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antileishmanial immunity to be able to explore the therapeutic potential.

Another potential target would be the transcription factor HIF-1α, the master regulator to the response to hypoxia. HIF-1α alters myeloid cell functions during *L. donovani* infection, leading to inhibition of T cell responses. It is also required by Leishmania to survive inside macrophages. In the case of HIF-1α, the therapeutic window is very small, since this transcription factor is involved in many vital functions that are unrelated to the immune response. Similarly to B cells, we first need to dissect specific pathways of activation and downstream effects of HIF-1α to better exploit the therapeutic potential of this target.

**What is the current status of visceral leishmaniasis? Which pressing steps should be taken to reduce it?**

Leishmaniasis is on the WHO list of neglected tropical diseases, despite the fact that the disease is spread over 98 countries and, in the case of visceral leishmaniasis (VL), is still taking a human toll. Over a billion people living in endemic areas are at risk of infection worldwide. There are about one million estimated cases of cutaneous leishmaniasis each year, and 300 000 of visceral leishmaniasis. Some 90% of the VL cases occur in India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia. Leishmaniasis mainly affects poor people in tropical and sub-tropical regions of the world, and is also associated with factors that are independent of genetics, such as malnutrition, poor housing, and population migration. Hence, an effective vaccine would be an ideal solution for controlling disease and limiting transmission, especially for species that can be transmitted from humans to humans, such as *L. donovani*. Unfortunately, to date there is no vaccine available on the market. Another important point is the constant emergence of drug resistance and consequently the urgent need to develop new drugs. Better epidemiological data are also required to thoroughly understand various reservoirs of infection, and gather information on vectors and Leishmania strains and species affecting a determinate region in correlation with disease outcome and severity. Finally, we certainly need more information on disease transmission and the role of asymptomatic carriers in parasite transmission.

The WHO aims to reduce the overall burden of these diseases by 2020. In your opinion, are we on track to achieve those goals? Why?

A reduction in the overall burden could definitely be feasible for some countries. Unfortunately, as I mentioned before, leishmaniasis is a disease that is associated with poverty, malnutrition, poor housing, and population displacement. The increasing incidence of conflicts and political and economic instability in certain regions of the world has actually worsened the disease burden in those areas in the past decade. A vaccine would be a cost-effective and a long-term solution to limit the overall spread of the disease in endemic countries. Unfortunately, I think that we are still far away from having one. Leishmanization (subcutaneous immunization with live parasites) has actually conferred a good degree of protection against cutaneous leishmaniasis. However, this method has never been officially approved because of the possibility that large scars may remain after immunization in certain individuals. Moreover, the efficacy of leishmanization against visceral strains has not yet been proven in humans. For the moment, control of infection relies on the systemic treatment of patients and on reservoir and vector control. This method will definitely reduce disease burden in the short term, but only until the emergence of drug-resistant strains. A long-term solution is warranted.

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**Science & Society**

**A Call to Introduce Structured Zika Surveillance in India**

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India has the climatic conditions conducive to year-round transmission of Zika virus, and a structured disease surveillance program should be implemented to prevent an outbreak. Such a program should (i) start screening before an outbreak arises; (ii) collect baseline data to assess future disease risk and monitor potential birth defects; and (iii) provide new insights into the ecology of the disease and inform public health policy following the one health concept.

**Zika in India**

The recent outbreak of Zika in South America brought Zika under the spotlight and led the World Health Organization (WHO) to declare it an international public health emergency in 2016, with an immediate need to prepare for, and respond to, any future epidemic. The WHO has since declared the end of the public health emergency, but Zika, a mosquito-borne flavivirus, remains a cause of global concern, particularly in resource-limited countries in Africa and the Asia-Pacific region. In these countries, the presence of competent mosquito vectors and suitable climatic conditions could support local transmission of the virus.

In India, four cases of Zika have now been reported in the media. In November 2016 and February 2017, three cases of Zika were confirmed from a densely populated area (Bapunagar) in the city of Ahmedabad in western Gujarat state, India.

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Whilst the three patients (two pregnant women and an elderly man) showed no complications, and had not travelled outside the country, the government confirmed these cases only several months later[1]. More recently, in July 2017, a fourth case, reported from Tamil Nadu state, was confirmed in a man[2]. These are not the first cases of Zika virus reported from India; a study conducted in 1953 detected the neutralizing antibodies of Zika virus in one-sixth of blood sera samples, suggesting exposure to the virus [1]. As I discuss below, India has the ideal ecological conditions to support transmission and to host a Zika outbreak; however, no measures were taken to combat future outbreaks and there is as yet no structured disease surveillance program.

Vectors, Human Movement, and Zoonotic Transmission

Zika is thought to be primarily transmitted by *Aedes* mosquitoes[3] at elevations <2000 m above sea level [2], but the scientific literature is dichotomous on whether *Culex* mosquitoes can transmit the virus. In India, *Aedes aegypti* is the only mosquito species that is screened for Zika, albeit at a low frequency (>24 000 *Aedes* mosquito samples screened until September 2017[4]. Recently, a controversial report originating from China [3], and a second report from Brazil [4], suggested that *Culex quinquefasciatus* could also potentially transmit the virus. *Cu. quinquefasciatus* is widely distributed and prevalent in India and should be screened for Zika virus to increase the likelihood of predicting any potential outbreak. Furthermore, vector control is key to prevention and control of mosquito-borne infections and, in India, Wolbachia-based vector control strategies for *Aedes* mosquitoes are currently being proposed and prioritized for Zika [1].

A recent study [5] further emphasises the Zika transmission potential of *Aedes* mosquitoes, but also highlights sexual transmission via travellers returning from Zika virus-affected areas. The role of human movement has clearly been central to disease transmission following a string of recent outbreaks, including Ebola, H1N1 influenza, and severe acute respiratory syndrome (SARS). The same study [5] reported that global human mobility places large populations at risk of mosquito-borne Zika virus infection. In India, an estimated 1.2 billion people (67 422 air travellers arriving per year from outside the country) are susceptible to Zika virus exposure at the time of peak seasonal risk, which coincides with dengue outbreak in the rainy season (August). Even though these predictions are based on very conservative estimates [5], such modelling efforts may offer useful insights to time-sensitive public health decision-making in India.

In Africa and South East Asia, many other host species may support Zika virus infection; forest-dwelling birds [6], horses, goats, cattle, ducks, and bats [7] have been reported based on serology, but were not verified by viral isolation. The question of whether birds transfer the virus over long distances remains unanswered. In India, infectious disease ecology with respect to wildlife is completely neglected and, consequently, the potential for the Zika virus as spillover infection from other animals to humans has never been considered. Furthermore, assessing future zoonotic disease risk requires baseline data – information about where infectious diseases are distributed geographically, taxonomically (with respect to animal reservoirs), and in relation to human populations. This is illustrated in Figure 1; the key ecological drivers of Zika virus and seasonality in India, where warm temperatures prolong the mosquito season – high mosquito density, densely populated areas, and a continuous flow of airline travellers – are likely to facilitate virus transmission by increasing human–mosquito–human contact. The Indian Zika cases have been reported outside the monsoon season and August, which is modelled as month of peak exposure to Zika virus [5]. In addition, the presence of wild animals, such as *Rhesus* macaques and other animals within human habitation, blurs the boundaries between reservoir hosts and spillover infections, further complicating the prediction of zoonotic disease events in India.

The Zika Puzzle

Of the four cases of Zika reported in the media, none has been published in a scientific journal. A confirmed case of Zika virus disease requires laboratory confirmation of infection by either the presence of Zika virus RNA or of antigen in serum or other samples (e.g., saliva, tissues, urine, whole blood); or alternatively the detection of IgM antibodies against Zika virus. The four cases of Zika reported in the media in India seem to have been caused by the Asian strain of the virus, the same strain that caused the Zika outbreak in Brazil[8]. However, without a confirmed record, it is difficult for the international scientific community to understand the extent of the disease or even to acknowledge the Indian government’s reluctance to report these cases. Furthermore, flavivirus genetic data are key to understanding the parasite’s movement and can be used to infer whether a given strain is the result of locally acquired transmission or importation based on relatedness to locally isolated strains (e.g., [5,8]). Without these data we cannot learn (i) if there has been an outbreak of Zika virus at the reported sites in India, and (ii) why the extent of outbreak was not as amplified as in South America or French Polynesia.

The ecological modelling study [5] predicts August as the time of peak seasonal risk for transmission of Zika in India; however, three of the four reported Zika cases in India appeared between November and February in areas with relatively poor access to health services. This discrepancy could be due to an extended mosquito transmission season or delayed appearance of symptoms. Malone et al. [9] reported that up to 80% of cases of adult human infection with Zika virus
remain asymptomatic, and it is reasonable to assume that the total number of cases in India is likely to be underestimated as they can easily be confused with chikungunya and dengue [10]. The sporadic and small number of Zika cases from India probably suggests either apparent immunity to the virus or a lower number of susceptible hosts to maintain transmission between humans and mosquitoes. A similar trend is now being observed in the Americas where transmission persists at low but steady levels with no symptoms [11]. However, the virus may persist in acute or chronic stages in humans or as zoonotic infection in reservoir hosts, which can be a source for future outbreaks.

Call for a Surveillance Program
The concept of disease ecology, or the idea that host–pathogen interactions can be studied within the context of their environment, which is central to understanding the epidemiology and to preventing future outbreaks, has remained neglected in India. In fact, India is an ideal place to explore the coevolutionary dynamics of this host–parasite system because of several factors: (i) the high volume of human movements [5], (ii) the apparent immunity to Zika from circulating strains of the virus [1], and (iii) the possibility of transmission in less immunocompetent hosts, such as pregnant women and the elderly [9], and (iv) adults with a prior history of malaria or dengue infections, which may help facilitate transmission and pathogenesis of Zika, potentially resulting in a positive feedback loop [12]. If there is a positive feedback loop, it may result in a malaria or dengue outbreak and/or the spread of strains of Zika adapted to hosts with prior infections. Whilst there is no influence of pre-existing immunity to dengue in the genetics of Zika infection in macaques [13], this work does not rule out a possible role of preimmunity in the immunologic enhancement in Zika-associated neurological and congenital abnormalities. These factors place India in a privileged place to better understand Zika evolution in the Asian context and whether reported microcephaly cases are Zika-linked or not. But to achieve this, there is an urgent need to collect data on microcephaly and other birth defects [9]. Finally, to reduce the risk of a Zika outbreak in the future, India needs a structured disease surveillance program that will go beyond sample screening post-outbreak; the program should include widespread serosurveillance for underlying population immunity, predictive sampling to collect baseline data to assess future disease risk, and should follow the ‘one health’ approach with the collaboration of public health officials, clinicians, social scientists, and local government. Such work, together with enhanced health care infrastructure aimed at improved diagnostics and maternal care, could provide novel insights into the ecology of the disease and protect women of childbearing age from the potential devastating fetal complications.

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Resources
www.who.int/mediacentre/news/statements/2016/zika-fifth-ec/en/
Spotlight

Targeting Plasmodium Proteases to Block Malaria Parasite Escape and Entry

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Proliferation of malaria parasites in a host requires mechanisms to spread between red blood cells (RBCs). We discuss here the implications for biology and antimalarial drug development of companion studies that establish the requirement of two Plasmodium spp. proteases of the plasmepsin family in parasite egress from, and invasion into, RBCs.

Protozoan parasites of the Plasmodium spp. cause malaria through cyclic waves of replication within the RBCs of infected individuals, followed by infection of new host cells resulting in large numbers of parasites within circulation. The mechanisms that guide malaria parasites through egress from infected host cells, and invasion into uninfected RBCs, are a subject of intense interest, not least because this critical step in proliferation has been little explored for the development of new drugs for the treatment of malaria and combatting the emergence of drug resistance. Plasmodium spp. employ intracellular proteases to break down the surrounding vacuolar and RBC membranes to initiate egress into the bloodstream [1]. Two recent papers identify essential roles in egress and invasion for two previously uncharacterized members of the plasmepsin (PM) family of aspartic proteases, PMIX and PMX [2,3]. The studies further demonstrate the potential for PMIX and PMX as drug targets to block malaria infections in vivo.

Plasmodium spp. express multiple plasmepsin protease genes during the blood stage that regulate distinct functions through the cell cycle, including hemoglobin digestion and the export of parasite proteins for remodeling of the host cell (e. g., [4,5]). PMIX and PMX, two homologs of a subclass of the plasmepsin family [6], are specifically expressed during the schizont stage of development late in the asexual cell cycle, and had not been previously characterized. In parallel, Nasamu et al. [2] and Pino et al. [3] studied the biological functions and potential drug susceptibility of these proteases in Plasmodium parasites. The researchers employed a combination of reverse genetics and chemical biology to discover novel, essential functions for these plasmepsin genes in egress and RBC invasion.

The two studies use either conditional knockdown [2] or conditional knockout [3] in Plasmodium falciparum to show that PMIX is specifically required for RBC invasion. PMIX is enriched at rhoptries [2,3], organelles released into the RBC during host cell attachment to initiate formation of a parasitophorous vacuole (PV). PMV is required for efficient proteolytic processing of rhoptry factors in vivo [2,3], with biochemical evidence in vitro for direct activity toward rhoptry-specific substrates [3]. Based on morphology, proteolytic processing by PMIX appears to mediate biogenesis of functional rhoptry organelles [2].

Nasamu et al. employed conditional knockdown in P. falciparum to identify an essential function for PMX in RBC egress, with evidence for function also in invasion [2]. PMX is targeted to exosomes [2], among the earliest parasite organelles discharged during egress, and is required in vivo for proteolytic maturation of the serine protease

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