The recent Zika virus (ZIKV) epidemic has created an urgent need for a safe and effective vaccine. There is still a dearth of knowledge about ZIKV immunity, but years of investigation into the immunobiology of other flaviviruses has helped to accelerate the development of a ZIKV vaccine. Although the humoral immune response generates the primary correlate of protection from disease, robust T cell responses could enhance ZIKV vaccine efficacy. Additionally, pre-existing immunity to related flaviviruses could generate cross-reactive T cells that may affect immune responses upon vaccination. In this review, we summarize the key discoveries in the area of flavivirus T cell immunity and postulate on how these findings can inform ZIKV vaccine strategies for inducing protective immunity.

Towards a Zika Virus Vaccine
Until the recent public health emergency in the Western Hemisphere, ZIKV was rarely reported as a cause of human disease [1–3]. ZIKV was found to be unique within the mosquito-borne flaviviruses, because it is also transmitted by other modalities, most notably by sexual contact and vertical transfer [2,4,5]. It usually causes an acute infection with a transient low viremia; however, in some cases, viral RNA can be detected for longer periods of time in serum and other bodily fluids, such as urine, saliva, breast milk, and semen, in humans and nonhuman primates [2,6–11]. The ability of ZIKV to replicate in the host for long periods of time in immune privileged sites, and to cause persistent infection in the central nervous system (CNS) presents unique challenges for preventing and controlling infection [11–13]. Although most cases of ZIKV infection are asymptomatic or mild in presentation, reports of neurologic sequelae, such as Guillain–Barré syndrome in adults, microcephaly, and other fetal development anomalies have heightened the attention paid to this virus [14–19]. The global research community has quickly mobilized to elucidate the underlying mechanisms of ZIKV disease and to develop safe and effective countermeasures; yet, there is still an incomplete understanding of ZIKV immunity. However, the existing body of knowledge on the immunobiology of other flaviviruses provides an opportunity to inform a better understanding of ZIKV immunity and its application to the development of a ZIKV vaccine.

Past efforts to develop and license vaccines against flaviviruses aimed to elicit high titers of neutralizing antibodies (Nabs), which typically correlated with protection from clinical disease [20,21]. Several ZIKV vaccine candidates, shown to be efficacious in different animal models, have correlated protection with the induction of Nabs (reviewed in [12,22]). In addition to antibodies, licensed flavivirus vaccines, such as those against yellow fever virus (YFV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV), have been shown to elicit strong T cell responses [21,23–25]. For example, the live-attenuated 17D yellow fever vaccine induces strong CD4 and CD8 T cell responses that correlate with antibody responses [26]. By contrast, Dengue virus (DENV) vaccine candidates have elicited Nabs titers that only
weakly correlate with protection, leading the field to explore cell-mediated immune responses as an additional parameter of flavivirus vaccine immunogenicity [21,27]. The quality of T cell responses against ZIKV may also be critical for determining protective versus pathogenic immunity and, therefore, may also influence ZIKV vaccine efficacy. In this review, we discuss the importance of T cell responses during the natural history of flavivirus infections and how this knowledge can inform the rational design and development of an effective ZIKV vaccine. We report recent data on T cell immunity in the context of ZIKV infection and the knowledge gaps that remain. Given concerns about the impact of pre-existing immunity on subsequent flavivirus infections, we also discuss the potential interaction of flavivirus memory immune responses on new ZIKV T cell responses and the strategies to enhance ZIKV vaccine efficacy through T cell immunity.

T Cell Responses to Flavivirus Infections

For neuroinvasive flaviviruses, T cell-mediated immunity is important for protection from disease and for viral clearance from the CNS [28–33]. CD4 T cells, although of lower frequency than CD8 T cells during flavivirus infection, are important in providing help to cytotoxic T cells and B cells and are required for antibody maturation. In one murine model, CD4 T cell depletion impaired IgG production and CD8 T cell activation after West Nile virus (WNV) infection [34]. CD4 T cells have also been shown to be essential for DENV clearance in mice [35]. In humans, the presence of multifunctional CD4 T cells has been suggested as an indicator of protection against severe DENV disease and JEV [23,36]. Moreover, a subtype of CD4 T cells with cytotoxic function was found in DENV-immune individuals carrying HLA alleles associated with protection from severe disease, suggesting a protective role for this T cell subtype [37]. For ZIKV infection, CD4 T cell responses have also been detected in infected nonhuman primates [6,7,11], and characterized in wild-type C57BL/6 mice, where antigen-experienced CD4 T cells showed a typical Th1 cytokine profile, with a high degree of polyfunctionality in which most cells produced IFN-γ, TNF-α, and IL-2 [38]. In human donors with a history of ZIKV infection, memory ZIKV-specific CD4 T cells have been detected in the CXCR3+ Th1 compartment [39]. These studies demonstrate that, similarly to other flaviviruses, ZIKV infection induces effector CD4 T cell responses. More studies on its contribution to the production of Nabs and cytotoxic T cell responses could help identify novel correlates of protection that might be useful for flavivirus vaccine development.

There are more data available on CD8 than CD4 T cell immunity against ZIKV and other flavivirus infections. In one mouse study, Nabs were able to clear WNV viremia in CD8 T cell-depleted animals, but virus persisted in the CNS for several weeks, suggesting a role for CD8 T cells in purging the viral reservoir from tissues [40]. Similarly, in mice infected with JEV or DENV, viral clearance from the brain and spinal cord tissues was shown to be primarily mediated by CD8 T cells [32,41]. Given the neurotropism of ZIKV and its presence in fetal brain tissues and cerebrospinal fluid in both humans and animal models [6,17,18,42–44], CD8 T cells may have a key role in clearing ZIKV from the CNS and, thus, in preventing or mitigating neurological complications. A recent study in nonhuman primates showed that no antibodies are present in the CNS during persistent ZIKV infection and the decrease in viral loads in CNS coincided with initiation of cellular immune responses [11]. New studies in animal models suggest a protective role for CD8 T cell responses against ZIKV. Wild-type as well as IFNAR−/− mice develop robust, polyfunctional, cytotoxic CD8 T cell responses after ZIKV infection [38,45]. Depletion of CD8 T cells in these mice before challenge with ZIKV resulted in higher ZIKV titers in both serum and tissues, while adoptive transfer of memory ZIKV-specific CD8 T cells into naive susceptible mice resulted in decreased viral loads after infection [45,46]. In HLA-transgenic mice, immunization with immunodominant ZIKV peptides elicited CD8 T cells responses that lowered ZIKV titers in the serum, livers, and brains of mice after viral challenge [47]. Activation of CD8 T cells after ZIKV infection was found to be diminished in pregnant mice, which might facilitate viral
Box 1. Immunopathogenesis of Dengue Infection

Epidemiologic data have shown that DENV primary infection generates serotype-specific immunity that results in protection against re-infection with the same DENV serotype. However, a secondary infection with a heterologous serotype can result in a more pathogenic outcome [50]. The higher risk for severe disease in secondary infection has been attributed to both humoral and cellular immunity, which may predispose to an immunopathology, contributing to the development of DENV hemorrhagic fever and DENV shock syndrome. Antibody-dependent enhancement (ADE) is hypothesized to occur when antibodies specific for a heterologous DENV serotype are unable to neutralize viral particles and, instead, may facilitate virus entry into target cells through antibody-virus complexes binding to Fcγ receptors on the cell surface. On the cellular immunity arm, cross-reactive T cells are preferentially activated during a secondary DENV infection with a heterologous serotype, recalling memory from the primary infection instead of activating naïve T cells that may express higher avidity receptors, a phenomenon called ‘original antigenic sin’ [110]. These cells usually show low avidity for the secondary serotype resulting in poor-quality cytotoxic function, which leads to a delay in viral clearance, resulting in higher viral loads [56]. These suboptimal cross-reactive T cells produce a different pattern of cytokines, predominantly inflammatory [54,111,112]. Thus, it is believed that a protective cellular immune response to DENV should stimulate high-avidity, homologous responses against all serotypes [99].

spread to the fetus [48]. These preliminary studies in animal models point to a protective role of CD8 T cells in ZIKV infection that should be further investigated in humans.

While the protective role of CD8 T cell immunity has been demonstrated in animal models of flavivirus infection [33,49], both protective and pathogenic roles have been suggested for DENV infection (Box 1) [50,51]. Strong multifunctional CD8 T cell responses have been associated with protection against DENV infection [52]. However, secondary DENV infections can reactivate memory CD8 T cells generated after the primary DENV infection that recognize a different DENV serotype with suboptimal avidity, which results in the proliferation of poor-quality T cells that may contribute to more severe outcomes. Some studies have found a positive correlation between the magnitude of DENV-specific CD8 T cell response and the severity of disease, showing that, although occurring in higher frequencies, DENV-specific T cells in severe disease showed lower levels of degranulation and produced more inflammatory cytokines than in less-severe DENV fever [53–55]. However, these features could be restricted to some HLA-restricted epitopes, since other studies involving genetically different populations and other immunodominant epitopes did not confirm this correlation [36,56].

Harmful CD8 T cell responses could also be implicated in the mechanism of ZIKV-induced Guillain–Barré syndrome, because cross-reactive immune responses induced by the virus may target antigens on host neural tissues [57–59]. Accordingly, common peptides between ZIKV and human proteins associated with Guillain–Barré syndrome and congenital brain malformations have been found, indicating a potential autoimmune process [60].

T Cell Specificity for ZIKV and Cross-Reactivity with Other Flaviviruses

A comprehensive understanding of ZIKV T cell specificity and cross-reactivity with other flaviviruses is critical because ZIKV is spreading in regions where DENV is endemic, YFV outbreaks are increasing, and first responders may have been vaccinated against flaviviruses, if not previously infected with them. ZIKV proteins share approximately 55–58% amino acid identity with other flaviviruses, including JEV, WNV, DENV, and St Louis encephalitis virus (SLEV) [61]. The flavivirus genome encodes a polyprotein precursor that generates three structural proteins: C (capsid), prM (pre-membrane), and E (envelope); and seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NSS. Circulating ZIKV strains worldwide belong to a single serotype [62], although genetic variation has been noted between African and Asian ZIKV lineages. The limited diversity among ZIKV strains (approximately 99% amino acid identity) and the ability of ZIKV-immune sera to broadly neutralize different strains [62–64] suggests that immunity to one ZIKV strain should protect against heterotypic ZIKV infection, as has been shown in a mouse model [65]. The risk of increased severity of disease in secondary ZIKV infections may be less of a concern than for DENV,
which shows more variability in intra- and interserotypes. In addition, a few segments of the ZIKV proteome are conserved across flaviviruses that co-circulate in the same regions, especially DENV, and may permit interactions of immune responses by cross-reactive recognition (Figure 1) [64]. These cross-reactive responses could be protective if they result in the boosting of efficient responses [66], but could also raise the possibility of antibody-dependent enhancement (ADE) and the phenomenon of original antigenic sin, in which suboptimal antibodies and T cells would contribute to an increased risk of severe disease. While DENV-immune sera have been shown to promote ADE of ZIKV pathogenesis in vitro and in immunodeficient mice [67,68], less is known about the interaction of T cell responses to different flaviviruses.

There is a limited understanding of T cell immunity against flaviviruses in humans; some epitopes have been identified, mainly in DENV and WNV (because of their public health importance) [52,54,69,70], but comprehensive mapping data are lacking for other flaviviruses. Some ZIKV CD8 T cell epitopes have been mapped in the murine model for almost all viral proteins, although predominantly for the E protein (Table S1 in Supplemental Information) [45]. An immunodominant CD8 T cell epitope for H-2b mice was identified in E294–302 by two independent studies, regardless of the viral strain used for infection [38,45]. Additional CD8 immunodominant epitopes in H-2b mice were found in E297–305, prM169–177, and NS52783–2792 [45]. In nonhuman primates infected with ZIKV, CD4 and CD8 T cells directed against capsid, E, and NS5 proteins have been detected [6,7]. These data seem to be in contrast to responses documented for other flaviviruses where CD8 T cell responses to nonstructural proteins, particularly to NS5 and NS3, appear to be more frequent (Figure 2A) [36,52,71–73]. Whether
there is a real distinction in CD8 T cell epitope targeting between ZIKV and other flavivirus infections will need to be confirmed in humans. A study using DENV2 peptides showed that CD4 T cells target proteins present in the viral particle (mainly E and C proteins) and the secreted NS1 protein, which are also targeted by B cells [69]. DENV-specific CD4 T cell responses targeting NS3 and NS5, in addition to C protein, have also been demonstrated in individuals vaccinated with a live-attenuated DENV vaccine candidate [74]. Mapping immunodominant epitopes of different flaviviruses will help to screen targets for specific or cross-reactive T cell responses and to establish the potential consequences of pre-existing immunity.

Data are still scarce on dominant human CD4 and CD8 T cell epitopes in ZIKV infection. CD4 T cell responses to NS1 and E proteins were detected in ZIKV-immune individuals, and showed low levels of cross-reactivity to DENV, even in individuals exposed to both viruses [39], although immunodominant epitopes could not be mapped, because T cells were stimulated with whole proteins. CD8 T cell epitopes for ZIKV have been mapped in HLA-transgenic mice, revealing CD8 T cell epitopes in the E protein for both HLA-B*0702 and HLA-A*0101 transgenic mice,
but a larger number of epitopes in nonstructural proteins for HLA-B*0702 transgenic than for HLA-A*0101 transgenic mice [47]. This study also demonstrated changes in immunodominance patterns between naïve and DENV-immune mice, in which previous immunity to DENV modulated CD8 T cell responses towards conserved epitopes that stimulated cross-reactive responses, leading to a reduced breadth of CD8 T cell responses. Importantly, the induction of these altered immunodominant cross-reactive CD8 T cell responses was equally protective against ZIKV challenge compared with ZIKV-specific responses. A similar change in T cell hierarchy was also described for secondary DENV infection with heterologous serotypes [75]. Additionally, in the presence of subneutralizing anti-DENV antibodies, CD8 T cells were shown to be required for protection and to prevent ADE in mice subjected to secondary heterologous DENV infection [49,76]. These findings suggest that the generation of cross-reactive T cell responses by a secondary flavivirus infection (following a primary natural infection or vaccination) is protective even in conditions permissive to ADE, and might be key for preventing antibody-mediated severe disease.

Crossprotection between JEV and DENV has been demonstrated in mice and in humans. Mice vaccinated with both inactivated and live-attenuated JEV vaccine showed overlapping immune responses and crossprotection against all serotypes of DENV [77]. Conversely, in humans pre-exposed to DENV who were subsequently infected with JEV, the presence of cross-reactive T cell responses was associated with better clinical outcomes [23]. Little is known about the crossprotection between ZIKV and other flaviviruses. A study in nonhuman primates infected with ZIKV showed that pre-existing immunity to DENV or YFV led to a higher magnitude of CD4 T cell activation and higher titers of anti-ZIKV IgG, which might indicate that ZIKV-specific responses benefit from pre-existing flavivirus immune memory. However, this flavivirus pre-immunity did not result in crossprotection against ZIKV infection, since no difference was observed between flavivirus-immune versus flavivirus-naive macaques, regarding ZIKV viral replication or in clinical and pathological analyses [78]. Whether crossprotection between other flaviviruses and ZIKV can be detected in humans is still an open and important question, the answer of which may guide a ZIKV vaccine.

**T Cell Responses to Flavivirus Vaccines**

Licensed vaccines against flaviviruses already exist, including live-attenuated YFV vaccine (LAV), the purified inactivated virus (PIV) for TBEV (only in Europe), and multiple platforms for JEV, including LAV, PIV, and a live-chimeric vaccine [20,79]. The development of an effective vaccine against DENV has been challenging; however, a chimeric vaccine with an attenuated YFV backbone has recently been licensed in some countries [80]. T cell responses elicited by these vaccines have been studied and provide insights into the type of T cell immunity that could be induced by a ZIKV vaccine.

The live-attenuated YFV vaccine that was initially developed in 1937 is still one of the most successful vaccines ever licensed, with an effectiveness of 90% and durability of several decades [81]. This attenuated virus is able to infect antigen-presenting cells [82] and stimulate a robust adaptive immune response, characterized by the generation of high titers of Nabs, a polyfunctional CD8 T cell response, as well as activation of CD4 T cells exhibiting a balanced Th1 and Th2 profile [25,83–86]. Early activation of CD4 T cells precedes activation of CD8 T cells and B cell maturation [87], and is associated with higher titers of Nabs [88]. Activated CD8 T cells, which peak 2 weeks after vaccination, are detected in blood at time-points that coincide with a viral load decline [83,89,90]. YFV-specific effector CD8 T cells exhibit a CD45RA⁺PD-1⁺ phenotype that transitions to a CD45RA⁻PD-1⁻ phenotype on memory YFV-specific CD8 T cells [87]. A similar phenotype of fully differentiated memory CD8 T cells has also been shown in DENV-specific responses [91] and the transient expression of PD-1 in effector cells was also detected in CD8 T cells from individuals bearing HLA alleles associated with resistance to DENV disease,
suggestion that this marker could be a surrogate of a protective T cell response [92]. Under a specific viremia threshold, the magnitude of the YFV-specific CD8 T cell response correlated with viral load (although there was no association with higher viremia), highlighting that the antigen load can influence the magnitude of response [89]. The frequency of YFV NS4B-specific CD8 T cells also correlated with YFV Nabs titer, suggesting that activation of cellular and humoral responses induced by vaccination depends on common factors [26]. This latter study also demonstrated that an activated immune microenvironment before vaccination impaired both arms of adaptive immune responses and shortened the duration of immunological memory [26].

Due to its success, the YFV vaccine has been the basis for developing chimeric vaccines against other flaviviruses, such as DENV, JEV, and WNV, in which the structural genes encoding prM and E are inserted in place of homologous genes in the attenuated YFV backbone [93]. The chimeric tetravalent DENV vaccine (CYD-TDV), comprising the four DENV serotypes, stimulates CD4 and CD8 T cell responses towards the structural proteins E and prM of all four serotypes, which show a Th1-biased profile [94]. Phase III clinical trials revealed higher vaccine efficacy for recipients who were DENV seropositive at the time of vaccination and, in general, the vaccine reduced the risk of severe disease, except for the 2–5-year-old group, which had a higher incidence of hospitalization [95]. Although age could be a covariant for vaccine efficacy, it is likely that DENV-naïve individuals predominated in this younger age group. Thus, in DENV-naïve recipients, the vaccine could prime immune responses equivalent to a primary infection that, after decay of optimal levels, would increase the risk of severe disease upon reinfection, while in DENV-immune individuals, the vaccine would boost pre-existing immune responses contributing to protection [96]. Interestingly, this vaccine boosts CD8 T cell responses towards DENV NS3 epitopes in DENV-immune individuals [94], demonstrating cross-reactive T cell activation, since the vaccine contains the gene encoding YFV NS3. This is an interesting finding, given that no cross-reactive NS3-specific CD8 T cell responses were detected in flavivirus-naïve vaccinated individuals [97]. Given the absence of DENV nonstructural proteins in this chimeric DENV vaccine, the lack of DENV-specific T cell stimulation in response to these main targets might have accounted for the low efficacy observed for the vaccine in flavivirus-naïve individuals [98]. Conversely, the reactivation of memory T cells that cross-reacted with YFV nonstructural proteins present in the chimeric vaccine may have been partly responsible for the higher efficacy in DENV-immune individuals. Moreover, pre-existing immunity against YFV and JEV resulted in quicker and broader T cell responses and higher antibody titers [39]. In mice, it has also been shown that sequential immunizations with different YFV chimeric vaccines stimulated cross-reactive T cell responses directed to E protein epitopes and also boosted YFV NS3-specific CD8 T cells [100].

Another DENV vaccine candidate that is in clinical trials (TV003/TV005) comprises a tetravalent formulation of an attenuated DENV. Individuals vaccinated with only one serotype component of this vaccine showed T cell responses to both structural (10–37%) and nonstructural (63–90%) proteins. However, when individuals were vaccinated with the tetravalent formulation, 97% of T cell responses were directed to nonstructural proteins, predominantly to the most conserved epitopes [101]. Tetravalent vaccination was also shown to elicit T cell responses similar in magnitude and breath to those after natural DENV infection [74].

All these data were generated from live-attenuated vaccines that induce T cell responses against the entire viral proteome. However, for ZIKV, other vaccination strategies are prioritized over the live-attenuated approach, thus limiting the likelihood that a ZIKV vaccine that is licensed will induce potent T cell responses. Since inactivated vaccines or virus-like particles are nonreplicative, they only present structural proteins as antigens, which are principally targets for neutralizing antibodies, in contrast to replicative vaccines, which contain structural
and nonstructural proteins (Figure 2B) [24]. The lack of viral protein production inside host cells and the absence of nonstructural proteins, which would be potent targets for CD8 T cell stimulation, might account for the weak stimulation of T cell response by inactivated vaccines and, thus, may potentially result in the poor induction of long-term protection. This could be overcome by the incorporation of potent T cell epitopes in the vaccine formulation. For instance, incorporation of the helicase domain of DENV NS3 protein into a PIV vaccine against DENV-2 was shown to enhance CD4 T cell responses as well as antibody production in mice [102].

**Development of an Effective ZIKV Vaccine**

The development of a vaccine against ZIKV has advanced rapidly from preclinical to clinical testing in the year since a public health emergency of international concern was declared. Nonreplicative vaccine strategies are favored for their safety profile, especially in the population likely to be targeted for vaccination, namely, women of child-bearing age [103]. Several vaccine approaches have been tested in animals, including nucleic acid vaccines [104,105], purified inactivated virus, vector-based vaccines [106,107], and live-attenuated ZIKV [12,108]. A plasmid DNA vaccine encoding the prM and E gene sequences from a Brazilian isolate (strain Brazil BeH815744) and an alum-adjuvanted PIV vaccine were able to provide complete protection in mice and rhesus monkeys against ZIKV challenge [106,107]. In addition to neutralizing antibody production, these vaccines also induced modest T cell response towards E protein; however, the subset of T cells activated was not discriminated. Depletion of CD4 and CD8 T cells before viral challenge did not abrogate protection [106], and vaccine efficacy was attributed to antibodies, because the titer of E protein-specific binding antibodies and Nabs correlated with protection. Additionally, adoptive transfer of purified IgG from mice vaccinated with plasmid DNA provided near-complete protection to nonvaccinated mice, depending on the dose administered [107]. However, purified IgG from PIV-vaccinated monkeys was only partially protective for naïve monkeys, suggesting that antibodies alone will not be sufficient to achieve complete protection against ZIKV challenge. Importantly, no enhancement was observed in IgG-treated animals that became infected because they exhibited lower viremia than nontreated control animals, an important reassurance regarding PIV vaccine safety [106]. The same prM/E insert used for the plasmid DNA vaccine, administered through a single-shot recombinant rhesus adenovirus (serotype 52) vector, elicited higher levels of T cell responses and neutralizing antibodies than the DNA plasmid vaccine and also completely protected rhesus monkeys against ZIKV challenge [106]. These optimistic results of vaccine protection were obtained upon intravenous and subcutaneous challenge. Since other routes, such as vertical and sexual transmission, have also been implicated in ZIKV epidemic, other routes of challenge as well as protection of the fetus by challenging pregnant animals should also be explored.

The ZIKV vaccine platforms being tested in human trials (PIV, plasmid DNA, mRNA, and viral vector platforms [109]) will likely induce weak T cell responses limited to the structural proteins (Figure 3). PIV are promising candidates for the rapid control of the ZIKV epidemic, because the platform has a well-established profile of safety and efficacy for other flaviviruses (JEV and TBEV). In a rational design to promote T cell responses, several avenues can be taken. The use of adjuvants to formulate these vaccines could enhance the magnitude of T cell responses. These vaccines can also be formulated to include nonstructural proteins or peptides containing immunodominant epitopes for T cells that would increase the breadth of T cell responses to nonstructural proteins. Finally, a combination of these ZIKV vaccines with other flavivirus vaccines could be a good strategy to take advantage of the pre-immune cross-reactive memory to broaden immune responses and increase vaccine efficacy. Moreover, the vaccination of individuals having been infected by other flaviviruses in the past could also benefit from the cross-reactive T cell responses for conserved epitopes with ZIKV.
Figure 3. Strategies to Improve Zika Virus (ZIKV) Vaccine Efficacy. The ZIKV vaccine platforms currently being tested in clinical trials will likely induce weak T cell responses limited to the structural proteins. Different approaches can enhance the efficacy of these vaccines by inducing higher frequencies and broader T cell responses. While vaccine formulation with T cell stimulator adjuvants and addition of T cell immunodominant epitopes are strategies focused on T cell responses, pre-immunity to flaviviruses acquired from natural infection or vaccination would also induce the production of cross-reactive antibodies and, therefore, the consequences of which on the risk of generating immunopathology by antibody-dependent enhancement (ADE) should be investigated.

Concluding Remarks
ZIKV vaccine studies have greatly benefited from previous knowledge and technologies applied to other flaviviruses. Although the assessment of T cell responses has not been a priority in flavivirus vaccine studies, a better understanding of such responses could help define the determinants of effective immunity and, ultimately, correlates of protection and immunopathology. The first studies available on T cells in ZIKV infection appear to indicate that the epitopes targeted are mostly in structural proteins and differ from those seen with other flaviviruses, where mostly nonstructural proteins are dominant, although these preliminary results were from studies performed in mouse models and may not reflect what occurs in humans. This will need to be confirmed in future studies in humans. The unique route of
transmission, pathogenesis, and tropism might also create specific challenges for ZIKV protection compared with other flaviviruses and might require eliciting a different quality of T cell responses. Cross-reaction among co-circulating flaviviruses must be taken into account, and more studies considering both humoral and cellular immunity should elucidate the protective or detrimental role of pre-existing flavivirus immunity to ZIKV infection and vaccination. The vaccine candidates under development have shown promise to quickly control the ZIKV epidemic. DNA and PIV vaccines have the advantage of providing a safe approach ideal for women of child-bearing age, an important factor for preventing ZIKV-associated microcephaly and other congenital abnormalities. Further studies will show whether these types of ZIKV vaccine could take advantage of additional strategies to enhance T cell immunity in addition to generating a strong humoral response or from the pre-existing immunological memory induced by natural flavivirus infection or by heterologous flavivirus vaccination (see Outstanding Questions). The unique challenges of the recent ZIKV outbreak have produced a collaborative and integrative environment where both scientific advances and clinical development are conducted together at a record pace to resolve these questions.

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