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.

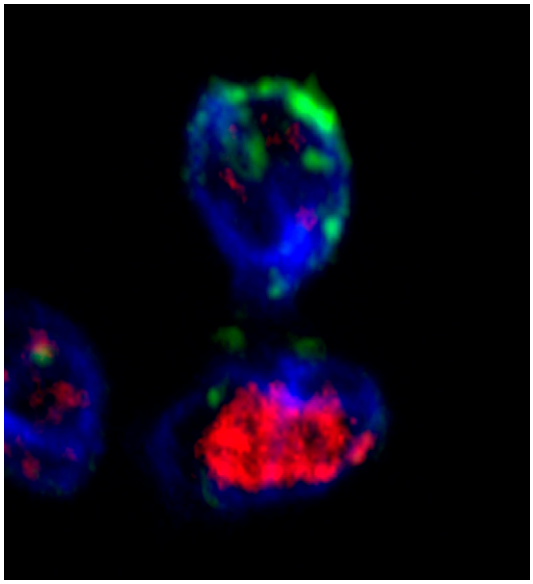
These cells had a whole host of polarity and effector cell determinants on one side of the cell, next to the immune synapse, and other polarity and memory cell determinants on the other side. These localizations were maintained through the first mitosis of the transferred T cells. Sorting the cells based 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.