STUDY ON BRAZILIAN ARBOVIRUSES AND RODENT RELATED VIRUSES

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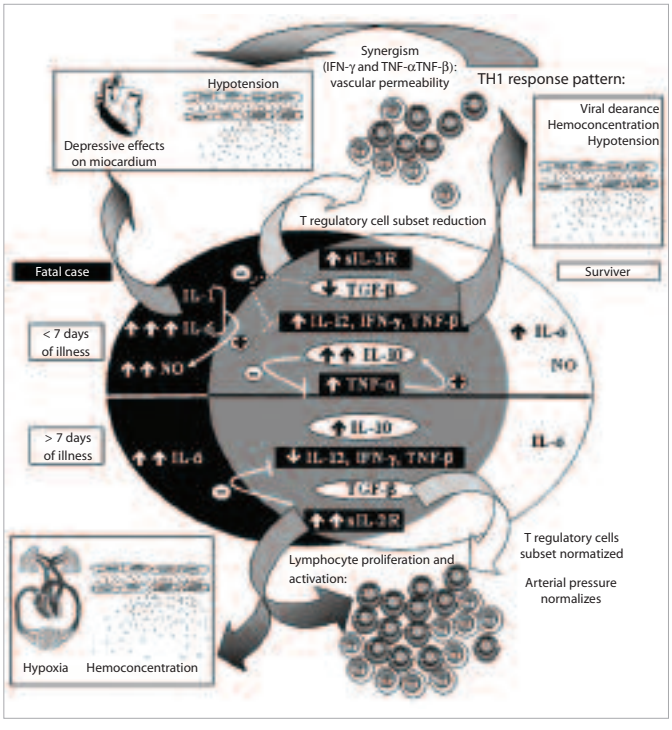
This thematic research project includes 13 sub-projects on arboviruses and rodent related viruses that cause human diseases in Brazil. This project will be carried out in 4 years. The flavivirus dengue type 1 was introduced in Rio de Janeiro in 1986 and spread in Brazil causing large outbreaks. In 1991, dengue type 2 virus caused an outbreak in Rio de Janeiro where dengue hemorrhagic fever/dengue shock syndrome (dhf/dss) cases were first reported. Dengue became endemic in Brazil with circulation of 2 serotypes in cities infested by *Aedes aegypti*. After 1998, hundreds of thousands of dengue cases have been yearly reported in Brazil and the number dhf/dss cases have increased. In 2001, dengue type 3 virus was introduced in Rio de Janeiro. More than 800,000 dengue cases were reported in 2002 including more than a thousand dhf/dss cases, and about 100 fatalities. The situation of dengue in Brazil is worsening, and it probably will become a serious public health problem as observed in the southeast of Asia. As part of this research project, molecular biology diagnostic methods of dengue, studies on dengue pathogenesis and genomic markers of dengue virus virulence will be studied. Sylvatic yellow fever is expanding in Brazil related to a zoonosis of primates transmitted by haemagoggus mosquitoes. In the past 4 years, dozens of severe human cases of yellow fever, many of them lethal, have been reported among people living close to the highly populated areas of Brasília, Goiânia and Belo Horizonte, as well as in the north of the state of São Paulo. The urbanization of yellow fever threats Brazil, especially in cities infested by the *Aedes aegypti* vector. The attenuated 17dd yellow fever vaccine is highly immunogenic and it is a suitable tool in order to avoid urbanization of the disease. However, some rare fatal cases did occur after vaccination, which could impair an intensive use of the vaccine. As part of this project, the structure and functional mechanism of the ns5 rna polymerase of yellow fever will be studied in order to allow further studies on specific antiviral drugs. The



Schematic model of cytokine participation in HCPS pathogenesis based on results obtained in the present study. The black circle includes findings in fatal cases while the white circle includes findings in survivors. Findings common to both are in intersection (gray area). Cytokines in black boxes are pro-inflammatory whereas cytokines in white boxes are anti-inflammatory. The gray arrows indicate inflammatory effects and their consequences, whereas white narrows indicate anti-inflammatory effects and their consequences.

Orthobunyavirus oropouche is the causative agent of the second Brazilian arboviral disease in number of reported cases. The virus causes outbreaks of acute febrile illness and encephalitis in towns at the borders of rivers of the Amazon region. Oropouche virus is maintained in urban cycles involving *Culicoides paraensis* vector and man. About 500,000 Brazilians were infected by the Oropouche virus in the last decades. We aim to test antiviral drugs against the Oropouche virus; to study a hamster experimental model of Oropouche fever, virus propagation in cell cultures, and mechanisms of virus replication. A cold-adapted virus strain is to be used for creating a vaccine. The rodent related hantaviruses are known to cause cardiopulmonary syndrome (HCPS).

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The Hantavirus Cardiopulmonary Syndrome (HCPS) is an emerging syndrome in the Americas resulting from multiple pathogenic factors associated with intense immune activation and changes in vascular permeability. The aim of this study was to determine the profile of serum cytokines in HCPS patients and their correlation with clinical parameters, severity and outcome of illness. By studying 21 HCPS patients we found that IL-6 probably has a more important role in the pathogenesis of HCPS than TNF-α, being associated with a fatal outcome. We have also shown that the immune response in HCPS follows a TH1 pattern, and that the magnitude of TH1 response effector cytokines is correlated to HCPS severity. The decreased levels of TGF-β observed in HCPS patients suggest that the immunoregulatory activity could be damaged in an early stage of illness. HCPS was first described in Brazil in 1993, and about 240 cases have been reported with a 40% fatality rare. The cases we studied presented prodromic fever that involved after 3 to 5 days to dyspnea, respiratory failure and shock. Thrombocytopenia and elevated hematocrit were also observed in these cases. We have also developed an RT-PCR diagnostic method for hantavirus, and detected the virus genome in the blood of 11 hcps patients. Nucleotide sequences of RT-PCR amplicons from these patients showed a 96.5 to 87.7% homology with the Araraquara hantavirus genome, thus showing that these cases were caused by this virus. We also performed a serologic survey for hantavirus in 2001 in Jardinópolis, São Paulo State, including 818 participants, and 14.3% of them presented IgG antibodies against the Andes hantavirus as detected by ELISA. Soropositive participants were not associated to sex, age, previous contact with rodents or severe pneumonia. These results suggest that hantaviruses may be causing undiagnosed asymptomatic or clinically minor infections in Brazil. It entails important questions such as whether more than one hantavirus strain would be circulating in Jardinópolis, causing mostly benign infections. Could hcps be associated with some predisposing condition in the infected individuals? As part of this project, a search for benign infections will be carried out in order to study the disease and the infecting hantavirus. Virus-reli interactions and the pathogenesis of HCPS will be studied based on the detection of cytokines and genetic markers. Additionally, a recombinant N protein of the Araraquara virus will be produced for diagnostic methods and as a vaccine candidate.

MAIN PUBLICATIONS

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