

## Non-human primates and *Leishmania* immunity

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### ARTICLE INFO

#### Keywords:

Leishmania  
Monkeys  
Immunity  
T cells  
Tfh  
CXCR5  
Cytokines

### ABSTRACT

In the context of infectious diseases, non-human primates (NHP) provide the best animal models of human diseases due to the close phylogenetic relationship and the similar physiology and anatomical systems. Herein, we summarized the contribution of NHP models for understanding the immunity to leishmaniasis, which are a group of diseases caused by infection with protozoan parasites of the genus *Leishmania* and classified as one of the neglected tropical diseases.

### 1. Introduction

The leishmaniasis are a group of diseases caused by infection with protozoan parasites of the genus *Leishmania*, affecting more than 98 countries, and are classified as one of the neglected tropical diseases (NTD). Worldwide, the population of 350 million is at risk with an annual incidence of 0.7–1.2 million cases of cutaneous leishmaniasis (CL) and 0.2–0.4 million cases of visceral leishmaniasis (VL). VL is the most severe form of leishmaniasis and is caused by *Leishmania infantum* or *L. donovani* leading to the infection of spleen, liver and bone marrow. The disease is transmitted to various mammals through the bites of infected sandflies. Thus, the domestic dog is the main urban reservoir for VL and they have been used as model for the study of VL disease [1–3]. However, the immunological tools to study canine VL are limited. Inbred mice strains have been useful to investigate the mechanisms of adaptive and innate immune responses but invariably control infections with viscerotropic *Leishmania* species and develop a life-long latent infection [4]. Moreover, mice and humans differ in number of effector molecules such as defensin [5,6] and granulysin [7] and the capacity of human phagocytic cells to produce NO appears to be under much tighter regulation [8,9]. Furthermore, no infection of intestine is observed in mouse model [10].

Therefore, despite the usefulness of these models, new insights into the immunopathogenesis of VL would benefit from a more frequent employment of alternative animal models such as non-human primates

(NHP) that constitute powerful experimental models for understanding host-pathogen interactions in humans.

#### 1) Non-human primates and human infectious diseases

The phylogenetic closeness of non-human primates (NHP) to humans makes them attractive models for assessing the pathogenicity related to infectious diseases, as well as for the development of vaccine and drug therapies. New World primate species include the families of *Cebidae*, *Callitrichidae*, *Aotidae*, *Pitheciidae*, and *Atelidae* (Fig. 1). Apes (*Hominoidea* and *Hylobatidae*) and Old World monkeys are native to either Africa or Asia. The latter are subdivided into two distinct sub-families: *Colobinae* and *Cercopithecinae* [11]. Of interest, experimental microbe infections have demonstrated distinct susceptibility of non-human primate species suggesting host-factors associated [12–14]. In the context of infectious diseases, the use of great apes has been discontinued, mostly due to ethical reasons. Macaques (member of the *Cercopithecinae* family) have been extensively explored over the past century as models of infections caused by bacteria, virus, parasites or prions. A major boost in the use of macaques for studies on the immunology of infection was driven by the emergence of the AIDS pandemic in the mid-80's. Soon after, it was discovered that macaques infected with the HIV closely-related simian immunodeficiency virus (SIV) developed a progressive immunodeficiency similar to AIDS [15,16].

#### 2) Non-human primate models and leishmania

a) Visceral leishmaniasis: In 1924, Shortt *et al.* reported an

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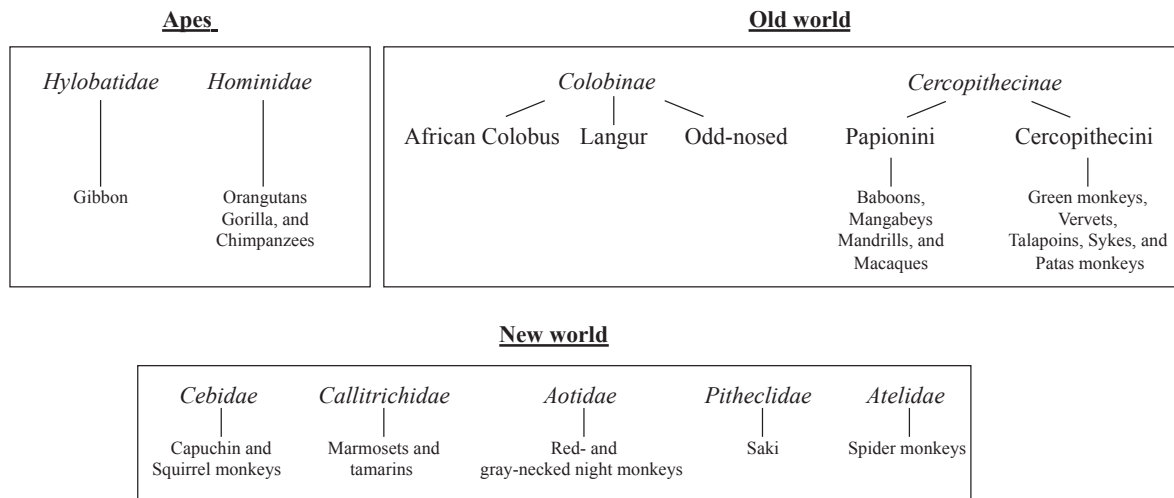


Fig. 1. Primate phylogeny.

experimental infection of Old World primates by intradermal inoculation of splenic puncture material from an Indian patient with Kala-azar [17,18]. This was reproduced two years later by Greig *et al.* [19]. Experimental infections of vervets also cause visceral organ enlargement and parasitosis following intraperitoneal, intranasal or intradermal *Leishmania* inoculation [20,21]. Experiments performed thereafter clearly demonstrated that various monkeys living in Africa including sykes and baboons can be infected experimentally with *L. donovani* or *L. infantum*, showing low grade infections for periods ranging between four and eight months followed by evidence of spontaneous cure [22–24]. Furthermore, infection of (*Presbytis entellus*) a langur monkey was demonstrated to be a model for *L. donovani* [25–27]. In contrary, experiments performed in New World monkeys including Aotus monkeys [28,29], squirrel monkeys [30] and marmosets [31], demonstrated fulminating VL after intravenous or intraperitoneal inoculation of amastigotes. Due to the rapid disease development, New World species have been useful for drug testing [32,33]. Finally, disease mimicking human VL was established in macaques. These animals developed a systemic disease showing characteristic features of human VL such as fever, diarrhea, body weight loss, anemia, hypergammaglobulinemia and transient lymphocytosis, as well as lymph node, liver and spleen enlargement [34]. Thus, although macaques were less susceptible to develop fulminant infections as compared to new world species, they are considered a model for pre-clinical evaluation of novel chemotherapeutics or vaccine candidates for human VL.

b) **Cutaneous leishmaniasis:** Vervet monkeys can be also animal model for studying *L. major* infection [35–38]. Like infected individuals with leishmaniasis, vervets have been shown to undergo spontaneous cure following experimental infection with *L. major*. Furthermore, *L. braziliensis* infection of macaques resulted in localized skin ulcerations, but complete spontaneous clinical healing occurred in infected animals. The lesion development was variable depending on the challenging parasite [39–43], dose and route of exposure [39,44]. Furthermore, monkeys infected with *L. major* transmitted by *Phlebotomus papatasi* [45] developed skin lesions longer than infections induced by needle inoculation [40]. Hence, monkeys have been considered to be useful for studying the interactions between parasite and host determinants for leishmania infection as well as for the evaluation of new drugs and candidate vaccines for human disease [46].

### 3) Immune responses in *Leishmania*-infected NHP

Primate models have been used to study host responses to *leishmania* [47–53]. Of interest, studies with the vervet model for cutaneous leishmaniasis have demonstrated that resistance is correlated with increased production of gamma interferon (IFN- $\gamma$ ) and strong delayed type hypersensitivity (DTH) responses similar to those seen in human

patients with cutaneous leishmaniasis [54,55]. Macaques infected with *L. major* developed a typical T helper (Th) cytokine profile related to type 1-mediated immunity (Th1 cells expressing IFN- $\gamma$ ) in which IL-12 was observed to improve immunity [40,56–58]. It has been also shown that experimental infection of macaques with *L. braziliensis* induced the recruitment and activation of inflammatory mast cells, granulocytes, mononuclear phagocytes, and lymphocytes at the site of infection. Longitudinal characterization of immune response in macaques revealed in the chronic phase, persisting parasites induced a Th1 profile associated with granulomatous reaction. In addition, less differentiated macrophages are observed, forming mature tissue granulomas, which are then substituted by fibroblasts resulting in fibrosis [43]. After 8 weeks, CD8 T cell numbers increased in healing lesions and expressed both TNF- $\alpha$  and IFN- $\gamma$ , contributing to the clearance of parasites. Furthermore, IL-10-producing CD4<sup>+</sup>CD25<sup>+</sup> T cells also accumulate in self-healing skin lesions [43,59] and promote lesional granuloma maintenance [60]. Whereas the immune parameters associated with immune protection in NHP with cutaneous leishmaniasis (CL) remains elusive [57,61], it has been shown that macaques immunized with the sand-fly PdSP15 salivary protein are protected against cutaneous leishmaniasis [62]. The administration of pentavalent antimonial sodium stibogluconate (20 mg/kg for 20 days) in infected macaques, reduced the lesion severity and accelerated healing, which was associated with the modulation in the expression of hundred genes in the skin biopsies from the lesion site [63]. These findings are in agreement with data from human biopsies, which demonstrated that treatment failure was linked to the excessive activation of the cytolytic pathway activated during infection [64,65]. Additional studies in macaques could also address the role of tryptophan-2,3-deoxygenase (TDO), that has recently been described to inhibit parasite burden in human lesions and cultured macrophages [65].

Whereas most of these monkey studies are informative about CL, few of them have addressed immune dysregulation following visceral leishmaniasis. It has been shown that vaccine responses reduce inflammation and structural changes of the splenic white pulp caused by *L. donovani* infection in macaques [66]. Recently we provided major advances regarding immune dysregulation associated with *L. infantum* in macaques [67]. Thus, the infection was associated with the differentiation of splenic CD4 T cells, which became more sensitive to FasL-mediated cell death [68–70]. Early after infection, CD4 T cells were Th1 polarized, switching thereafter to an IL-10 dominated profile consistent with observations in humans and rodents [71–77]. Most importantly, associated with the disruption of splenic architecture, we found that a population that expressed CXCR5 and PD-1, named T follicular helper cells (Tfh) [67,78–82], were abnormally differentiated and not

sustained during the chronic phase. The expression of the master transcriptional factor Bcl-6 of Tfh and the cytokine IL-21 that is critical for B cell activation [83,84], were lowered during the chronic phase. This is of crucial interest given that concomitantly with abortive Tfh differentiation, B cells failed to mature and the circulating levels of parasite-specific IgG and IgM were low, despite the chronic persistence of hypergammaglobulinemia [67,70].

In addition, we observed in macaque infected with *L. infantum* an expansion of splenic CD8 T cells. During experimental VL in mice, the splenic CD8 T cell population expands several-fold at two months post-infection [85–87]. Whereas the role of IFN- $\gamma$  in promoting the killing of intracellular amastigotes is well documented, the role of effector cytotoxic molecules remains less clear, particularly during VL. Thus, the cytotoxic molecule granzysin that kills intracellular parasites in association with granzyme and perforin is absent in mice [7], which further calls for the need of more accurate models of human VL, such as NHP where granzysin is expressed [88]. Thus, NHP may constitute powerful experimental models for understanding host-pathogen interactions that are not directly accessible in human patients, particularly concerning deep tissues in which immune dysregulation may favor parasite persistence as well as the early events after infection, which are usually poorly characterized in infected individuals. They also offer the opportunity to evaluate immunotherapies based on interleukins or antibodies directed against molecules that contribute to the regulation of immune system. Thus, the development of the tools for monkeys in the context of HIV-infection may represent an opportunity for assessing novel strategies for VL/CL diseases.

## 2. Conclusions

Anti-leishmanial drugs have been demonstrated to control parasite infection. However, the observation that the relapse rate is increasing in Asia [89,90] and in Africa, particularly in patients co-infected with HIV [91–93] indicates the requirement for an effective immunity in the control of leishmania. Parasite breakthrough increases the potential for the emergence of resistant parasites [94–96]. Therefore, monkeys represent attractive models for assessing pathogenicity, processes associated with parasite relapse and novel therapies for leishmaniasis, which is one of the main tropical neglected diseases.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was supported by the European Community's Seventh Framework Programme under grant agreement No.602773 (Project KINDRED) and Infect-ERA ERA-NET (Project InLeish) to JE. JE is funded by the Agence Nationale de la Recherche ANR (ANR-16-IFEC-0002 project InLeish). JE also thanks the Canada Research Chair program for financial assistance. SA is supported by a post-doctoral fellowship granted by Fédération pour la Recherche Médicale (FRM: SPF20160936115) and by Infect-Era (ANR-16-IFEC-0002). VR by a post-doctoral fellowship granted by KINDRED. This work was also supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (NORTE-01-0145-FEDER-000013) funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and the Fundação para a Ciência e Tecnologia (FCT) (contract IF/00021/2014 to RS).

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