Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

have identified a variety of new and intriguing genes necessary for survival. A combination of different methodologies will be required for comprehensive identification of virulence and survival factors – no single technique will provide all the answers. Careful controls will reduce the remaining perceived STM-specific limitations. Finally, the validity of extracellular complementation, proposed by authors to explain the absence of known extracellular virulence factor genes in STM studies, will remain unresolved until a comprehensive STM survey is performed.

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On viral epidemics, zoonoses and memory

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Inevitably, perhaps, we see the world from a human point of view. Microorganisms are bad guys and human epidemics rivet our attention. When microorganisms devastate crops and animals their impact is keenly felt, yet there is a myriad of 'lesser' microorganisms that do much less damage or, indeed, none whatsoever. Although this reservoir attracts few headlines, many are but one event (mutation, plasmid or pathogenicity island) away from a pathogenic form. However, because it lacks economic or immediate public health impact, this pool is poorly described. For example, the recent fatal cases of human hendravirus infection in Malaysia must be seen in the light of only a handful of Medline citations (e.g. Refs 1,2).

Restricting the debate to viruses still leaves us with an impressive Hall of Fame, including names such as variola, 'flu A, yellow fever and the neophyte – HIV – among many others. Notice that all have non-human counterparts. Variola, which can proudly be discussed in the past tense, was particularly devastating when introduced into the Americas following discovery of the New World by the Old. One can read about these events in many recent books with 'plague' in the title. Yet this is but a variation on the theme of high virulence following introduction into a naive population. What does this mean in terms of immunity and memory? Why should a new pathogen be so devastating? Can one believe in 'holes' in the immunological repertoire and keep immunologists as friends?

'Novel' viruses

By definition, a 'novel' virus for a species must always come from a different species, for nobody seriously believes the panspermia theories advocating that life arrived on Earth from elsewhere. Armed with PCR, it is increasingly evident that there are huge numbers of viruses lurking in non-humans[3–5]. As no systematic search has been undertaken, it is difficult to know exactly what fraction of viruses is known. Hence, the question becomes: how frequently do non-human viruses become established in man?

The problems are obvious. Firstly, we do not know the spectrum of viral candidates and, secondly, most funding agencies are interested, understandably, in pathogenic viruses or those of economic importance. Yet, Jenner observed that milkmaids did not develop smallpox because they were immunized by cowpox virus. Every case represented a new species jump as there were no milkmaids' trade union conferences to aid spread between them. The majority of pulmonary hantavirus syndrome cases described in the Four Corners region of the USA in 1993 were primary infections from rodents to humans[6,7]. The same is true for the fatal cases of Hong Kong H5N1 influenza A in 1997, when an avian virus turned up in humans[8,9]. With no disrespect to Pasteur, rabies is a dead-end disease in humans. Although the answer to the question of the number of non-human viruses that become established in humans is open, it is probable that such zoonosis is far more frequent than we would like to believe.

Although some dead-end infections are fatal, cowpox infection confers protection against variola. Given our lack of interest in non-pathogenic infections, it is arguable that many of these infections are sub-clinical. However, even an abortive infection will prime the immune system to some extent. Perhaps the reason why the milkmaids usually got off scot-free is that they were immune as a result of repeated infections. This is

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likely to be the case with the microorganisms around us – those that can infect once can probably infect again, with transmission being density dependent. This process has probably been especially intense since the domestication of animals started 10 000 years ago. The development of agriculture would also have changed the habitat for non-domesticated animals, particularly rodents. In this light, ever-increasing urbanization and battery farming represent a step towards isolation from animal microorganisms and priming of the immune system.

Crossreactivity

Immunological crossreaction between different strains of the same virus is extremely common – influenza A, coronaviruses, hepatitis E virus and HIV-1 and HIV-2 are but a few examples[10–17]. Indeed, HIV-2 was identified precisely because, using western blots, sera lacked reactivity to the HIV-1 surface envelope protein. Peripheral T cells recognize foreign peptides presented by major histocompatibility complex proteins on the surface of surrounding cells. Although the peptides are no more than nine or ten residues long, perhaps only four or five are recognized by the T-cell-receptor (TCR) complex. This means that the information is stored in a relatively simple manner, in contrast to B-cell memory, which is frequently conformation dependent. If the appropriate antigen-presenting cells are present, T cells can be stimulated to proliferate and some will enter a memory state. Crossreactivity then depends on the promiscuous recognition of related microorganisms by a given TCR. In fact, TCRs show a high level of crossreactivity[18].

Can crossreactivity between related microorganisms be induced, and how related do microbial antigenic epitopes have to be to allow crossreactivity to be maintained? A good example is influenza A infection of mice. Immunization with one type of hemagglutinin/neuraminidase confers protection against other types, with the protection being mediated by CTLs (cytotoxic T cells)[16,19–21]. Importantly, it was shown that as little as one specific amino acid residue within a peptide antigen was sufficient to expand the population of memory CTLs (Ref. 22). An extreme (and deleterious) case is the cellular crossreaction between the 60-kDa, cysteine-rich outer membrane proteins of *Chlamydia* and murine-heart-muscle-specific α-myosin heavy chain protein[23]. From an evolutionary point of view, immunological crossreactivity allows memory to be maintained in the absence of the specific antigen as long as crossreactivity towards self remains rare, at least up to reproductive age.

New encounters

Given this, what might have happened when Christopher Columbus *et al.* and attendant microorganisms travelled into virgin territory? As American Indians had been geographically isolated from the conquistadors for tens of thousands of years, much of the local human and animal microbial fauna, particularly the rapidly mutating RNA viruses, would have been antigenically very distinct from those aboard the Santa Maria. Eurasians had harnessed the horse, dog, pig, goat and cow to mention just a few and, unbeknown to them, they would have been used to the infections originating from these animals. Not so the American Indians, who had only domesticated the llama and dogs and, we may imagine, their microorganisms. Perhaps the reason why variola and measles were so lethal was not because they were new, but because the American Indian immune systems had never encountered anything similar. Therefore, the problem was not with the American Indians but rather with the European populations, which were not entirely naive. Not to belabour the point, the same logic goes for the White Man's grave – sub-Saharan Africa – for the local microbiology here was very different from far-off Western Europe.

Notice that this argument pertains to domesticated animals and local insect fauna and concerns particularly RNA viruses and many retroviruses, which fix amino acid substitutions at rates of 1% per year. Probably a mere thousand years between any two human communities could be enough for some RNA viruses to appear totally different. One might point out that smallpox is a DNA virus and therefore fixes substitutions at a slower rate. Indeed. However, as American Indians had only domesticated the llama and dogs, for which there are no reported orthopoxviruses, this might explain why they were so vulnerable (cowpox virus has not been isolated in the Americas[24], although orthopoxviruses have been described for the racoon and skunk). The parallel with antigenic drift and the shift of influenza A virus is not lost. Antigenic drift represents incremental changes in the viral surface proteins, which are advantageous to the virus yet not enough to prevent considerable restriction of viral replication by existing host immunity. Antigenic shift usually results from reassortment between very different strains and leads to the introduction of a novel hemagglutinin for which there are no pre-existing antibodies. The severity of disease is much greater following antigenic shift.

What can be said of societies with good public hygiene and highly sophisticated animal husbandry employing fewer and fewer personnel? Apart from pets and perhaps horses, their animal populations rarely see fellow mammals. It is probable that our immune systems are becoming relatively focused on a few microorganisms and lack memory to a wide variety of microorganisms living but a few fields away. This is not to criticize good public health measures, the merits of which are unchallenged. But with more and more adventure seekers ploughing into jungles in four-wheel drives, who knows what they will find? Perhaps it is time to make an inventory of mammalian and insect microorganisms. More importantly, we should invest heavily in field-based microbial ecology and control of zoonoses. Greater investigation of immunological crossreactions and

disease susceptibility would also not go amiss – something that working with a microorganism and specific pathogen-free mice cannot resolve.

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Vibrio cholerae TCP: a trifunctional virulence factor?

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Cholera, a disease characterized by severe watery diarrhea, is caused by specific strains of *Vibrio cholerae*[1,2]. *V. cholerae* are Gram-negative bacteria that persist in aquatic environments and infect humans via ingestion of contaminated water or food.

TCP: a colonization factor

Cholera pathogenesis requires that the bacteria colonize the intestine and secrete cholera toxin (CTX). The action of CTX alters epithelial ion transport, causing a massive flux of fluid into the intestinal lumen. Bacterial colonization requires toxin-coregulated pili (TCP), which are type IV pili composed of TcpA subunits that form bundled filaments at the bacterial surface. TCP might not mediate the binding of *V. cholerae* to epithelial cells[3,4]; instead, it might protect the bacteria from being exposed to and killed by host factors in the intestine by causing the bacteria to aggregate[5]. Other surface factors appear to provide more classical adherence and colonization functions.

TCP: the CTXΦ receptor

In 1996, Waldor and Mekalanos[6] reported that the genes encoding CTX – *ctxA* and *ctxB* – reside on the cholera toxin phage (CTXΦ). Many of the open reading frames encoded by CTXΦ are homologous to those on filamentous coliphage such as M13 and fd. Analogous to the filamentous coliphage, transduction of CTXΦ requires that recipient bacteria express a pilus receptor, in this case TCP. Because the *tcp* locus is essential for colonization and also encodes a transcriptional regulator that activates both *tcp* and the *ctx* genes during infection, it has been speculated that important evolutionary advantages are conferred to CTXΦ by its choice of receptor[2,6]. Acquisition of the *ctx* genes by a TCP^+ recipient automatically links CTX expression to a virulence regulon and allows the transductant to grow within the host intestine. Interestingly, although there are over 150 serotypes of *V. cholerae*, it is primarily strains of the O1 and O139 serotypes that encode the *tcp* genes[7,8]. The limited distribution of *tcp* genes and the requirement of CTXΦ transduction for TCP might explain why CTX^+ TCP^+ strains of *V. cholerae* are predominantly of these two serotypes.

TCP: a transducing phage?

Soon after the discovery of CTXΦ, Kovach *et al.*[9] proposed that the *tcp* locus also might be a mobile genetic element. They showed that the *tcp* genes are inserted in the *V. cholerae* chromosome at a site that is analogous to the CP4-57 prophage integration site in the *Escherichia coli* chromosome. Karaolis *et al.*[10] have now shown that the *tcp* locus can, in fact, be mobilized and appears to be encoded on another filamentous bacteriophage, the *V. cholerae* pathogenicity island phage (VPIΦ). By using traditional methods for purifying bacteriophage from

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