Out with the bad:

Studying autophagy to fight infectious disease

By Leah Shaffer

Herbert (Skip) Virgin in his lab.

Cells, just like people, could sometimes use help taking out the garbage. This idea is the basis for a movement in infectious-disease research that hopes to exploit a cellular process known as ‘autophagy,’ a process by which cells recycle needed nutrients, to eliminate dangerous pathogens from the body.

During autophagy, double-membrane vesicles called autophagosomes encircle matter inside the cell and fuse with a waste-disposing organelle called a lysosome to break up its cargo. This “garbage disposal is pretty important,” says microbiologist Beth Levine, who studies autophagy at the University of Texas Southwestern. Cell ‘garbage’ in this case can include anything from old organelles and decaying bits of cell matter to—importantly—pathogens.

In recent years, the role of autophagy in immunity has become of great interest. In 2013, Levine published results¹ showing how autophagy could be enhanced to treat viruses such as West Nile. In the study, Levine’s team first created an autophagy-enhancing peptide called Tat-beclin 1 from a subset of amino acids found in an autophagy-related protein called beclin 1, and then administered it to mice. They found that muscle cells in mice receiving Tat-beclin 1 contained an increased number of

autophagosomes, as compared to those in mice injected with a control peptide.

In an attempt to understand how Tat-beclin 1 may induce autophagy, Levine’s team searched for proteins that are bound by Tat-beclin 1, and found an autophagy inhibitor called GAPR1 (also known as GLIPR2). The researchers think that by binding GAPR1, Tat-beclin 1 frees up beclin 1 to increase autophagosome formation.

In the same study, the scientists then looked at how Tat-beclin 1 would affect mice infected with West Nile virus and found viral concentrations in nerve cells declined dramatically within six days of treatment. Moreover, 20% of Tat-beclin 1-treated mice survived the infection, whereas none of those in the control group did. The team extended their study to other viruses and found that Tat-beclin 1 reduced mortality of neonatal mice infected with chikungunya. The peptide also inhibited the replication of HIV in human white blood cells, and decreased intracellular survival of a listeriosis-causing bacterium in bone-marrow derived white blood cells in mice. The work thus suggested that an autophagy-enhancing drug could potentially help to treat infections caused by a variety of viruses and bacteria.

Expanding our knowledge of autophagy-related genes could also play a part in addressing the rising threat of Zika virus, which is being

studied for a possible link to a birth abnormality known as microcephaly, in which the head is unusually small. Zika belongs to the same family of viruses as West Nile and dengue, and beclin 1 seems to have a role in both autophagy and the stabilization of cell organelles called centrosomes, which help to coordinate healthy neural development².

As such viruses continue to emerge, and as the threat of antibiotic-resistant bacteria grows, some researchers are beginning to view autophagy enhancement as a potent tool for fending off infectious disease. The US National Institutes of Health (NIH) has lent its support to the exploration of this notion. Two years ago, Levine’s university, along with the Broad Institute and Massachusetts General Hospital, began collaborating with the Washington University School of Medicine, a Centers of Excellence for Translational Research (CETR) awardee. The NIH tasked the group with finding other autophagy enhancers in addition to Tat-beclin 1. Since then, scientists involved in the NIH-supported endeavor have discovered new properties of and genes related to the autophagy pathway that could change how we fight many diseases—but findings published in December on tuberculosis suggest that these efforts might be more complicated than initially expected.

Beyond beclin 1

The term autophagy, which means ‘self-eating’ in Greek, was coined at a conference in 1963 by Christian de Duve, one of the scientists who first studied how lysosomes degrade cell debris³. In the subsequent years, most researchers viewed autophagy as a basic metabolic process. The first autophagy-related genes were discovered in experiments with starved yeast in the 1990s⁴. Autophagy is an old, well-conserved cellular pathway, which means that it has had many years for its underlying genes to develop into multi-taskers that do not just handle processes of cell metabolism, such as recycling cell bits during times of starvation, but also serve a role in cellular defense mechanisms. Autophagy is a “primordial defense mechanism,” says Washington University School of Medicine immunologist Herbert Virgin. “There’s an evolutionary battle of wills where the pathogen is blocking the pathway, and the pathway is blocking the pathogen.”

When a cell needs fuel, it activates a process that brings cell material such as old mitochondria or ribosomes to a lysosome, which then degrades these parts into needed nutrients, such as amino acids and essential proteins. But as it turns out, this is not an all-or-nothing process. The autophagy machinery can be selective in what bits of debris get designated for this fate. One of the main goals of the CETR’s work is to identify the pathways of autophagy that selectively target intracellular pathogens.

When seeking out ways to increase autophagy to fight pathogens, scientists’ biggest focus is to refine the Tat-beclin 1 peptide into a “drug-like molecule that can advance through preclinical development,” Levine says. But, she adds, they are also on the lookout for the ‘next’ Tat-beclin 1, by doing several genome-wide RNA-interference screens to identify autophagy proteins that respond to different viral infections. Tat-beclin 1 is a good general-autophagy enhancer, but these screens could lead researchers to a peptide that selectively targets specific types of pathogens even more effectively.

“In the past decade or so, there’s been an explosion of work on selective autophagy,” says Levine. Such research has drawn in scientists from a variety of backgrounds. Virgin, for instance, was chasing after the proteins that controlled interferon- γ , an immune signaler, when he found that some of the same genes also controlled autophagy.

He then developed mice with a depleted version of a core mammalian autophagy-related gene called *Apg16l1* (later changed to *Atg16l1*). This gene was first discovered⁵ in 2003, as the mammalian version of the yeast autophagy-related gene *Atg16*. The mutated gene expressed lower than normal levels

of its autophagy protein. At a conference a decade ago, Virgin met Ramnik Xavier, a gastroenterologist at Massachusetts General Hospital, who was studying the same autophagy gene and its role in Crohn’s disease⁶. The two started to collaborate. They found that mice with a mutated *Atg16l1* gene produce malfunctioning Paneth cells, which normally secrete a lysozyme to help limit and control the types of bacterial growth in the lining of the gut. The same mutation and abnormal Paneth cells can be found Crohn’s disease patients. “The study of autophagy is this amazing thing, where you start studying one thing and if you keep an open mind, you discover that there’s relationships between autophagy and a lot of different kinds of biology,” Virgin says.

Xavier now heads one branch of the CETR project, focusing on the genetic mechanisms behind selective bacterial autophagy. In unpublished work, he says, his team has been able to take advantage of the link between Crohn’s disease and autophagy by using the disorder as way to screen for even more autophagy-related genes.

Tuberculosis twist

Even in selective autophagy, the process can get more selective still. Certain genes within the autophagy pathway function independently from other autophagy-related genes, says Virgin, principal investigator for the NIH’s CETR project. “So you’ve got different parts or cassettes of the autophagy machinery being involved in different processes,” Virgin explains.

For the past decade, scientists have thought that some form of selective autophagy is required to control the replication of the tuberculosis-causing bacterium, *Mycobacterium tuberculosis*, inside macrophages, or white blood cells. The disease has been connected to 1.5 million annual deaths worldwide, so there is an immense incentive to find a way of exploiting autophagy to fight the infection.

In previous studies, scientists assumed that autophagy had a crucial role in tuberculosis regulation, because when they deleted an autophagy-related gene called *Atg5*, mice became very susceptible to the bacterium. But new findings published in December suggest that simply enhancing bulk autophagy may not work to fight tuberculosis⁷. In their deep dive into the autophagy machinery, Virgin and Washington University Medical School microbiologist Christina Stallings found an entirely new pathway through which the inflammation caused by tuberculosis is controlled.

The protein that *Atg5* encodes, ATG5, seems to have a role in the initial formation and elongation of pre-autophagosomal structures that fuse together to form an autophagosome

membrane, according to Stallings. She decided to test how mice would fare against tuberculosis when other autophagy-related genes besides *Atg5* were deleted. The mice were barely affected—only the deletion of *Atg5* caused the mice to succumb to the disease, mostly because the immune system overstimulated the inflammatory response to the invading bacterium. The data suggest that the role of ATG5 is not to reduce bacterial replication, but instead to tamp down inflammation, Stallings says. This also meant that it was not autophagy doing the work to control tuberculosis, “because if autophagy was required, then all those genes [beyond *Atg5*] would be necessary,” she adds.

Instead, the group had stumbled onto a new immune-system pathway, or some offshoot of autophagy, that has taken on a role in immune function, she says. Targeting or finding a way to enhance the function of ATG5 could be a therapeutic option for tuberculosis.

The new findings suggest that exploiting autophagy directly to combat tuberculosis might not work as hoped, but there remains great enthusiasm among scientists to learn more about this cellular process for other infections. Just because ATG5 might be involved in another pathway does not mean that scientists discount the role of canonical autophagy to fight disease, according to Stallings. The next step is to explore how or whether *M. tuberculosis* might be blocking autophagy. And the enthusiasm extends to areas beyond infectious disease: some scientists have put forward some autophagy related proteins as potentially druggable targets in autophagy for diseases such as cancer. In that case, they’re looking for ways to inhibit instead of enhance autophagy.⁸

Researchers have three more years of CETR funding to further dissect the role of autophagy in infectious disease. The “blue-sky” scenario would be to find a compound that could potentially work for viral, bacterial and parasitic infections, Virgin says. He concedes that not everyone thinks that this could be possible—but he remains steadfast. “If it was easy to do, someone would already have done this.”

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