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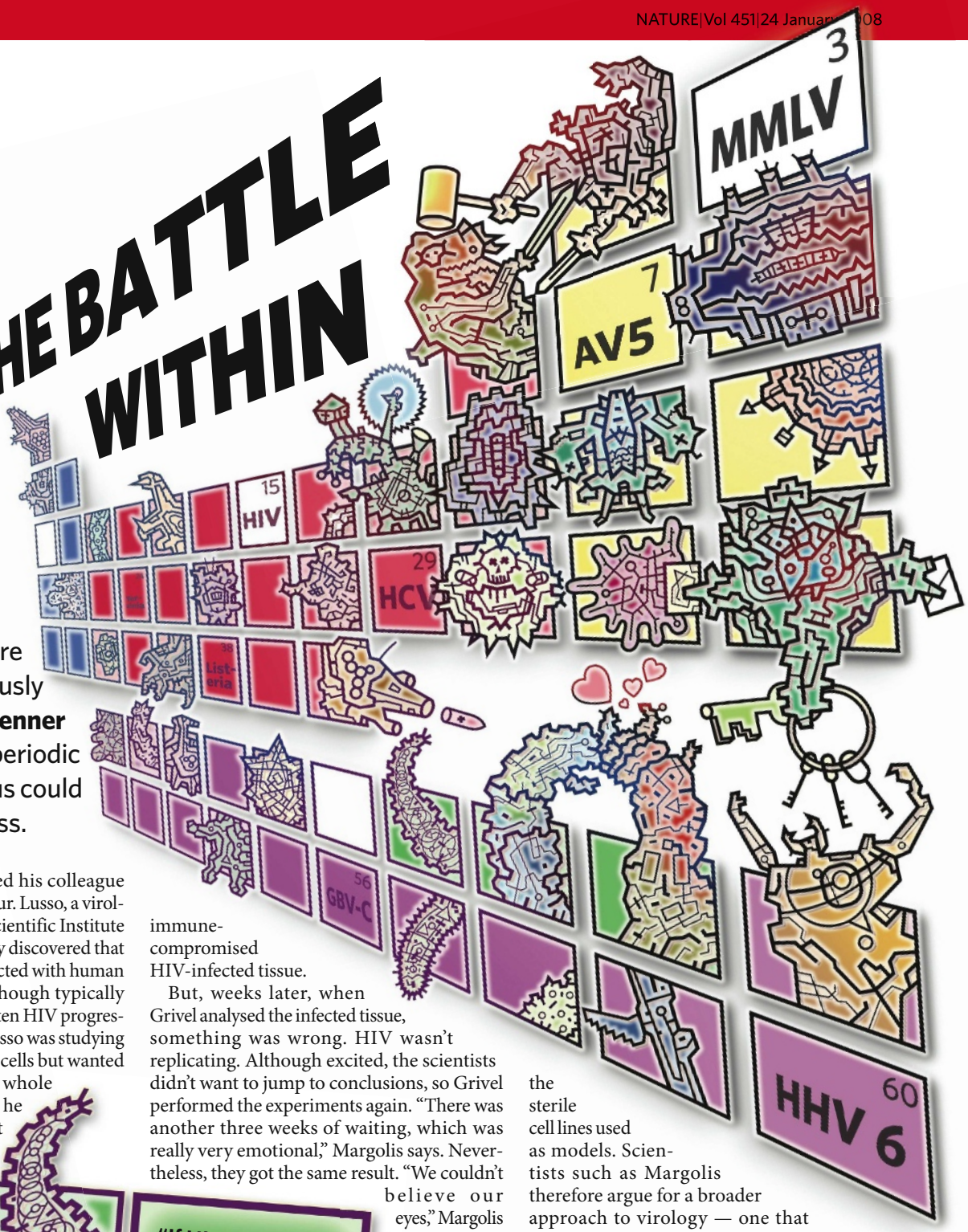
THE BATTLE WITHIN



J. DEPCZYK

Viral and microbial interactions within living tissues are more complex than previously thought. **Melinda Wenner** explores whether a periodic table of the infectious could help sort out the mess.

In 2001, Paulo Lusso asked his colleague Leonid Margolis for a favour. Lusso, a virologist at the San Raffaele Scientific Institute in Milan, Italy, had recently discovered that HIV patients are often co-infected with human herpesvirus 6 (HHV-6). Although typically benign, HHV-6 seemed to hasten HIV progression, and no one knew why. Lusso was studying HHV-6's effects on lymphoid cells but wanted to see what the virus did to whole pieces of lymphoid tissue. So he asked Margolis, a virologist at the US National Institute of Child Health and Human Development in Bethesda, Maryland, and an expert on three-dimensional-tissue, to perform some experiments for him.

Margolis agreed. Human lymph-node tissue was hard to come by, but tonsils, which doctors remove from patients all the time, are also lymphoid tissue — and Margolis had developed an experimental tonsil-tissue system to study HIV pathogenesis. Because HHV-6 infection was often found alongside HIV, Margolis and his colleague Jean-Charles Grivel co-infected tonsil tissue with both viruses. He predicted that the herpesvirus, normally suppressed by the immune system, would be free to replicate in immune-compromised HIV-infected tissue.

But, weeks later, when Grivel analysed the infected tissue, something was wrong. HIV wasn't replicating. Although excited, the scientists didn't want to jump to conclusions, so Grivel performed the experiments again. "There was another three weeks of waiting, which was really very emotional," Margolis says. Nevertheless, they got the same result. "We couldn't believe our eyes," Margolis says. HHV-6, at least in this situation, seemed to protect against HIV[1].

> "If HIV patients had GBV-C, they were three times more likely to be alive at follow-up."
> — Jack Stapleton

So infectious agents interact with each other and with hosts in unpredictable ways. An average human body is rife with viruses, and benign and not-so-benign bacteria as well as 'endogenous retroviruses', which buried themselves in the human genome eons ago. This crowded house is a different beast from the sterile cell lines used as models. Scientists such as Margolis therefore argue for a broader approach to virology — one that involves studying infections in a more true-to-life context, predicting their interactions and sometimes taking the unexpected good with the usual bad that infections bring.

That microbes can benefit their hosts is by no means new. For example, bacteria living in the human gut are known to influence immune function, and help our body absorb nutrients. But only recently have scientists suggested that infectious viruses could provide their hosts with benefits as well. Viruses influence the host immune system in significant — and occasionally beneficial — ways, a concept that isn't surprising when one considers that they have been interacting with immune systems for millions of years.

Recently, Herbert 'Skip' Virgin, an immunologist at Washington University School of Medicine in St Louis, Missouri, infected mice with dormant viruses genetically similar to human Epstein–Barr virus and human cytomegalovirus. These viruses, he found, protected the mice from the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis*. Virgin and his colleagues suggest that the viruses upregulate the production of immune factors that prevent further infection rather than interacting directly with the microbes[2].

Helping or hindering?

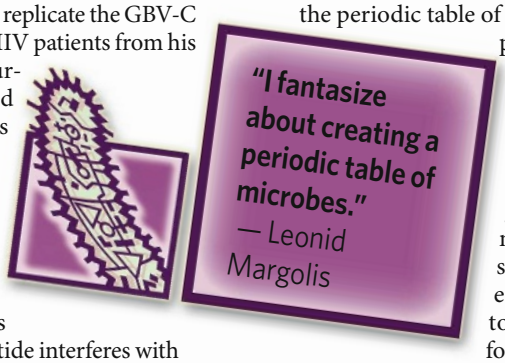
The endogenous retroviruses that cemented their place in human history by infecting the eggs and sperm of our ancestors account for more than 8% of our genome, and some report that as much as half of our genome is composed of fragmented viruses. These viruses seem to influence immune function; for example, the susceptibility of mice to Friend virus, a strain of murine leukaemia virus, is controlled by two genes derived from endogenous retroviruses[3]. Some have proposed that endogenous retroviruses, long fixed in mammalian genomes, provide the immunosuppression that allows a fetus to develop in its mother's body, despite the differences between the immune systems.

Viruses also interact with each other directly, as Margolis discovered for himself in 2001. Similar viruses sometimes compete with each other, causing one to eventually 'win over' a cell and literally block infection by others. Viruses that are different enough from each other can co-infect cells cooperatively; in a process known as complementation, one virus provides another with a useful protein that it co-opts for its own use. Occasionally, viruses become dependent on other viruses. One such example is hepatitis D, which requires the presence of hepatitis B in order to replicate.

Margolis uncovered why HHV-6 prevents HIV replication under certain conditions. A subtype of HIV, most often found in early infection, generally gains entry into the cell by binding the receptor CCR5. When HHV-6 infects first, however, it instigates production of an immune chemical that binds to CCR5 receptors, blocking HIV's access. HIV can develop resistance to this chemical over time and HHV-6 co-infection may exert selective pressure on HIV to become immune-resistant, or switch to a different co-receptor — a change accompanied by increased HIV virulence. This explains the often poor prognosis of patients infected by both viruses.

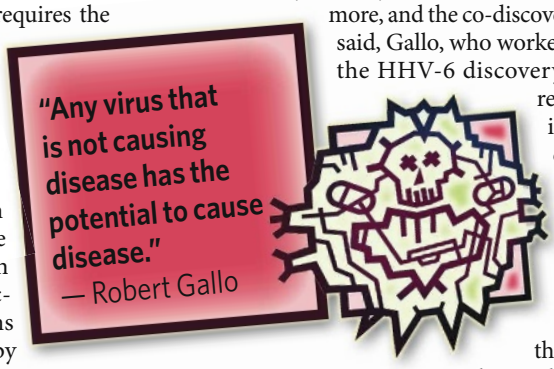
Other human viruses influence HIV replication. Margolis found that human herpesvirus 7 (HHV-7) inhibits HIV replication, albeit via a different mechanism from HHV-6 (ref. 4). In 1998, two groups independently reported that HIV patients infected with a seemingly innocuous hepatitis-like virus called GBV-C live longer, although neither group knew why. The studies piqued the interest of Jack Stapleton, director of the University of Iowa's Helen C. Levitt Center for Viral Pathogenesis and Disease in Iowa City, who was, at the time, running an AIDS clinic while studying hepatitis C–HIV interaction.

"We thought it was a statistical fluke and that it wouldn't hold up in a larger study," Stapleton recalls. With access to hundreds of samples, Stapleton decided to replicate the GBV-C study on a group of 362 HIV patients from his clinic. What he found surprised him and confirmed the findings: "If patients had [GBV-C], they were three times more likely to be alive at follow-up," he says[5]. Stapleton's subsequent research, along with work completed by a group in Germany, has shown that a GBV-C peptide interferes with early replication of HIV, and that GBV-C, like HHV-6, increases production of an immune chemical that blocks HIV's entry into the cell.



Always dangerous

No one would suggest purposefully infecting individuals with a persistent infection to ward off HIV. After all, one of the hallmarks of viruses is that they evolve quickly. "Any virus that is not causing disease has the potential to cause disease," says Robert Gallo, director of the Institute of Human Virology at the University of Maryland School of Medicine in Baltimore, and the co-discoverer of HIV. That said, Gallo, who worked with Lusso on the HHV-6 discovery, and his team recently tested the immune chemical produced during HHV-6 infection as a vaginal microbicide in macaque monkeys and found that it significantly lowered risk of contracting a monkey-infecting version of HIV[6].



To make further progress, scientists need to expand which viruses they study, and how they study them, Margolis says. They need to use tissue systems that preserve immune function and cellular communication, because cells within tissues communicate with one another differently and have a different architecture than do cells that are cultured *in vitro*.

Moreover, Margolis suggests, scientists should study infections in tissues harbouring the same persistent viruses present in humans. "If you study a pathogen such as HIV in a cell-culture model where there is no immune system, there is no effect from other microbes that are in the normal state in an infected human," says Stapleton. "You're really missing important factors that will influence the pathogenesis."

What would be ideal, says Margolis, is a table, not unlike Mendeleev's table of the elements, for infectious agents. "I fantasize about creating a periodic table of microbes," he says. Each cell in the table might feature the name of the microbe, the immune factors it affects, the receptors it uses and the signalling systems it incorporates, he says. Just as the elemental version predicts how two substances react with one another, the periodic table of microbes would predict how two microbes interact in the human body.

"It's off the wall, but it could generate enormous insights," says Michael Lederman, the director of the Center for AIDS Research at Case Western Reserve University in Cleveland, Ohio. Other scientists say that although such a table may have some practical value, the concept is potentially more interesting as a catalyst for scientific ideas and approaches. Philip Murphy, chief of the Laboratory of Molecular Immunology at the US National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, says that scientists can only get so far with existing tissue and animal models. "Pathogens are very host-limited, so there is a whole range of human pathogens that you could never do an experiment with in mice models," he says. For example, although Margolis's lymphoid-tissue model is incredibly useful, he says, there are a number of pathogens that will never infect lymphoid tissue. Scientists will, in other words, need to get creative.

Expanding the study of virology in these ways is challenging for other reasons, too. It is difficult, for example, for a scientist with expertise in DNA viruses such as herpesviruses to study their interactions with RNA viruses such as HIV. But collaboration can help, and although broadening the context of virology might make experiments more complicated, it will also make them more realistic, Margolis says. "In a more complicated system, you probably can understand less," he admits. "But what you understand is really relevant." ■

Melinda Wenner is a science writer based in New York City.

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