themselves become differentiated subtypes of T cells but had to divide first. He gained evidence for this obligate division theory but did not know exactly why the division was needed.

The other clue was that stimulation of T cells never produced a clean population of just one subset of T cells, no matter what cocktail of cytokines was used to coax the cells in a single direction. "We as a field swept that under the carpet, just like we swept the delay under the carpet," says Reiner. "If you stimulate a T cell, it seems to have a complex fate."

The breakthrough came when researchers took a closer look—literally. "The imaging field really gave us the epiphany of how it could be deterministic," says Reiner. When researchers watched T cells roaming around lymph nodes, they saw that T cells initially bounced on and off APCs. But after 8–10 hours, just when the T cell is committing to cell division, it laid itself down on and made prolonged contact with an APC. There were conflicting claims about whether the contact lasted until the T cell divided, but that distinction "probably doesn't matter that much," says Reiner. "As long as the polarity is set up, it can persist through the division."

"The hard part was trying to prove it," says Reiner. "It worked miserably in vitro." After a wasted year, Reiner and colleagues tried an in vivo system instead. They labeled T cells and injected them into immunized mice. After a pause to allow for activation, T cells that had not yet divided could be spotted based on their full (rather than halved) level of fluorescence.

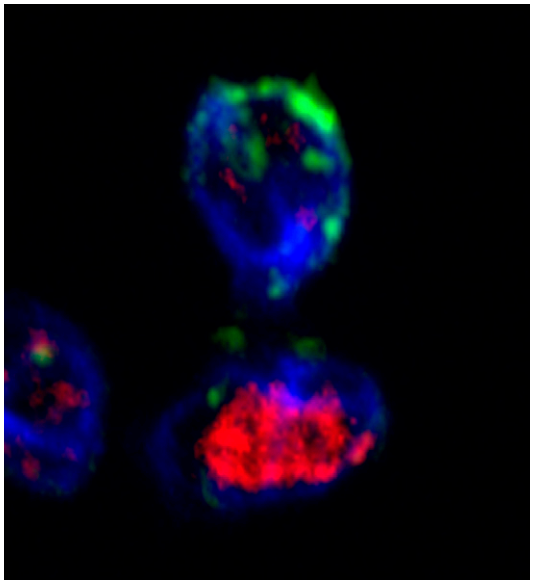
These cells had a whole host of polarity and effector cell determinants on one side of the cell, next to the immune synapse, and other polarity and memory cell determinants on the other side. These localizations were maintained through the first mitosis of the transferred T cells. Sorting the cells based on these markers revealed that the two resulting cell types had bona-fide effector and memory functions, respectively.

By roping in the synapse, explains Reiner, a mobile cell can take advantage of asymmetry pathways that are commonly used by stationary cells. For researchers studying mobile immune cells, "our paradigm for signaling was very prokaryotic—a cell responds uniformly to a signal from outside," says Reiner. "But lymphocytes should know how to diversify a division."

A single antigen-stimulated T cell can create two daughters with two fates as long as it doesn't differentiate before the cell division. And that may not be the end of the story. Some T cells make repeated visits to APCs. Each visit may repolarize the cell and lead to a division that further doubles the lineage's functional diversity.

Reiner admits that some diversity may rely on maturation rather than asymmetry pathways. But the logic of the asymmetry pathway is certainly appealing. "Many people have been pretty blown away by it," he says. He is busy studying how long the asymmetry persists after the first division, but is also enjoying the initial discovery. "I can't imagine topping this one," he says. "It's been fun." JCB

Reference: Chang, J., et al. 2007. *Science*. doi:10.1126/science.1139393.



REINER/AAAS

Polarity and fate markers (red and green) segregate during the first division of stimulated T cells.

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Destroying the messenger

An E2 ubiquitination enzyme is meant to shuffle ubiquitins through its active site and on to substrates so that the substrates are marked for destruction. But a polyubiquitin chain on the active site of Ubc7 can result in the downfall of this E2, according to Tommer Ravid and Mark Hochstrasser (Yale University, New Haven, CT). The result gives clues about how polyubiquitin chains are built.

If Ubc7 strays away from its binding partner Cue1 on the yeast ER, it is destroyed. The Yale team found that this destruction required both polyubiquitination on the active site cysteine of Ubc7 and release from Cue1's grip. Similar in vitro evidence that polyubiquitin chains can form on an E2 active site was recently presented by Li et al. (*Nature*. 2007. doi:10.1038/nature05542).

The reaction may work via a seesaw mechanism between dimeric E2s. In this model, one E2 receives first a single ubiquitin and then on top of that the entire growing polyubiquitin chain from the other E2. This frees up the active site of the second E2 to receive another single ubiquitin. Eventually the fully grown chain can be transferred to another substrate.

The seesaw model contrasts with the original model of sequential addition. In the sequential model, it was not clear how the enzyme would reach out to the distant end of a substrate's growing ubiquitin chain to add additional ubiquitins. JCB

Reference: Ravid, T., and M. Hochstrasser. 2007. *Nat. Cell Biol.* doi:10.1038/ncb1558.

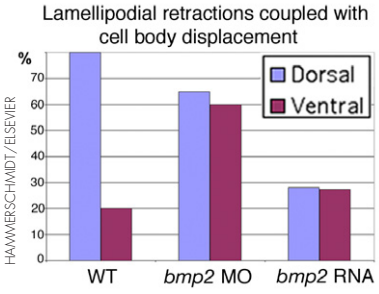
Migrating toward adhesion

A morphogen can determine the direction of cell movement by creating an adhesion gradient, according to Sophia von der Hardt, Matthias Hammerschmidt (Max-Planck, Freiburg, Germany), and colleagues.

The bone morphogenetic proteins (Bmps) are better known as factors that determine cell fate decisions. Bmps appear to affect migration, but this might have been a side-effect of changes in cell fate.

The German group therefore implanted a Bmp-containing bead on the opposite side of a fish embryo from Bmp's normal source. Cells responded by moving away from the bead. Bmp receptors were needed not in the migrating cells but in the surrounding cells on which they migrated. This suggested that the surrounding cells might be creating a gradient of adhesion that was guiding the migrating cells.

Sure enough, the migrating cells showed equal numbers of protrusions at the front and back, but only the protrusions facing away from a Bmp source were able to grab on securely enough to pull the cell body forward.



Based on an adhesion gradient, dorsal but not ventral retractions are productive.

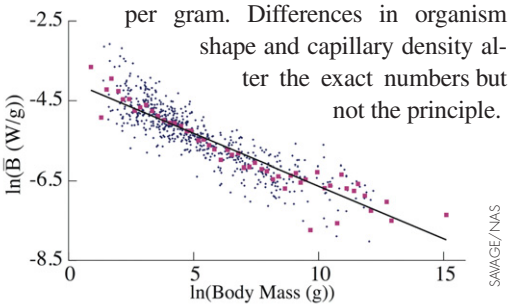
Other developmental pathways also regulate adhesion. This has been presumed to affect cell survival or cohesion of migrating masses of cells, but the creation of an adhesion gradient is another possibility. JCB

Reference: von der Hardt, S., et al. 2007. *Curr. Biol.* doi:10.1016/j.cub.2007.02.013.

Big mammals have big (or slow) cells

Cells use two basic strategies to adapt to the size of the organism in which they reside, say Van Savage (Harvard Medical School, Boston, MA), Geoffrey West (Santa Fe Institute, Santa Fe, NM), and colleagues. Depending on how often they divide, comparable cells in a mouse and an elephant differ in either metabolic rate or cell volume, but usually not both.

The need for such adaptation stems from simple geometry. As body volume increases, surface area increases more slowly. So an elephant radiates and loses less energy per gram than a mouse and thus requires less replacement energy per gram. Differences in organism shape and capillary density alter the exact numbers but not the principle.



Bigger animals have lower metabolic rates (B).

Thus, what Savage and others call the "cell is a cell is a cell" theory cannot hold. With energy consumed per unit volume decreasing with increasing animal size, average cell volume and average cellular metabolic rate cannot both remain constant.

There are at least two possible solutions. Under theory one, average cell volume stays constant but each cell in the larger organism consumes less energy. Theory two keeps energy consumption per cell constant but the cells in the larger organism are larger so that there are fewer of them per unit volume.

Digging through the literature, the researchers found that rapidly dividing cell types were a close fit to theory one. Slower metabolism in these cells in larger organisms may explain why these animals accumulate damage and age more slowly.

Cells such as neurons and adipocytes, however, divide infrequently and must maintain their structural integrity using a constant energy supply. Their variation fit theory two.

The findings reflect the extent to which organisms also affect cells, says Savage. "For a cell type to exist in an organism it has to adapt to an organism," he says. He plans to study the phenomenon in yeast that can be manipulated to grow at different sizes and metabolic rates. JCB

Reference: Savage, V.M., et al. 2007. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0611235104.