Antibodies not needed

By Lev Osherovich, Senior Writer

Italian and American researchers have mouse data showing that the most important function of B cells in fighting some viral infections is not making antibodies but rather stimulating an innate immune response led by macrophages.[1] The findings could point to new vaccine adjuvant strategies for viruses such as rabies and West Nile, assuming the mouse data translate into humans.

A team co-led by Matteo Iannacone and Ulrich von Andrian made the discovery while studying the early steps in the immune response to vesicular stomatitis virus (VSV), a veterinary pathogen that serves as a model for neurotropic viruses.

Iannacone is group leader in the division of immunology at the **San Raffaele Scientific Institute**. Von Andrian is professor of immunopathology at **Harvard Medical School**.

Iannacone said the researchers were trying to understand the precise sequence of immunological events in VSV infections, in which the virus gravitates to the CNS if unchecked by a combination of innate and adaptive immune responses in the lymph nodes.

In a previous study the team showed a successful immune response against VSV required macrophages, a type of innate immune cell.[2] Ordinarily, macrophages in the lymph nodes ingest viral particles, deliberately become infected by the virus and then launch the type I interferon (IFN) response that turns on other immune cells and helps neurons ward off the infection.

"In our previous paper, we showed that the macrophages in the lymph nodes capture the virus," said von Andrian. "We depleted those macrophages and infected the mice, which caused the mice to die from CNS penetration of the virus."

Von Andrian noted that a particularly intriguing finding from the paper was that macrophage-deficient mice often had high titers of antibodies against VSV but died nonetheless. Based on this, the team suspected that "making the antibodies is not sufficient for protection," said von Andrian. "We thus asked whether the antibodies were even necessary."

Ain't got no (anti)body

Von Andrian and Iannacone began their new study by confirming prior observations by other researchers that mice engineered to lack B cells succumbed to VSV infection while similarly treated wild-type controls survived.

The surprise came when the team repeated the experiment using a mouse strain engineered with B cells that were unable to splice antibody genes but were otherwise functional. Those mice proved as resistant to VSV as wild-type controls despite having no detectable antibodies.

VSV-infected mice with antibody-deficient B cells had levels of lymph node macrophage activation and type I IFN response similar to those in wild-type controls.

"We found that it wasn't the antibodies that were needed," said Iannacone.

The next step was finding out what the B cells were making that was really needed to combat the virus. The researchers suspected the key was lymphotoxins, a family of cytokines that promote macrophage proliferation in lymph nodes.

Indeed, mice treated with a decoy receptor that binds and inactivates lymphotoxin-α (Lta) and Ltb (p33) had lower macrophage activation and type I IFN response than untreated controls and succumbed to VSV infection.

Altogether, the findings suggest uptake and infection of macrophages by VSV is an essential step in launching an innate immune response against the virus. B cells help this process by secreting lymphotoxins, which help the macrophages take up and replicate the virus, resulting in a protective type I IFN response.

Results were reported in *Immunity* and were not patented.

Adjuvant opportunity

Von Andrian and Iannacone's findings challenge the notion that B cells are purely antibody factories. The key question is whether, as in mice, the human immune system uses B cell–derived lymphotoxins to increase antiviral immunity.

"This paper shows that there is a non–antibody-mediated effector mechanism" for B cells, said Patrick Iversen, SVP of research and innovation at **AVI BioPharma Inc.** "The vaccine world may now understand that antibodies aren't the only important thing."

AVI has antiviral oligonucleotides in Phase I testing for Ebola virus and Marburg virus.

"This is somewhat of a novel finding," said Jim Tartaglia, VP and new vaccine project head in North America at **Sanofi**'s Sanofi Pasteur vaccine division. "The results are intriguing and worth following up to understand the relevance in other viruses and human pathogens."

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> ***—Matteo Iannacone, San Raffaele Scientific Institute***

Tartaglia cautioned that many features of the mouse and human immune systems differ, so it will be necessary to replicate the findings in cell culture or mouse models of human viral infection.

Iannacone and von Andrian agreed but said first efforts are to repeat the VSV experiments in mice with humanized immune systems.

Iannacone suspects lymphotoxins could be used to improve immunity to human viruses that resemble VSV, such as rabies virus or West Nile virus. He noted that although "there is a vaccine for rabies that works beautifully," timely delivery of that therapy—a combination of neutralizing antibodies and a prophylactic vaccine—is a challenge.

"VSV doesn't infect humans, but it's in the same family as rabies, so we think [these findings] could apply to rabies," said Iannacone. "This study might suggest that passive prophylaxis for rabies could be boosted with lymphotoxins."

Von Andrian wants to determine whether lymphotoxins also contribute to the immune response to influenza or HIV, which do not infect the CNS but do infect macrophages. For those viruses, it's unclear whether macrophage infection ultimately helps or harms the immune response.

Tartaglia thinks the lymphotoxin mechanism described by von Andrian and Iannacone may be relevant to other viral infections but wants to see more data.

"I see this [mechanism] as being potentially useful for broader vaccine development, but I couldn't say right now for which vaccines because I'd like to see it replicated in human immune cells," said Tartaglia.

One concern about using lymphotoxins as adjuvants is the proteins' proinflammatory effects. For example, treating patients with lymphotoxins runs the risk of triggering autoimmunity. If so, further study of the downstream pathways activated by lymphotoxins could help identify ways to separate the antiviral and autoimmune effects.

Rather than using lymphotoxins themselves, Tartaglia said that it may "make sense to develop small molecules that trigger desirable antiviral effects as part of an adjuvant formulation or to engineer an immunogen that triggers these mechanisms" without eliciting full-blown autoimmunity.

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