promise of chemical-based mitochondrial uncoupling as a therapeutic strategy for obesity and obesity-associated diseases. Recent progress has been made toward developing liver-specific DNP derivatives or milder mitochondrial uncouplers that are effective in treating obesity and obesity-associated diseases but with minimal side effects (8–10). The identification of N-acyl amino acids as endogenous mitochondrial uncouplers would not only advance our understanding of adaptive thermogenesis, but also might present safer alternatives to chemical uncouplers if a direct role of N-acyl amino acids as mitochondrial uncouplers can be established. This may require the development of N-acyl amino acid mimetics, given the higher hydrolyase over synthase activity of PM20D1. An immediate question that should be addressed is that in cell culture, N-acyl amino acids take 20 to 40 min to initiate uncoupling, which is much longer than the time taken by known chemical uncouplers such as DNP. In addition, N-acyl amino acids such as N-arachidonyl glycine (C20:4-Gly) have a wide range of biological functions via their interactions with G protein–coupled receptors and ion channels in brain and other tissues (II), which in vivo could contribute appreciably to the food suppression and weight-loss phenotypes observed in the treated mice. Furthermore, because the uncoupling effect of N-acyl amino acids is UCP1-independent and thus not limited to brown and beige fat, their role in mitochondrial ATP production in highly energetic tissues such as heart, brain, and kidney needs to be explored.

The findings of Long et al. open a door on a new class of endogenous mitochondrial uncouplers and present a new mechanism of adaptive thermogenesis via a secreted enzyme and its products. However, every open door reveals more questions than it answers, and follow-up studies are required. We are left to ponder the hope of a magic pill offering effortless and consequence-free fat burning.

**REFERENCES AND NOTES**

3. J. Z. Long et al., Cell 166, 424 (2016).
8. R. J. Perry et al., Cell Metab. 18, 740 (2013).

**ACKNOWLEDGMENTS**

We thank C. S. Lin for help with this manuscript.

10.1126/science.aah6189

**Virology**

**Diagnostics for Zika virus on the horizon**

The immune response to Zika virus informs antibody-based diagnostics and therapeutics

By Scott D. Speer and Theodore C. Pierson

Zika virus (ZIKV) is a mosquito-transmitted flavivirus that is related to other pathogens of clinical importance, including yellow fever and dengue (DENV) viruses. Although once infrequently associated with human disease, ZIKV has emerged as a global health threat with its introduction into South America during 2014 and 2015. Of concern, recent ZIKV outbreaks are linked to severe neurodevelopmental complications in the children of women infected while pregnant, as well as Guillain-Barré syndrome in adults (1). Management of this epidemic has been complicated by extensive serological cross-reactivity among flaviviruses and the cocirculation of ZIKV and DENV in regions experiencing the greatest disease burden. Current serological diagnostics have a limited capacity to distinguish between DENV and ZIKV. On page 823 of this issue, Stettler et al. (2) characterize monoclonal antibodies (mAbs) isolated from ZIKV-infected humans that hold promise as diagnostics or therapeutics, and advance our understanding of the repertoire of antibodies elicited by ZIKV infection.

Flaviviruses are assembled from three viral structural proteins [capsid, premembrane (prM), and envelope (E)], a host-derived lipid envelope, and the genomic viral RNA (3). Flavivirus-infected cells also secrete a nonstructural protein 1 (NS1), which has multiple roles in viral replication and pathogenesis in vivo (4). Both NS1 and the structural proteins are immunogenic. Virus-neutralizing antibodies most commonly target the E protein, may be highly protective in vivo, and are a correlate of protection for many flavivirus vaccines (5). NS1 antibodies are non-neutralizing, yet they contribute to protection via antibody heavy chain–mediated effector functions (6). Whereas the functional characteristics of antibodies in ZIKV-immune individuals have been studied (7), human ZIKV mAbs have not been reported.

Accordingly, Stettler et al. have now isolated a panel of 119 human mAbs from the memory B cells of four ZIKV-infected donors; two of these subjects had been infected previously by DENV (2). Roughly two-thirds of the mAbs produced bound epitopes within the E protein. Antibodies specific for the immunoglobulin (Ig)–like domain III (DIII) had considerable neutralizing activity and were largely specific for either ZIKV or DENV. Numerous cross-reactive mAbs with modest neutralization capacity mapped to E protein domains I or II. Domain II is the location of the highly conserved fusion loop frequently targeted by antibodies elicited by other flaviviruses. Although more study is required, cross-reactive fusion loop–specific antibodies may also be common in ZIKV-immune individuals. Of considerable interest, Stettler et al. found that the most potent neutralizing mAbs bound efficiently to intact virions but not to soluble forms of the E protein, which suggests that antibodies that bind quaternary epitopes composed of more than a single ZIKV E protein may be desirable. In agreement, three recent studies detail the recognition and functional properties of neutralizing mAbs that bind a quaternary epitope shared by ZIKV and DENV (8–10).

E protein antigens

19 AUGUST 2016 • VOL 353 ISSUE 6301

**Eur. J. Immunol.** 46, 9 (2016). Both NS1 and the structural proteins are immunogenic. Virus-neutralizing antibodies most commonly target the E protein, may be highly protective in vivo, and are a correlate of protection for many flavivirus vaccines (5). NS1 antibodies are non-neutralizing, yet they contribute to protection via antibody heavy chain–mediated effector functions (6). Whereas the functional characteristics of antibodies in ZIKV-immune individuals have been studied (7), human ZIKV mAbs have not been reported.

Accordingly, Stettler et al. have now isolated a panel of 119 human mAbs from the memory B cells of four ZIKV-infected donors; two of these subjects had been infected previously by DENV (2). Roughly two-thirds of the mAbs produced bound epitopes within the E protein. Antibodies specific for the immunoglobulin (Ig)–like domain III (DIII) had considerable neutralizing activity and were largely specific for either ZIKV or DENV. Numerous cross-reactive mAbs with modest neutralization capacity mapped to E protein domains I or II. Domain II is the location of the highly conserved fusion loop frequently targeted by antibodies elicited by other flaviviruses. Although more study is required, cross-reactive fusion loop–specific antibodies may also be common in ZIKV-immune individuals. Of considerable interest, Stettler et al. found that the most potent neutralizing mAbs bound efficiently to intact virions but not to soluble forms of the E protein, which suggests that antibodies that bind quaternary epitopes composed of more than a single ZIKV E protein may be desirable. In agreement, three recent studies detail the recognition and functional properties of neutralizing mAbs that bind a quaternary epitope shared by ZIKV and DENV (8–10). E protein antigens
and immunogens that include the antiparallel dimers found on virions may be required to capture the full complexity of the humoral immune response when used as diagnostics and vaccines, respectively.

Neutralizing mAbs have potential as therapeutics capable of preventing or limiting disease, when administered after infection. Stettler et al. demonstrated that at least one neutralizing ZIKV type-specific DIII-reactive mAb, genetically modified to prevent antibody-FcγR interactions, was protective when administered 1 day before or after lethal challenge of immune-compromised mice. Similar murine ZIKV DIII–specific mAbs with therapeutic potential were also recently reported (11). In this second study, mAbs were produced from mice immunized with ZIKV and recombinant DIII. Structural studies revealed that the two most potent mAbs were specific for a DIII lateral ridge (DIII-LR) epitope on the surface of intact mature virions. Strikingly, this same DIII-LR epitope is also the target of a potent neutralizing West Nile virus–specific antibody that was developed as a potential therapeutic (12). Whether robustly neutralizing mAbs will have utility for limiting infection and disease in the unborn requires further study in recently developed animal models.

NSI-reactive mAbs were also present in the panel of mAbs described by Stettler et al. Binding studies with recombinant NSI from ZIKV and the four DENV serotypes revealed that most of these mAbs were virus type–specific. Antibody competition studies identified two sites on NSI recognized by ZIKV-specific mAbs. While cross-reactive antibodies that target NSI exist (2), the use of NSI as a more specific antigen in diagnostics has been proposed (13).

The extensive serologic cross-reactivity among flaviviruses, and especially with DENV, poses an obstacle for the specific diagnosis of infection and management of disease, particularly in pregnant women. Existing diagnostic assays have limitations. Multiple commercial real-time reverse transcription–polymerase chain reaction assays for the detection of ZIKV RNA are now in use but are only effective during the brief window when viral RNA is detectable in blood, urine, or other fluids. Serological assays, such as the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA, with virus particles as antigen) and the plaque reduction neutralization test, have the potential to detect prior antigen recognition patterns of recognition in the virus-specific patterns of recognition in the antiparallel dimers found on virions may be required to capture the full complexity of the humoral immune response when used as diagnostics and vaccines, respectively.

The complex serology of ZIKV virus infection

The envelope proteins of ZIKV and DENV share a considerable degree of amino acid homology. Both ZIKV and DENV infection result in the production of antibodies specific for only one of these two viruses [type-specific (TS) antibody] as well as cross-reactive (CR) antibodies capable of binding both viruses to varying degrees. The presence of CR antibody complicates the development of specific and sensitive serological tests to diagnose ZIKV infection in populations frequently exposed to other flaviviruses such as DENV.
Diagnostics for Zika virus on the horizon
Scott D. Speer and Theodore C. Pierson

Science 353 (6301), 750-751.
DOI: 10.1126/science.aah6187