housed inside mitochondria (i.e., all four subunits of succinate dehydrogenase are encoded by nuclear genes and are imported into the organelle). Complex II is also the entry point for electrons derived from FADH₂ (instead of from NADH) into the ETC. To elucidate the central role of complex II in the antibacterial response, Garaude *et al.* focus on fumarate, the product of the succinate-dehydrogenase reaction, which they show has bactericidal properties that are independent of acidification, analogous to itaconic acid, another toxic mitochondrial product derived from the tricarboxylic-acid cycle⁴. Thus, we are tempted to speculate that a core component of the antibacterial response is direct killing mediated by mitochondria surrounding bacteria-containing phagosomes, whereby a toxic 'witch's brew' of mitochondrial ROS and mitochondria-derived anti-microbial metabolites can be delivered at high concentrations locally to kill the invaders without collateral damage to cellular components (Fig. 1b). However, inhibition of complex II also leads to alterations in cytokine production (for example, less pro-inflammatory IL-1 β and more anti-inflammatory IL-10) in infected mice. This indicates that mitochondrial ETC rearrangement and the resulting downstream metabolic responses have other anti-microbial signaling consequences that remain to be delineated.

Mitochondria and NADPH oxidase (NOX) are major sources of ROS that promote oxidative damage to cellular components, but they are also important mediators of redox-signaling pathways with a wide range of physiological effects^{6,7}. In fact, ROS produced by both mitochondria and NOX enzymes have direct roles in bacterial killing, as well as important signaling roles in a variety of immune cells, including neutrophils and macrophages^{6,7}. Garaude *et al.* find that an unusual mixture of ROS responses

drives bacteria-induced macrophage mitochondrial adaptations⁴. The main response requires phagocytic NOX, which they propose might lead to an oxidative-damage-induced decrease in complex I activity. They also find that mitochondrial antioxidants inhibit complex II activity, suggestive of a potential positive role for mitochondrial ROS in regulating the ETC. The phagocytic NOX-mediated ROS effects on mitochondria and the subsequent immune responses involve a series of apparently coordinated events, including activation of the kinase Fgr, which has been linked to metabolic adaptations and redox regulation of complex II, engagement of the NLPR3 inflammasome, known to sense mitochondrial signals³, and endosomal Toll-like receptor (TLR) signaling. The precise TLRs involved are not identified, but the requirement for the adaptors TRIF and MyD88 indicates that TLR signals are involved in the response to live versus dead bacteria, which the authors show is probably driven by bacterial RNA.

The study by Garaude *et al.* is focused on bacteria and connects mitochondrial ETC and metabolic changes to TLR and inflammasome signaling with no apparent involvement of cytoplasmic nucleic-acid sensors such as cGAS, STING or MAVS, which mediate anti-viral innate immune responses^{3,4}. However, mitochondrial DNA, which is of bacterial origin, has been shown to activate the cGAS-STING pathway and prime antiviral innate immunity when it gains access to the cytoplasm⁸. In addition, there are reports that bacterial DNA can stimulate these nucleic-acid-sensing pathways and interferon production⁹ or result in the release of mitochondrial DNA to promote inflammasome activation¹⁰. Finally, some bacteria produce cyclic dinucleotides, similar to the 2'-3' cGAMP produced by cGAS, that bind and activate STING¹¹. Such examples suggest that

the multitude of interactions among microbes, mitochondria and innate immune responses are only just beginning to be understood. As these new pathways continue to be identified and elucidated, the potential arises to target mitochondria and the associated immunological-metabolic interactions to enhance antibacterial and antiviral responses and/or prevent inflammation that can lead to autoimmune diseases and other pathologies. At the heart of this quest is much-needed additional research on the cell-type and tissue specificity of mitochondrial structure, function, dynamics and metabolism and, in turn, how this is affected by different pathogens and leads to idiosyncratic innate and adaptive immune responses mediated by various immune cells. One final frontier in this context is how this nexus of mitochondria and the immune system changes with age and contributes to immunosenescence and enhanced susceptibility to microbial pathogenesis¹², a looming issue as the world's population shifts to a much older demographic in the coming decades.

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Immunogenic cross-talk between dengue and Zika viruses

Stephen C Harrison

At least five recent papers have shown an unexpected antigenic relationship between Zika and dengue viruses, with potential implications for vaccines and therapeutics.

Flaviviruses are plus-sense RNA viruses transmitted between infected humans by mosquitoes or ticks. This group includes the

recently prominent Zika virus and such well-known human pathogens as dengue virus and yellow fever virus (after which the group is rather oddly named). Two collaborative papers, one by Screaton and colleagues in this issue of *Nature Immunology*¹ and another in *Nature*², and at least three other reports published concurrently^{3–5} show an unexpected

antigenic relationship between Zika and dengue viruses, with implications for vaccines and perhaps for therapeutics. Other related studies are undoubtedly in the press.

Infectious flavivirus particles are compact, icosahedrally symmetric assemblies, with a lipid bilayer membrane smoothly coated with 180 copies of a three-domain envelope

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Figure 1 Antibodies that neutralize both dengue virus and Zika virus. In the arrangement of 180 E-protein subunits on the surface of a flavivirus particle (top left), the subunits associate as dimers, packed in a 'herringbone' pattern¹⁴; a single E-protein dimer (far left) is oriented to show how it relates to the whole (right). An expanded view of the ectodomain dimer of E protein (PDB accession code, 1OAN (dengue virus type 2)) (bottom left) shows the three domains (I (red), II (yellow) and III (blue)) and the fusion loop at the tip of domain II (orange), plus glycans and disulfides ('sticks') and the amino (N) and carboxyl (C) termini of each subunit. A single-chain Fv from an antibody to EDE1 (isolated from cells of a patient infected with dengue virus (top right): dark green, heavy chain; gray, light chain) is shown bound to a Zika virus E-protein ectodomain dimer (top right; 'ribbons' in colors as at left); outlined area indicates the Fv–E protein interface and shows how the epitope spans a dimer contact. The surface of the Zika virus E dimer (bottom right), presented as if viewed from above at top right, shows the footprint of Fv (green outline) and conservation of the exposed residues. Right half of figure is from ref. 2.

(E) protein (**Fig. 1**). E protein has three functions associated with viral entry and host defense: cell attachment, membrane fusion and antigenicity. Antibodies that recognize E protein, when present in sufficient titer, neutralize infection in cell culture and protect against disease in human hosts. Dengue and West Nile viruses can proliferate in cells of the immune system, many of which bear FcγR receptors. Sub-neutralizing concentrations of antibody can promote infection in such cells by facilitating Fc-receptor-mediated entry, a phenomenon called 'antibody-dependent enhancement' (ADE)⁶.

The four distinct and (in recent evolution) relatively stable serotypes of dengue virus have made vaccine development much more complicated than it was for the vaccine against yellow fever—still one of the most successful vaccines ever made. The antigenic differences among the four types are great enough that robust immunity to one conferred by recovery from infection does not confer immunity to the other three. Instead, previous exposure to one serotype increases the risk of severe disease after infection by virus of a second serotype. It was proposed many years ago that ADE, mediated by low titers of cross-reactive antibodies from the primary infection, might

account for the enhanced severity of the secondary infection⁷. Studies of infants who had passively acquired antibodies to dengue virus from their mothers and results obtained with subsequently developed mouse models support that hypothesis⁶. Thus, to avoid risk of enhancement, a safe vaccine against dengue virus will need to confer protective immunity against all four serotypes.

In principle, conservation across serotypes of certain surface patches on E protein suggests that it should be possible to find broadly cross-reactive and cross-protective antibodies and hence that a goal of vaccine research should be to elicit these. One such patch is the so-called 'fusion loop', critical for viral entry⁸. This hydrophobic loop, at the tip of domain II of E protein (**Fig. 1**), interacts with the endosomal membrane when low pH triggers a fusogenic conformational change in E protein. On a mature virus at a neutral pH (its state when an antibody might bind), the interface with the dimer partner subunit buries the fusion loop, making it inaccessible. In 2015, a collaboration between the groups of Screaton (at Imperial College) and Rey (at Institut Pasteur) showed that recently identified human antibodies recognize another conserved patch on the surface of the same

interface that buries the fusion loop^{9,10}. These antibodies neutralize all four serotypes quite effectively. Structures of the antibodies bound to E protein from each of the four serotypes show that the antibodies fall into two classes, with overlapping contacts. The researchers called these contact regions EDE1 ('E-dimer epitope 1') and EDE2, because they bridge the dimer interface (**Fig. 1**).

In the two papers now published, the same two groups have now shown that the EDE1-binding antibodies also neutralize Zika virus^{1,2}. They illustrate by phylogenetic analysis based on the amino-acid sequence of E protein that for this protein, Zika virus is closer to dengue virus than to any of the other flaviviruses and indeed is almost close enough to think of it as a fifth serotype², although the rest of the genome places Zika virus closer to some of the other mosquito-borne flaviviruses. A structure of one of these antibodies in complex with Zika virus E protein shows the reason for the cross-neutralization: nearly all the contact residues are the same in Zika virus and in all four serotypes of dengue virus (**Fig. 1**). The EDE2-binding antibodies do not neutralize Zika virus at levels that would protect *in vivo*, but because they do bind weakly, they instead promote ADE in a cell-based assay, as the paper in this issue of *Nature Immunology* shows¹. Two other papers confirm these results^{3,4}.

The new data clearly influence potential strategies for vaccines against dengue and Zika viruses. The ADE observed *in vitro* does not show that immunity to dengue virus can enhance the risk of infection with Zika virus. That conclusion would be provided only by epidemiological analyses, together with studies in validated animal models. Moreover, it remains unknown whether enhanced infection of Fc-expressing cells would influence the course of infection with Zika virus in humans. Nonetheless, Screaton and colleagues¹, along with others^{3–5}, provide clear evidence of immunogenic cross-talk between Zika and dengue viruses. Four of the seven hospitalized, dengue-virus-infected patients from whom blood was obtained for the initial study had plasmablasts that encoded EDE-reactive antibodies at their rearranged immunoglobulin loci. Thus, B cells that make these antibodies are relatively common in patients with secondary infection. Moreover, plasma samples collected from patients 6 months following acute secondary infection with dengue virus all showed the presence of antibodies that bound Zika virus (as well as dengue virus) but neutralized Zika virus either marginally or not at all. Instead, most of the plasma from convalescent patients enhanced infection in an assay for ADE.

Given the current data, the epitopes of the Zika-virus-reactive antibodies in plasma from convalescent patients remain unknown. Antibodies directed against EDE1 and EDE2 might account for some of the activity, but antibodies directed against the fusion-loop epitope (FLE) can also enhance infection through a mechanism that depends on the presence of a second surface protein: precursor of M (prM)^{11,12}. Flaviviruses assemble by budding into the endoplasmic reticulum, where their core structures (genome plus a nucleocapsid protein) acquire both a lipid-bilayer membrane and 180 copies each of E protein and prM. The precursor domain of a prM subunit covers the fusion loop of each E-protein subunit, which projects outward rather than lying flat on the membrane surface¹³. This domain, cleaved from the rest of the protein by furin in the trans-Golgi network, dissociates from the virus when it emerges from the cell, leaving only a residual, membrane embedded segment called 'M' (Fig. 1) and allowing E protein to form the 'flat' dimers found on mature virions. The way the precursor domain of prM associates with the fusion-loop tip permits attachment of fusion-loop-directed antibodies. Because many viral particles produced during infection have incomplete cleavage of prM, antibodies to FLE can bind these partly immature particles and enhance the infection

of Fc-receptor-expressing cells. Exposure of FLE on partly immature particles might also account for the prevalence of antibodies to FLE elicited by infection with dengue virus².

Each of the five papers^{1–5} outlines some of the next steps for understanding how the potential cross-reactivity of human immune responses to dengue and Zika viruses can affect plans for the development of vaccines or antibody-based therapeutics. Clearly, it is essential to know whether there is any epidemiological evidence that immunity to one virus influences the risk of infection after exposure to the other. Cross-neutralization of Zika virus and all four serotypes of dengue virus by EDE1-directed antibodies also suggests the value of more extensive cross-protection studies in animal models to determine how worthwhile it would be to pursue proposals for vaccine immunogen design and especially to suggest whether antibody-based therapeutics are a sensible objective. As the inconclusive outcome of studies during the 2015 outbreak of Ebola virus illustrates, analysis of the efficacy (or lack thereof) of passive transfer after infection or after diagnosis should occur before an emergency, not once the need becomes acute.

These papers collectively offer both 'good news' and 'bad news'^{1–5}. They show that certain members of a group of well-characterized antibodies that neutralize all four serotypes of

dengue virus can neutralize Zika virus as well. These antibodies might indeed be useful for treatment or prophylaxis. The papers also show, however, that in the plasma of convalescent patients infected with dengue virus, these cross-neutralizing antibodies are present at a titer too low to neutralize Zika virus. In any case, the close antigenic relationship between Zika and dengue viruses that these papers illustrate adds a new dimension to the study of each.

COMPETING FINANCIAL INTERESTS

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HLA-Fatal attraction

Frédéric Vély, Rachel Golub & Eric Vivier

Open conformers of the non-classical and monomorphic major histocompatibility complex (MHC) class I molecule HLA-F are ligands for the activating receptor KIR3DS1 and trigger the activation of natural killer (NK) cells.

The activating receptor KIR3DS1 is expressed at the surface of some NK cells and T cells. Genetic epidemiologic studies have indicated an association of KIR3DS1 not only with resistance to several viral infections

and cancers and a better outcome after transplantation of hematopoietic stem cells but also sensitivity to various autoimmune disorders^{1,2}. However, how KIR3DS1 affects immunity remains unclear. In particular, the nature of the ligands recognized by KIR3DS1 has been disputed. In this issue of *Nature Immunology*, Garcia-Beltran *et al.* show that KIR3DS1 directly interacts with open conformers of the MHC class I molecule HLA-F, which leads to attacks on target cells by NK cells³.

The MHC class I family inarguably represents a major group of molecules involved in immunity. Beyond presenting antigens to T cells, cell-surface MHC class I proteins are also ligands for a series of cell-surface receptors, originally described on NK cells but also

expressed on T cells and myeloid cells. These MHC-class-I-specific receptors are members of the immunoglobulin-like superfamily, such as KIRs ('killer cell immunoglobulin-like receptors') and LILRs ('leukocyte immunoglobulin-like receptors') in humans, and lectin-like receptors, such as members of the Ly49 family in mice and CD94-NKG2 heterodimers in both human and mice (Fig. 1). Inhibitory receptors are characterized by an intracytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM)⁴. Most ITIM-bearing molecules have counterparts with highly homologous extracytoplasmic domains but divergent transmembrane and intracytoplasmic regions that confer activating properties⁴. These activating isoforms of ITIM-bearing molecules signal via

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