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Puzzling over privilege:

How the immune system protects—and fails—the testes

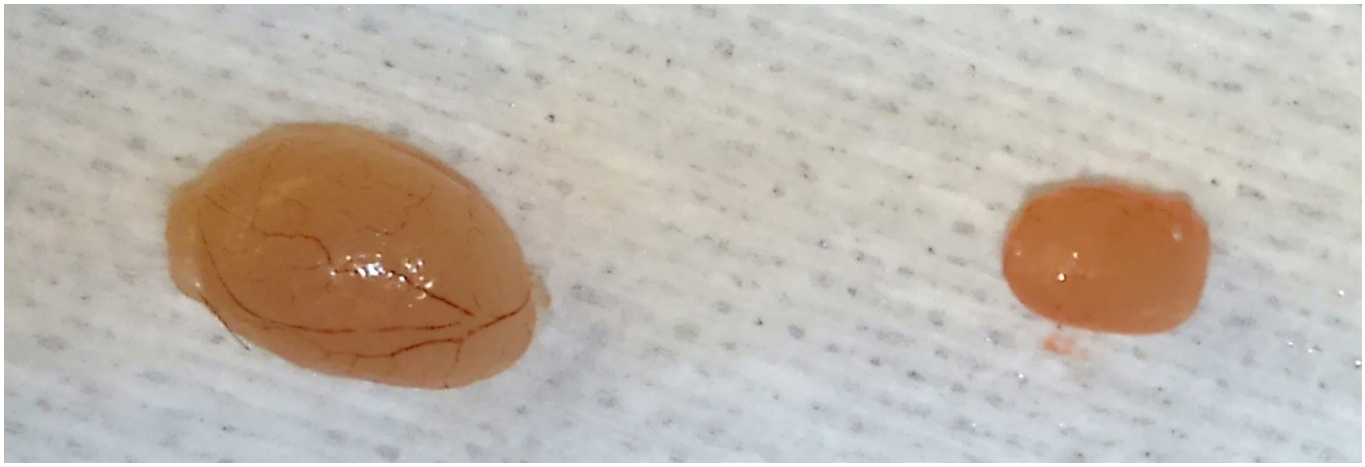
By Shraddha Chakradhar

It all started about two years ago, when a patient of Jean-Pierre Routy with HIV wanted to undergo a sex change. The individual was planning to go to GRS (for 'Gender Reassignment Surgery') Montreal, a center that facilitates over 400 such surgeries a year. At the time, it was the only center in Canada to offer the procedure, also known as gender affirmation surgery. According to Routy, an infectious disease clinician and researcher at McGill University in Montreal, getting human testicular tissue for study is extremely difficult, so after he received consent from the individual to obtain samples of the man's discarded testes for research once they were removed during surgery, Routy reached out to GRS Montreal. "I was probably the first person to ask GRS to share tissue," Routy says. The tissue from the patient was delivered to his lab, and since then, Routy has received close to 100 such testicular tissue samples from GRS Montreal, of which a tenth have come specifically from people who are HIV positive.

Routy's main research focus is on how and why viruses like HIV persist in the body. With the testicular tissue from the HIV-positive man, he was hoping to get closer to understanding this persistence. Testes are what are known as an 'immune-privileged' site, which allows sperm cells within testicles to be protected from the body's immune system. Most cells in the body are recognized by the immune system as 'self,' but mature sperm are a notable exception. Since sperm cells don't mature until puberty, the immune system doesn't recognize sperm as 'self,' but rather as a foreign agent. Immune privilege prevents molecular triggers, called antigens, expressed by sperm from inciting an autoimmune attack against them. Some 20% of male infertility is thought to be a result of a breakdown of immune privilege, in which sperm cells are attacked and destroyed by the body.

On the flip side, immune privilege allows foreign invaders, including viruses, to find protection from the immune system within the testes. This is because antigens from invaders also do not seem to set off an inflammatory response, unlike elsewhere in the body. Routy and his team observed this to be the case when they examined testicular tissue and blood samples of six patients who were HIV positive and receiving antiretroviral therapy. All six, despite being treated with anti-HIV medication with such success that the virus was undetectable in their blood, had lingering viral DNA in at least one testicle[1]. The result indicated the virus could evade drug treatment by finding a home in the testes.

Exactly how HIV and other viruses manage to gain entry into the testes and stay, often for years on end, without being detected by the immune system is still being worked out by scientists. The recent Zika and Ebola epidemics brought further urgency to the issue of immune privilege. Reports emerged of patients who had these viruses in their semen



Ryuta Uraki

Sizable difference: An uninfected mouse testis (left) and a drastically shrunk Zika-infected testis (right).

months after they were originally infected even though the viruses had been cleared from elsewhere in their bodies. In one of the most extreme cases, Zika virus was detected in the semen of a man in Italy at least 134 days after symptoms of the disease (mainly a fever) first emerged, even though blood and saliva samples showed no trace of the virus. A semen test done at day 188 still showed the presence of virus (his blood and saliva weren't tested).

The testes seem to be a particularly understudied area among immune-privileged sites, which also include the eye and the brain. Human testes are less readily available than other tissue types donated to research, making it tough to validate findings in animal studies. But the dedicated few scientists in the field working with testicular tissue say that the reach of insights gleaned from studying infectious diseases that affect the testes could also help treat diseases affecting other organs, including cancer and diabetes. For now, though, much of the focus is on viruses. "If we don't learn how [viruses] persist in the testes, then patients will continue to transmit the virus to others and put people at risk," Routy says.

Immune insight

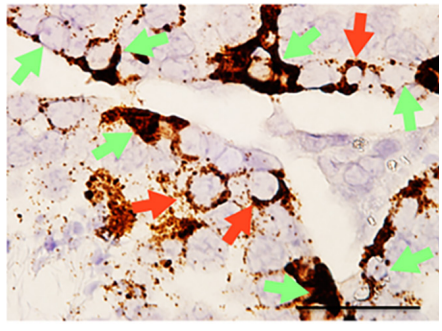
The Dutch scientist J.C. van Dooremaal first observed in the 1870s that mouse skin grafted onto the eyes of dogs was not rejected and managed to survive for a long time. However, it was not until the 1940s that the British transplant scientist Sir Peter Medawar coined the term 'immune privilege'. Medawar, who studied immunity needed for transplantation, realized that skin grafts transplanted into the area between the iris and cornea in the eyes of rabbits survived longer without rejection than those grafted onto other sites in the body[2]. In the several decades following Medawar's report, people thought that physical barriers, such as the blood–brain barrier, the blood–retina barrier or the blood–testis barrier, made immune privilege possible. The concept of immune privilege has evolved in this century to where scientists now seem to agree that not only are the eyes, testes and brain physically sequestered, but immune cells found at these sites also don't function in the same manner as they do elsewhere in the body. Even still, there is mounting evidence to suggest that cells that form the blood-testis barrier, for instance, secrete factors that suppress immunity to keep immune privilege in check[3].

Not all immune-privileged sites seem equal, at least when it comes to research. Compared to the brain and eyes, "our understanding of the immune mechanisms in testes is very limited," says Saguna Verma, an infectious disease researcher at the University of Hawaii in Honolulu. The brain and eyes have their own set of unique characteristics, but the testes set themselves apart in other ways. Testicles are the only gland to hang outside the body, and are about two degrees cooler than the rest of the body (higher temperatures kill off sperm).

Already, the Zika and Ebola emergencies have broadened our understanding of how viruses use immune privilege to their advantage. For instance, in October 2017, Verma's team published results outlining that the long gatekeeper cells that protect sperm cells, known as Sertoli cells, succumb easily to Zika virus[4]. The tightly held space between Sertoli cells—known as the blood–testis barrier—keeps immune cells from reaching sperm. But Zika-infected Sertoli cells attracted cell-eating immune cells known as macrophages that destroyed them, thereby breaking the barrier. Verma speculates that a breakdown of the barrier could mean that Zika from elsewhere in the testes could get to sperm cells. In Ebola, macrophages seem to be a reservoir for virus in the tightly coiled tube, known as the epididymis, that is present on the outside of testes and helps transport sperm to the ejaculatory ducts[5].

The importance of macrophages is underscored by Routy's work with donated testes. His study of samples from 16 donors who underwent surgery at GRS suggests that, in testicular tissue, macrophages make up a whopping two-thirds of the immune cells known as myeloid cells[6]. Routy speculates that the only other organ to have as many macrophages may be the lungs, where they have the important duty of filtering out dust and microbes.

Zika also has a detrimental effect on testes and their function. Zika-infected mice experience damage to the tubules that are lined with Sertoli cells, called seminiferous tubules[7]. The virus has also been known to shrink testes[8]. Yale University scientists infected mice testes with Zika and found that three weeks after infection, the testicles were 10%–40% shorter and weighed between 20% and 85% less than the testes of uninfected mice. What's more, testosterone-producing Leydig cells, which lie adjacent to the seminiferous tubules in the testes and help maintain fertility, seemed to be a target and



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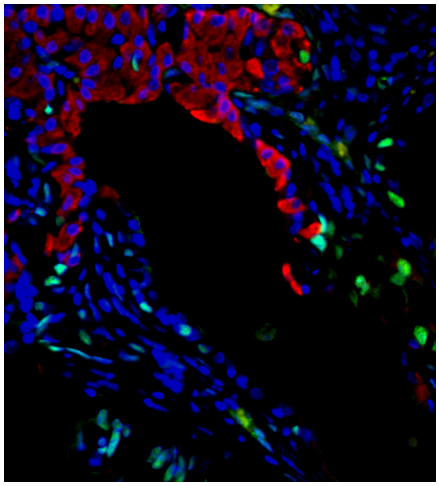
Viral load: Sertoli cells (green arrow) and germ cells (red arrow) infected with Zika (brown stains).



Gurvinder Kaur and Jannette Dufour.

Engineered solution: Sertoli cells (green) co-transplanted with insulin-producing islet cells (red).

potentially a reservoir for the virus. When male mice carrying Zika mated, they seemed to often infect their female mice partners, which produced fetuses that weighed between roughly 10% and 35% less than fetuses born of uninfected male mice[9].

The effects of immune privilege could have implications for diseases that manifest outside the testes. According to Routy, the pathways involved in protecting the testes are similar to those that allow tumors to escape the grips of the immune system[10]. "All the tools used by Mother Nature in the testes and eyes are similar to tools used in cancer," Routy says.

Routy adds that, historically, boys with leukemia were more likely than girls to experience a disease relapse because the cancer can hide in the testes. This gap has shrunk recently, he says, given the improvement in cancer treatment, but it raises an important issue about the use of immunotherapy to treat cancer. Immunotherapy has been a boon to cancer patients, but therapies like checkpoint blockade inhibitors could inadvertently break down immune privilege because the pathways that these drugs target are the same ones that help maintain immune privilege. "We have to be very vigilant," Routy says, of this potential concern as our reliance on immunotherapies increases. Using the testes he gets from GRS, "I want to learn about other ways to attack cancer," Routy says.

Some researchers have been looking into whether the protective power of Sertoli cells could be leveraged for use elsewhere in the body. Jannette Dufour, an immunologist at Texas Tech University Health Sciences Center in Lubbock, Texas, is hoping for a new way to treat diabetes. "We've had tremendous improvement in controlling diabetes with insulin pumps and drugs, but none of them adequately control insulin," Dufour says. And so she and others have been working on taking pancreatic islet cells, which are the body's natural insulin producers but are damaged in those with diabetes, and Sertoli cells to co-transplant the mixture elsewhere in the body. This is especially apt for type 1 diabetes, which is thought to be an autoimmune disease: "Sertoli cells already know how to prevent an attack from the immune system," Dufour says.

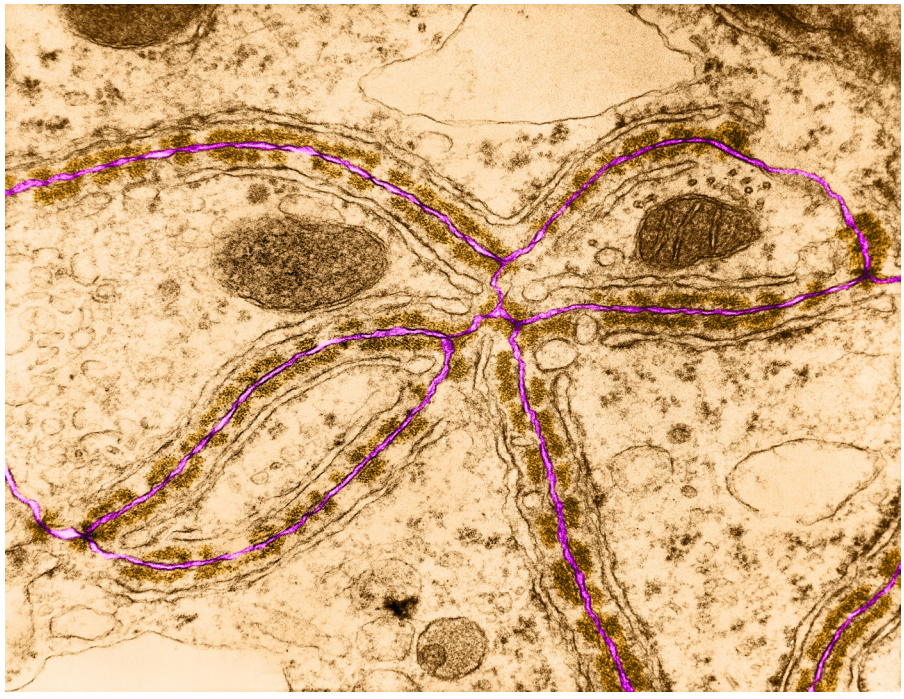
The group has shown that Sertoli cells may work well as a vehicle for delivering insulin. For instance, mouse Sertoli cells engineered with a viral vector containing insulin-producing DNA showed high survival rates after being transplanted underneath the outside lining of the kidneys of the mice: 75% survived 50 days after transplantation[11]. The method is a few years away from being tested in the clinic, but "if we can get this to work, then we'll have a way where insulin is produced and regulated within the body again," Dufour says. "Patients won't have to rely on injections or daily medications."

Breaking barriers

The path to understanding immune privilege is complicated by more than just scientific unknowns. Testicular tissue samples need to be fresh when researchers work with them, which poses a major problem because access to such tissue is lacking. Routy has managed to circumvent this difficulty through his partnership with GRS. Still, Routy doesn't get more than three or four samples a week because the cells contained within the tissue are short lived and tedious to preserve. Commercial vendors of testicular tissue are available, but they tend to be expensive, and donations of testicular tissue, following surgeries like for testicular cancer, are rare. "It's just not at the top of the list of priorities for surgeons," Dufour says of getting testicular tissue donations, since procuring more life-saving organs like the heart takes precedence. "Also, it's not really an easy subject you can bring up, like, 'Hey, can we have your testes?'"

Until such tissue becomes more readily available, Verma's team in Hawaii is collaborating with a group at Wake Forest University in North Carolina that has developed three-dimensional tissue models, or organoids, of testicles. The organoid model has many of the cell types present in human testes, including Sertoli and Leydig cells[12]. Her group has been working on getting Zika virus to replicate in the organoids derived from human cells in the same way as in humans. Although their work is unpublished, the team has observed that testicular organoids, likely like those in humans, produce less testosterone when infected with Zika. Verma hopes that setting up the organoid platform will allow studies not only of Zika but also of other viral diseases, including Ebola.

Verma says the community of researchers



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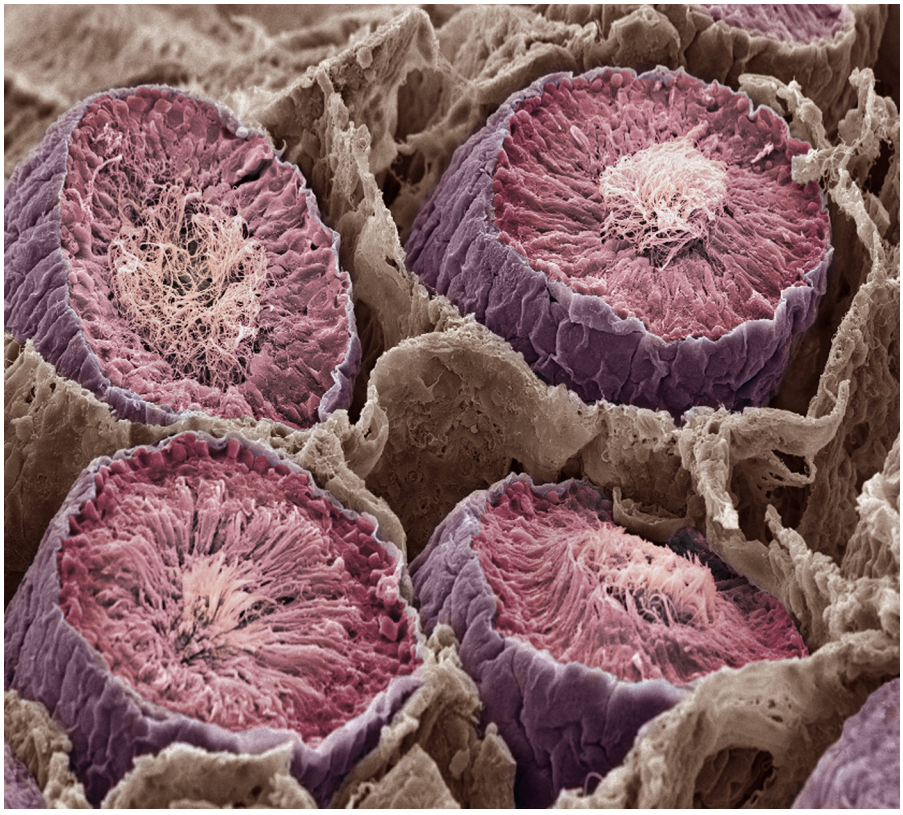
Infection control: Sertoli cells come together to form tight junctions (purple) that make up the blood–testis barrier.



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Tubular trappings: Seminiferous tubules with sperm tails clustered in the center and Sertoli cells on the outer edge of each tube.

interested in understanding immune privilege in the testes is also relatively small. But that may be changing, albeit slowly. A September 2016 workshop sponsored by the US National Institutes of Health (NIH) on immune privilege seems to indicate increased attention to the topic. Verma and Dufour, who were introduced to each other at the meeting, both say that this was the first such meeting they had attended.

As Routy indicated with his work on cancer, a major consideration that scientists are faced with is a practical dilemma: how can the benefit of immune privilege be maintained while also allowing the immune system to respond to infection in the testes? Except when viruses like Zika and HIV take refuge in the testes, immune privilege is a necessary benefit that protects sperm cells, and so targeting only the virus will be key. "What you don't want is for immune cells to break immune privilege," says Michael Diamond, an infectious disease researcher at Washington University in St. Louis. He adds that allowing the entrance of viruses into a testis is also not ideal because clearing virus in the absence of regular immune mechanisms is difficult.

A vaccine-based approach, in which the body is inoculated and gets rid of virus before it has a chance to enter the testes, may be a good solution. Diamond, for instance, is testing a Zika vaccine that was developed by collaborators at the University of Texas Medical Branch in Galveston, Texas. In September last year, his team reported that the live-attenuated vaccine was able to protect male mice from Zika-related damage to the testes[13]. Female mice that were vaccinated, mated with wild-type male mice, and then infected with Zika were monitored for Zika transmission to the fetus. A single dose of the vaccine reduced the viral load in 70% of the placenta and fetal brain samples to below detectable levels. Another group, based in Canada, has been testing a DNA-based vaccine for Zika and similarly found that mice were able to make antibodies in response to the vaccine, which prevented damage to mouse testes[14].

There may be room for pharmacological interventions, too. Nathan Cherrington, a pharmacologist at the University of Arizona, was also an attendee of the September NIH meeting, and in April last year, he was awarded a nearly $300,000 grant by the NIH to explore how drug molecules could circumvent the blood–testis barrier. His group has characterized how a family of drugs used against HIV and other viruses, the nucleoside analogs, cross the blood–testis barrier. The group has found several specific nucleoside transporter proteins that allow these compounds to go from the blood into epithelial cells lining the epididymis [15]. Beyond HIV, which can hide in the testes, Zika is "exactly the type of situation where our work could help," Cherrington says. "We know that sexual transmission happens," he says, so it's a matter of figuring out how to get compounds to infiltrate the testes.

No matter the route, researchers agree that an abundance of caution is necessary. "Messing with the immune system, especially when you don't understand it, is kind of a scary thing," Dufour says. The lack of constant headlines about Zika and Ebola have some scientists worrying that enthusiasm for conducting research into immune privilege could diminish. But the key to curing disease, whether Zika or HIV, will be to know how the testes preserve their unique position in the human body, according to Cherrington, adding, "If we can't understand the complexities of the testis, viruses and cancers that survive in that reservoir will continue to thwart our best efforts."

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Correction

In the December 2017 issue, the article "Drugs that made headlines in 2017" (*Nat. Med.* **23**, 1392–1393, 2017) inaccurately described the mechanism of the RNAi drug fitusiran. The drug does not fix defective RNA code or a defective protein, but rather suppresses the production of a functional protein. The error has been corrected in the HTML and PDF versions of the article as of 9 January 2018.