A Pas de Deux

Stuffy nose, body aches, chills, fever, we all know what this means… the flu. What you might not know is that it's not the virus making you feel lousy, it's your own body's defenses. Thankfully, these symptoms don't last forever. They die down as the infection wanes and your body recovers, or so it seems. The body's defense and its recovery are not two distinct performances; they're one routine performed throughout the infection.

After getting sick, your lungs become a stage for a violent performance, the set torn apart by both the virus and your own body. The flu's goal is to make more of itself. To do so, it takes over the epithelial cells of the lung. These cells are crucial, as they form the barrier separating your body from the outside world. Infection of the epithelial barrier results in death for these cells, breakdown of the barrier they make up, and the release of more virus into the lung. This chaos, however, does not go unchecked. It is sensed by your body's alarm systems and results in all cells close to the infected area deploying their leading virus-fighting cast of proteins: interferons (IFNs).

Like many proteins, IFNs are named after their function: they "interfere" with the infection. Their disruption of flu's choreography takes many shapes. IFNs prevent the virus from multiplying, they call immune cells to the infected area, and they shut down the growth-and-repair machinery of the cell, keeping it out of the flu's reach. This last function is the most established, yet the most paradoxical. When flu kills lung epithelial cells, it directly tears holes in the barrier keeping the infection out from the rest of your body. If those holes could never be repaired during an active infection, then even the mildest flu would turn into a tragedy. At first glance the lung seems caught between a rock and a hard place. Leave the cell's repair equipment running and the flu takes it over; shut it down with IFN and the wounds never close.

My thesis work finds that the cell does not have to make this choice. While it's true that sensing IFN does halt most of a cell's processes, it also sets the stage for the cell to become harder to infect by turning on internal anti-viral defenses. To do this it employs a range of intracellular protein messengers including members of the STAT family. Using flow cytometry, a technique that allows me to quantify the relative amounts of select proteins across hundreds of thousands of cells at once, I peered into the epithelial cells actively responding to IFN in the lungs of flu infected mice and unsurprisingly found them full of STATs. I was not expecting to find those cells proliferating. This ran counter to what was accepted as true in the field: sensing of IFN halts proliferation. My observation instead suggested a more nuanced performance and led me to a hypothesis: in preparing themselves to stand against the virus, epithelial cells that sense IFN build up intracellular messengers capable of both turning on antiviral defenses and of poising them to proliferate upon receiving a compatible cue. But where could this cue be coming from? Who is the epithelial cell's partner?

In addition to the immune cells called to the stage by IFN, the lung houses troupes of its own: chief among them, alveolar macrophages (AMs). Constantly exposed to the outside world, these cells serve as the lung's resident sentinels, patrolling for the first sign of trouble and keeping the peace. Earlier work shrugged them off as additional casualties of an infection. My work showed otherwise. By using genetically modified mice whose recruited immune cells glow with a fluorescent tag, I found that the original residents largely remain in the lung. Instead of dying, AMs adopt a guise like that of their recruited counterparts, but lack the fluorescent tag, revealing their true nature to the audience. I

theorized that if AMs change the way they look, and remain within the infected lungs, they could be playing a role in repair. To test this, I banished them using antibodies instilled into the lungs. When AMs were gone, I saw that epithelial cells failed to perform their proliferative routine in response to IFN. The epithelial cell's partner was thus cast as the alveolar macrophage.

Previously, we had shown that mice could not survive a mild flu infection in the absence of Oncostatin M (OSM; Hoagland et al., Science, 2025), a secreted signaling protein exclusively made by immune cells. Crucially, other work in the field had also observed OSM primarily signals through STAT proteins, the same intracellular messengers induced by IFN. This prior work led me to hypothesize that OSM was perhaps the permissive cue given by AMs to epithelial cells that allowed them to proliferate in the presence of IFN. To test this, I cast genetically modified mice whose macrophages lacked OSM and challenged them. This time, I found that, even if the macrophages were physically present, the epithelial cells could not perform their proliferative routine without macrophage-derived OSM. The permissive cue the epithelial cells had been waiting for had been found.

If the repair of the epithelial barrier runs on the same antiviral intracellular protein messengers built up by IFN, then the IFN responsive cells, the ones housing the most STATs, should answer the loudest upon hearing their proliferative cue: OSM. I tested this theory out by using a variation on flow cytometry that physically sorts discrete populations for subsequent culture. I then performed an OSM dose-response experiment on IFN-responsive and naïve epithelial cells from the same mouse lungs. This experiment showed me that the difference between these two populations was not in how well they could "hear" the cue. Both populations took the stage at the same threshold. The difference was in how large of a response they were able to mount once they did. The cells with more STATs, those that had responded to IFN, mounted a response roughly twice as large. The STATs that IFN had cast for defense were also called to the stage for repair.

With their macrophage partners providing their cue, the IFN responsive epithelial cells left waiting in the wings finally enact their routine, center stage. In this pas de deux, epithelial cells and macrophages link the body's recovery to its defense in one unified routine. When a step is missed, repair lags, and recovery does not happen. An infection the body should have survived turns deadly. By continuing to learn the choreography of repair in this way, we'll do more than recognize the steps we've missed. We'll improvise, recover, and receive a standing ovation with every rescued performance.

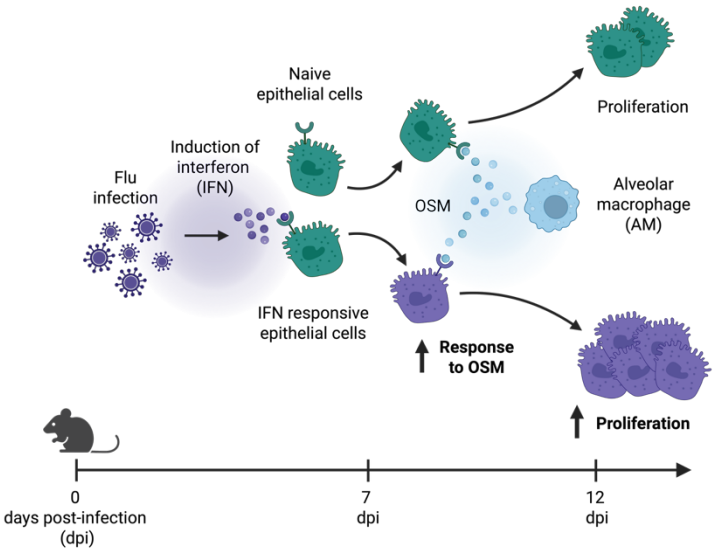

Figure 1. Interferon (IFN) primes alveolar epithelial cells to receive reparative signals from macrophages. Infection with the flu results in the induction of IFN. IFN is then sensed by local epithelial barrier cells who adopt an IFN responsive state. This state can mount a greater response to alveolar macrophage (AM)-derived Oncostatin M (OSM). This results in a larger subsequent proliferative response in the epithelial cells that experienced IFN vs. their naïve counterparts.