



003575

IN VITRO INTERLEUKIN-1 AND 2 PRODUCTION AND INTERLEUKIN 2 RECEPTOR EXPRESSION IN THE RHESUS MONKEY

Didier A. Schmitt,* Gerald Sonnenfeld,† David Husson,* Jean Tkaczuk,* Eric André,‡
 and Laurance Schaffar §

* Laboratoire d'Immunologie, Faculté de Médecine et CHU Rangueil, 31054 Toulouse, France,
 † Department of General Surgery Research, Carolinas Medical Center, Charlotte, NC, USA,
 ‡ Centre de Primatologie, Université Louis Pasteur, Niederhausbergen, France, and
 § INSERM U 210, Faculté de Médecine, 06107 Nice, France.

(Received in final form July 8, 1996)

Summary

Anti-human monoclonal antibodies were used to detect and quantify interleukins-1 and 2 and interleukin-2 receptor expression in peripheral blood mononuclear cells from a rhesus monkey. Interleukin-1 production could be induced by phorbol esters (PMA) and was potentiated by phytohemagglutinin (PHA). Interleukin-2 secretion could also be induced by the combination of PHA and PMA, but only weakly with PHA alone. Interleukin-2 receptor expression was present in a subpopulation of unstimulated lymphocytes and could be enhanced by PHA or PMA. These data show once again that the rhesus monkey immune system is cross-reactive with the human one and that rhesus macaque could be a good model to study interleukin therapy.

Key Words: primates, *Macaca mulatta*, lymphocyte proliferation, cross-reactivity, cytokines

As with other nonhuman primates, the rhesus monkey (*Macaca mulatta*) demonstrates marked similarities to humans in its anatomy and physiology (13) due to a close phylogenetic relationship. These similarities are of importance for medical research in many disciplines such as immunology. Thus, rhesus monkeys have been used for many years as a model for infectious diseases (16, 20, 22) and grafting (18). Moreover, the discovery of a simian immunodeficiency virus (SIV) capable of inducing an acquired immunodeficiency syndrome (AIDS)-like disease in rhesus monkeys (4, 12) contributed to AIDS research (2, 7, 16). Interestingly, more and more cross-reactivity of human immunological reagents has been described (16, 22), reinforcing the usefulness of this animal model as a human analogue in the immunological field. An increasing number of anti-human monoclonal antibodies (MAbs) against cell surface antigens can be used to determine lymphocyte subsets in the rhesus monkey (14, 18, 21, 25). As a consequence, functional activities of mononuclear cells from rhesus monkeys could be compared with humans (1, 9, 19).

In contrast, to our knowledge of cell membrane markers, little is known about cytokines or cytokine receptors in macaques (15), and especially rhesus monkeys (3, 8). Interleukin-1beta (IL-1) is a multifunctional polypeptide which is secreted by monocytes and participates in T lymphocyte blast transformation (26). Interleukin-2 (IL-2) is produced by activated T lymphocytes and participates in T lymphocyte proliferation once the interleukin-2 receptor (IL-2R) is expressed (26). The present study was undertaken to determine whether IL-1, IL-2, and IL-2R expression could be quantified in rhesus monkeys using anti-human MAbs.

Address correspondence and reprint requests to : Didier A. Schmitt, Laboratoire d'Immunologie, CHU Rangueil, 31054 Toulouse Cédex ; Tel.: (33) 61.32.28.53 - Fax : (33) 61.32.22.82

Materials and Methods

Animals and blood samples. Rhesus monkeys (*Macaca mulatta*) of various ages, housed at Centre d'Etudes et de Recherche en Médecine Aérospatiale (Brétigny sur Orge, France) and at Centre de Primatologie (Niederhausbergen, France) were used in this study. Monkeys were housed according to WHO criteria under the supervision of a veterinarian. Peripheral blood (5 ml) was obtained by veinipuncture from Ketamin (10 mg/kg) (Panpharma, Yvery/Seine, France) tranquilized animals using heparinized sterile vacutainer tubes (Becton Dickinson, San Jose, CA). The samples were shipped by express mail at ambient temperature to the laboratory within 24 h after sampling.

Peripheral blood mononuclear cell isolation. Peripheral blood mononuclear cells (PBMC) were separated on a density medium (MSL, Eurobio, Paris, France) by centrifugation for 25 min at 400 g. The PBMC were then washed twice in RPMI 1640 medium (Gibco BRL, Cergy-Pontoise, France) and resuspended at a concentration of 10^6 viable cells/ml of culture medium consisting of RPMI 1640 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% heat-inactivated fetal bovine serum (IBF, Villeuve-La-Garenne, France).

Interleukin production and ELISA assays. Duplicate or triplicate cultures containing 2×10^5 PBMC per well were set up in 0.2 ml of culture medium in U-bottom microtiter plates (Nunc, Roskilde, Denmark) and incubated for 48 h in the presence of activators in a 37°C, humidified, 5% CO₂ atmosphere. The IL 2 production was induced by stimulating PBMC with various concentrations (10 and 20 mg/l) of phytohemagglutinin-P (PHA-P, Sigma Chemical, St. Louis, MO) or 10 mg/l PHA plus 1 or 5 ng/ml of phorbol 12-myristate 13-acetate (PMA, Sigma). The IL-1 secretion was induced by incubating the PBMC in the presence of 1, 5 or 10 ng/ml of PMA or with 20 mg/l of PHA plus 1 ng/ml PMA. After incubation, the plates were centrifuged at 400xg for 10 min and the supernatants were collected and stored at -80°C until analysis. In order to minimize inter-experimental variations, all the experiments were performed using the same batches of reagents previously aliquoted and frozen. The IL1 and IL2 were measured by a non commercial enzyme immunoassays as previously described (5, 6). Secreted IL1 was measured in cell supernatants and total IL1 was measured by lysing the cells with detergent in the culture wells (5). Human recombinant IL1 and IL2 (Biogen, Geneva, Switzerland) were used as standards. For additional control experiments a sandwich immunoassay was used for both IL-1 and IL-2 (Immunotech, Marseille, France).

Labeling of IL-2 receptor (IL-2R)-expressing cells. Cultured PBMC stimulated with 5 µg/ml of PHA or 1 and 5 ng/ml of PMA for 12, 24, 36, and 48 h were resuspended in .05 ml of PBS-NaN₃. Next, .005 ml of FITC-conjugated anti-IL-2R alpha chain monoclonal antibody (10) (anti-CD25, hybridoma B1.49.9, Immunotech, Marseille, France) were added for 15 min at 4°C. After two washes in PBS-NaN₃, the cells were fixed in PBS containing 0.37 % formaldehyde and stored at 4°C in the dark until analysis within a maximum of 1 day after labeling. In order to test the specificity of our antibody monkey PBMC were stimulated with PHA (5ng/ml) for 36h and were then incubated for 1h at 4°C with 10 UI/ml human recombinant IL2 (Biotest, Dreieich, Germany). After two washes, half of the cells were incubated at 37°C for 1h in order to enable the internalization of the IL-2 receptor. The cells were then stained as described above. Cytofluorometric analysis was carried out using a FACScan Analyser (Becton-Dickinson) fitted with a single laser and filters allowing detection of light emission of fluorescein. Forward scatter and side scatter parameters were used to gate lymphocytes and monocytes and to gate out damaged cells and debris. For each sample, the fluorescence intensities of 10^4 cells were recorded, and the percentage of positive cells was computed through LYSYS II software (Becton-Dikinson).

Statistical Analysis. Measurements were compared to each other by means of a paired t test. Alpha was set a priori at $p < 0.05$.

Results

IL-1 was produced by resting PBMC, and its production was significantly enhanced when PBMC were triggered by PMA. Interestingly, as in humans, low doses of PHA potentiate drastically the IL-1 secretion (Fig.1). The optimal concentration of PMA was 1 ng/ml (Fig. 2).

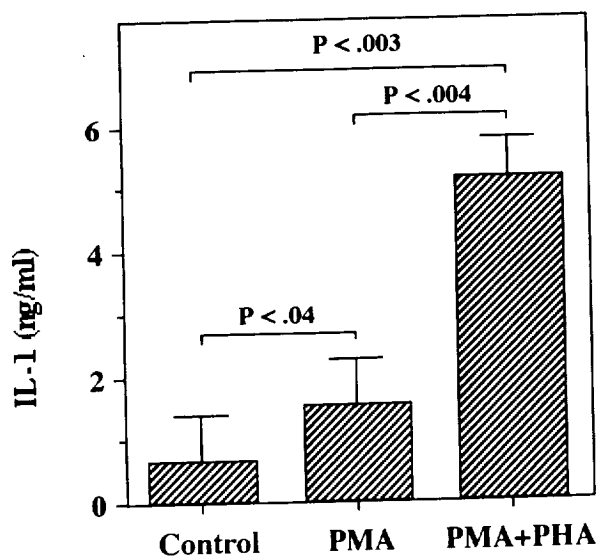


FIG. 1

IL-1 secretion in rhesus monkey PBMC. IL-1 secretion by PBMC was quantified in either unstimulated cells or in cells that received PMA (5 ng/ml) or PMA (1 ng/ml) plus PHA (20 mg/l) -stimulated cells for 48 h. Results are expressed as mean \pm SD, $n = 5$.

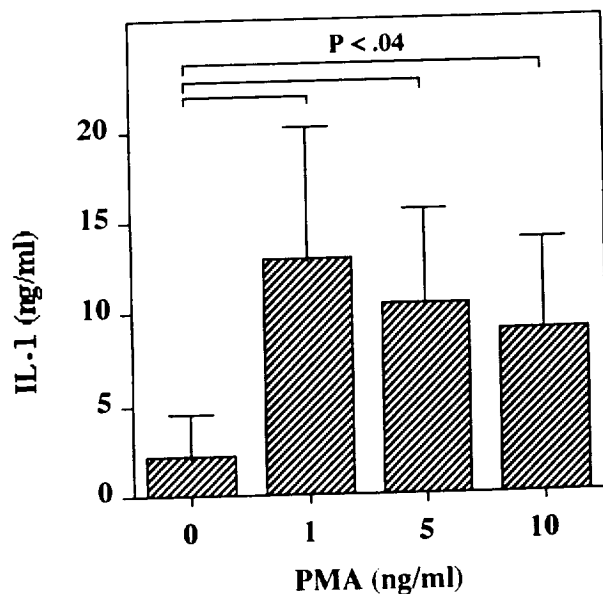


FIG. 2

IL-1 secretion in rhesus monkey PBMC. IL-1 secretion was induced in PBMC with various concentrations of PMA over a 24 h period. IL-1 concentrations decreased slightly when PBMC were stimulated with PMA concentrations higher than 1 ng/ml. Results are expressed as mean \pm SD, $n = 5$.

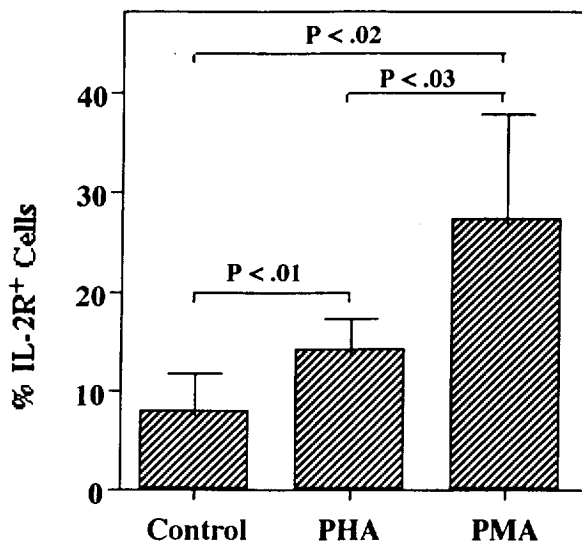


FIG. 3

IL-2R expression in rhesus monkey PBMC. IL-2R expression in unstimulated and PMA (5 ng/ml) or PHA (5 mg/l of PHA)-stimulated cells was determined by cytofluorimetry. The IL-2R was expressed on unstimulated T lymphocytes (IL-2R⁺ cells) and is increased in PHA (5 mg/l) stimulated cells. Results are expressed as mean \pm SD, n = 5.

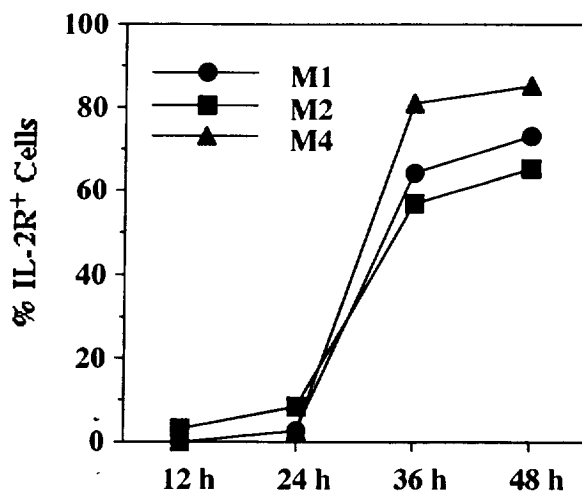


FIG. 4

IL-2R expression in rhesus monkey PBMC. IL-2R expression in PMA (5 ng/ml)-stimulated cells was determined by cytofluorimetry. The percentage of IL-2R positive cells reached a maximum after 36 h.

TABLE I

	IL-2 (U/ml)	SD
Control	0	0
PHA	0	0
PMA	.03	.025
PHA + PMA	.62	.24

IL-2 secretion in rhesus monkey PBMC. IL2 secretion (U/ml) by PBMC was undetectable in unstimulated or in PHA (10 mg/l) stimulated cells. A detectable amount of IL2 was produced when PMA (5 ng/ml) was used as the activator whereas, PHA (10 mg/l) largely potentiated the effect of the same concentration of PMA. Results are expressed as mean \pm SD, n = 7.

TABLE II

	IL-2 (U/ml)	
Monkey	Control	PHA + PMA
1	0	2.30
2	.23	4.96
3	0	.52
4	.15	2.19
5	.26	.52

IL-2 secretion in rhesus monkey PBMC. In this experiment, IL2 secretion (U/ml) was detectable in some monkeys in unstimulated cells. IL2 production was increased when PBMC were stimulated with PMA (5 ng/ml) plus PHA (10 mg/l).

IL-2 was nearly undetectable in unstimulated or in PHA-stimulated PBMC. Similar to IL-1, IL-2 secretion was induced by PMA and PHA potentiated strongly this activation (Table I). No IL-2 was detectable with PHA alone in one experiment (Table I) and, in another experiment, small amounts of IL-2 were detectable in unstimulated PBMC cultures in 3 animals out of 5 (Table II). These differences might be due to the different origin of the monkeys or to inter-laboratory variability between the experiments. In order to further demonstrate the cross-reactivity of anti-human MAbs raised against IL-1 and IL-2, we also used successfully commercially available sandwich immunoassays (data not shown).

In resting PBMC cultured for 48 h, approximately 9 % of the T-lymphocytes expressed the IL-2R (Fig. 3). The number of IL-2R positive cells increases in presence of PHA and PMA (Fig. 3). Similar to IL-1 secretion, the expression of the IL-2 R was at an optimum at 1 ng/ml of PMA. In fact, the percentage of IL-2R positive cells was in the same range with 1 and 5 ng/ml of PMA (data not shown). The time course of IL-2R expression showed a rapid increase in IL-2R positive cells after 24 h of culture and reached a maximum after 48 h with PMA or PHA. The same results were obtained with 5 ng/ml of PMA (Fig. 4) or with 5 mg/l of PHA (data not shown). The incubation of PHA-activated PBMC with human recombinant IL-2 induced a decrease in the mean fluorescence of IL-2R highly positive lymphoblastes by about 20% in one monkey and 29% in the second one. The decrease in the mean fluorescence is probably due to IL-2R internalization and, therefore, is an indirect argument for the specificity of the anti-CD25 antibody.

Discussion

Human T-cell proliferation involves IL-1 production by monocytes, followed by autocrine IL-2 secretion by a T-lymphocyte subpopulation, and IL-2R expression on T-lymphocytes undergoing proliferation / differentiation (10). In the present report, we showed that IL-1 and IL-2 secretion *in vitro* by PBMC from rhesus monkey and T-lymphocyte expression of IL-2R can be detected by MAbs directed against the corresponding human antigens. Moreover, IL-1 synthesis and secretion and, to a lesser extent, IL-2 synthesis, were inducible by PMA, and PHA potentiated the effect of PMA. However, the optimal concentration of PMA is much lower in the rhesus monkey than in humans. Unlike the human case, PBMC in the presence of PHA alone produced only a small amount of IL-2. Nevertheless, PHA-induced T-lymphocyte proliferation, evidenced by [³H] thymidine incorporation (27), was similar to what is observed in humans. Therefore, the IL-2 production might be underestimated if the anti-IL-2 antibody used has a lower affinity with the macaque IL-2. The cross-reactivity for these cytokines has also been demonstrated with commercial sandwich immunoassays showing that two opposite epitopes are recognized by anti-human MAbs. Because these ELISA assays are sandwich assays and because two different tests were used successfully for each cytokine, four epitopes have therefore been recognized on each molecule. Despite these data we can not exclude cross-reactivities with other proteins. The IL-2R was expressed on some T-lymphocytes and was enhanced in PHA or PMA-stimulated cells with a time-dependent increase similar to what is observed in humans. The incubation of lymphocytes with IL-2 decreased the amount of labeling with our antibody against the IL-2R. This is due to the internalization of the IL-2R and can be considered as an additional argument for the specificity of the antibody. In contrast to rhesus monkeys and humans, T-lymphocytes from Japanese monkeys (*Macaca fuscata*) respond less to PHA as shown by [³H] thymidine incorporation and they also express IL-2R to a lesser extent (15). Nevertheless, in our experiment, not all anti-IL-2R MAbs studied stained-activated T lymphocytes (unpublished results), demonstrating that some epitopes of the receptor are weakly or not recognized by certain anti-human MAbs.

Despite the fact that a number of anti-human MAbs have been used to characterise rhesus monkey surface antigens (14, 17, 21, 25), the interspecies similarities concerning cytokine secretion by and regulation of monocyte and lymphocyte functions are known poorly. Lymphokines such as interferon-gamma and tumor necrosis factor-alpha have been identified in several rhesus monkey tissues on frozen sections (25) and human MAbs directed against these lymphokines had immunosuppressive effects *in vivo* (24). Human B cell growth factor (BCGF) or human recombinant IL-4 were found to be stimulatory for macaque splenic B cells (3, 11) and recombinant human interleukin-3 and granulocyte/macrophage colony-stimulating factor promotes peripheral blood hematopoietic progenitor cells in the rhesus monkey (8). Messenger RNA from IL-2, IL-4, IL-5, IL-2R, and interferon-gamma could be detected with human cDNA probes in T cells from rhesus monkey intestinal lamina propria (11). Additionally, we have recently shown that interferon-gamma production can be induced and quantified with a human enzyme immunoassay (23). Therefore, a profile of rhesus monkey cytokine activities is now emerging.

Acknowledgments

The authors thank Frank Martin from CERMA for providing valuable help in organizing blood sampling and Martine Papais for typing the manuscript. This work was supported by French Centre National d'Etudes Spatiales, grants number 91/297 and 92/315 and by US NASA, grant number NAG2-933.

References

1. A. AHMED-ANSARI, A.R. BRODIE, P.N. FULTZ, D.C. ANDERSON, K.W. SELL, and H.H. MC CLURE, *Am. J. Primatol.* **17** 107 (1989).
2. A. AHMED-ANSARI, J.D. POWELL, P.E. JENSEN, T. YEHUDA-COHEN, H.M. MCCLURE, D. ANDERSEN, P.N. FULTZ, and K.W. SELL, *Aids* **4** 399 (1990).
3. E.A. CLARK and K.E. DRAVES, *Eur. J. Immunol.* **17** 1799 (1987).
4. M.D. DANIEL, N.L. LETVIN, N.W. KING, M. KANNAGI, P.K. SEHGAL, R.D. HUNT, P.J. KANKI, M. ESSEX, and R.C. DESROSIERS, *Science* **228** 1201 (1985).

5. B. FERRUA, C. AUSSEL, and M. FEHLMANN. *J. Immunol. Methods* **97** 215-220 (1987).
6. B. FERRUA, P. BECKER, L. SCHAFFAR, A. SHAW, and M. FEHLMANN, *J. Immunol. Methods* **114** 41 (1988).
7. M.B. GARDNER, *Adv. Exp. Biol. Med.* **251** 279 (1989).
8. K. GEISSLER, P. VALENT, P. MAYER, E. LIEHL, W. HINTERBERG, K. LECHNER, and P. BETTELHEIM, *Blood* **75** 2305 (1990).
9. N. GENGOZIAN, R.E. LONGLEY, J. FILLER, and R.A. GOOD, *Cell. Immunol.* **101** 24 (1986).
10. M.E. HEMLER, B. MALISSEN, N. REBAI, A. LIABEUF, C. MAWAS, F.M. KOURILSKY, and J.L. STROMINGER, *Human Immunol.* **8** 153 (1983).
11. S.P. JAMES, W.C. KWAN, and M.C. SNELLER, *J. Immunol.* **144** 1251 (1990).
12. M. KANNAGI, J.M. YETZ, and N.L. LETVIN, *Proc. Natl. Acad. Sci.* **82** 7053 (1985).
13. F. A. KING, H.C.J. YARBROUGH, D.C. ANDERSON, T.P. GORDON, and K.D. GOULD, *Primates Sciences* **240** 475 (1988).
14. N.L. LETVIN, N.W. KING, E.L. REINHERZ, R.D. HUNT, H. LANE, and S.T. SCHLOSSMAN, *Eur. J. Immunol.* **13** 345 (1983).
15. Y. MARAYAMA, A. NOGUCHI, and O. TAKENAKA, *J. Med. Primatol.* **16** 373 (1987).
16. P.R. MEYER, L.D. ORMEROD, K.G. OSBORN, L.J. LOWENSTINE, R.V. HENDRICKSON, R.L. MODLIN, R.E. SIMTH, M.B. GARDNER, and C.R. TAYLOR, *Hematol. Oncol.* **3** 199 (1985).
17. F.J.M. NOOIJ, J.G. BORST, G.J.E. VAN MEURS., M. JONKER, and H. BALNER, *Eur. J. Immunol.* **16** 975 (1986).
18. F.J.M. NOOIJ and M. JONKER, *Eur. J. Immunol.* **17** 1089 (1987).
19. G. RAPPOCCIOLO, J.S. ALLEN, J.W. EICHBERG, and T.C. CHANH, *Vet. Pathol.* **29** 53 (1992).
20. L.S. SAMMONS, R.H. KENYON, G.T. BURGER, C.E. PEDERSEN, and R.O. SPERTZEL, *Am. J. Vet. Res.* **37** 725 (1976).
21. G.E. SANDUSKY, P.J. HORTON, and K. A. WIGHTMAN, *J. Med. Primatol.* **15** 441 (1986).
22. H. SCHELLEKENS, P.H. MEIDE and K. VAN DER NOOTER, *Interferon studies in non-human primates* M. Revel (Eds). 285-306. Kluwer Academic Publishers, Boston, MA (1988).
23. G. SONNENFELD, L. SCHAFFAR, D.A. SCHMITT, D.A. KOEBEL, B.A. SMITH, and H.M. MC CLURE, *J. Interferon Res.* **13** 259 (1993).
24. H.P.J.D. STEVENS, T.H.K. KWAAT, P.H. VAN DER MEIDE, W.A. VAN DER BUURMAN, and M. JONKER, *Transplantation* **50** 856 (1990).
25. H.P.J.D. STEVENS, T. KWAAT, A. TIMMERNANS, M. STOUTEN, *J. Med. Primatol.* **20** 386 (1991).
26. A. THOMSON, *The cytokine handbook* A.Thomson (Eds). Academic Press, London, San Diego (1991).
27. B.J. WILSON, G. PORTER, H. KOCVARA, and G. LEO, *Primates* **19** 195 (1978).