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Research paper

Flow cytometry identification and characterization of mononuclear cell subsets in the neotropical primate *Saimiri sciureus* (squirrel monkey)

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Abstract

Background: The neotropical primate squirrel monkey is used in many areas of biomedical research including neuro-endocrinology, immunology and infectious diseases. However, research has been hampered by the lack of immunological tools for this primate.
Methods: A series of 67 commercially available monoclonal antibodies to human CD antigens or cytokines were tested on *Saimiri* mononuclear cells and the specificity was assessed by double staining using flow cytometry.
Results: Monoclonal antibodies defining the main mononuclear cells subsets (monocytes, B, T, including CD4 and CD8 T cells) as well as activation markers have been identified. The conditions to specifically identify the various cell subsets using two color flow cytometry and establish their relative proportions have been set-up. We also have established normal values of the main circulating mononuclear cell subsets for adult *Saimiri sciureus* monkeys from the breeding unit of Institut Pasteur in French Guiana. The distribution between spleen, blood and lymph nodes has been compared.
Conclusions: These tools allow documenting the phenotype of most Saimiri mononuclear cell subsets and assessing their activation level. This opens new perspectives for vaccinology and immunopathology research in this experimental non-human primate host, in particular for malaria research.

Keywords: Squirrel monkey; *Saimiri*; Leukocyte subsets; CD16; Flow cytometry

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1. Introduction

Among the new-world monkeys commonly used in biomedical research, the *Saimiri sciureus* is of particular interest. It is not a protected species, and can be easily bred in captivity for a relatively low cost due to its relatively small size. *Saimiri* has retained attention of scientists in biomedical areas because it represents a valuable experimental model for the study of stress physiology, as well as other functions of the Hypothalamo-Pituitary-Adrenal (HPA) axis (Reynols et al., 1999; Lyons et al., 2000; Ricaurte et al., 2002). It is also susceptible to many human infectious agents including parasites (Pung and Kuhn, 1991; Galland, 2000), bacteria (Stadtlander et al., 1998), viruses such as HTLV1 (Kazandji, 2000) and even the human spongiform encephalopathy prion (Gajdusek and Gibbs, 1971). It is recognized as a reference model for human malaria infection, because it is susceptible both to *Plasmodium falciparum* and *Plasmodium vivax* parasites, the two major human malaria parasites, to which most commonly used non-human primates are resistant (Collins, 2002). The use of *Saimiri*, in the field of malaria research, has been largely focussed on efficacy assessment of malaria vaccine candidates (Galland, 2000; Perraut et al., 2000). Unfortunately, these trials did not permit to draw optimal vaccine strategies, because of the inability to dissect the immune responses as well as the protective mechanisms induced by the vaccine candidate. Similarly, the early immune events induced by *P. falciparum* infection that lead to parasite clearance and recovery remain poorly explored because of the lack of appropriate and reliable immunological tools (Contamin et al., 2000). To fill this gap, we have screened a large panel of commercially available monoclonal antibodies reactive with human CD antigens for cross-reaction with PBMC from *S. sciureus* by flow cytometry. We have not only checked the positivity, but also have confirmed the antigen specific recognition by several double staining and determined optimal conditions for a routine use. Using these antibodies, we have established the normal values of the main mononuclear cell subsets in the peripheral blood and the spleen of adult monkeys. We also describe here the identification of monoclonal antibodies against activation markers to be used for immune response characterization.

2. Materials and methods

2.1. Animals and tissue sampling

Saimiri monkeys were from the breeding colony of the Pasteur Institute, French Guiana (de Toisy and Contamin, 1998). Adult 3–5 years old healthy *Saimiri* monkeys from the F1 and F2 generation were studied. Work was conducted with the approval of the institutional ethic committee and in accordance with the requirements of the laboratory biosafety rules. Each animal was anaesthetized by Imalgen at 10 mg/kg (Ketalar, Mérieux, France) for venipuncture or lymph node and spleen removal. All animal procedures were performed under the regulations of the Institut Pasteur ethical and animal use committee.

2.2. Cell isolation and activation conditions

All *Saimiri* blood samples were drawn from the femoral vein and poured into heparinized sterile tubes. Blood was then diluted 1/10 in RPMI, layered on a Ficoll gradient as described (Garraud et al., 1994). To immunophenotype lymphocytes in lymph node and spleen, the lymphoid tissues were minced to single cell suspension in RPMI, which was layered on a Ficoll gradient. For activation, 2×10^6 cells per well were delivered into a 24 well-plate in RPMI medium complemented with glutamine, pyruvate, and 5% FCS in the presence of PHA-L (Sigma-Aldrich, St Quentin, France) at 10 μg/ml. Human blood from Healthy donors was collected on heparin at the French Blood Bank.

2.3. Antibodies

The specificity, clone names, isotypes and supplier of the commercially available monoclonal antibodies against T and B lymphocytes, monocytes and NK cells surface are indicated in Table 1.