Immunology 1991 **73** 246–248

ADONIS 001928059100146B
BRIEF COMMUNICATION

In vitro simulation of immunosuppression caused by *Trypanosoma brucei*

M. SILEGHEM, A. DARJI & P. DE BAETSELIER *Instituut voor Molekulaire Biologie, Vrije Universiteit Brussel, Belgium*

Accepted for publication 27 February 1991

SUMMARY

Macrophage populations derived from *Trypanosoma brucei*-infected mice suppress both interleukin-2 (IL-2) production and IL-2 receptor (IL-2R) expression. A prostaglandin-independent mechanism accounts for the suppression of IL-2R expression, while the suppression of IL-2 is prostaglandin-dependent. A macrophage hybridoma cell line (i.e. 2C11-12) was used to mimic the parasite-induced immunosuppression *in vitro*. It was found that 2C11-12 cells acquired a suppressive potential *in vitro* following interaction with opsonized living parasites. *T. brucei*-pulsed 2C11-12 cells failed to block IL-2 secretion in the presence of indomethacin but still suppressed the IL-2R expression. In contrast, addition of living *T. brucei* parasites to the cultures caused a complete suppression of IL-2 secretion and IL-2R expression, even in the presence of indomethacin. Hence, the addition of excess living parasites to the cultures could cause a depression which was different from the suppression associated with infection, whereas the addition of parasite-pulsed 2C11-12 cells mimicked the situation occurring during infection. This model system can be adopted as a suitable tool to unravel the mechanisms underlying the suppression of IL-2R expression during *T. brucei* infections.

INTRODUCTION

It is well established that both T- and B-cell responses are profoundly suppressed in mice infected with African trypanosomes. However, no agreement has been achieved about the nature of the mechanisms underlying the immunosuppression. The reduced immunoresponsiveness has been attributed to suppressor T cells,[1] suppressive adherent macrophage-like cells[2,3] or combinations of both cell types.[4,5] Finally, Askonas *et al.* have demonstrated an increase in prostaglandin and hydrogen peroxide production by macrophages (Mϕ) from infected mice, thereby suggesting a key role of the Mϕ in the immunological unresponsiveness through the release of such suppressive agents.[6-8]

Examination of the T-cell responsiveness has recently shown that whilst concanavalin A (Con A)-stimulated lymph node cells derived from *Trypanosoma brucei*-infected mice could secrete macrophage-activating lymphokines and interferon, they were unable to secrete interleukin-2 (IL-2)[9] and failed to express receptors for IL-2 (IL-2R).[10] Thus, the inhibition of the T-cell proliferation could be attributed to a 'selective' suppression of the IL-2/IL-2R circuit. At least two mechanisms appeared to be implicated in this suppression of IL-2 release and IL-2R expression. The reduction of the T-cell proliferation was in part due to the generation of prostaglandin-secreting Mϕ; these 'suppressive' cells were entirely responsible for the suppression of IL-2 secretion, but were not the cause of the suppression of IL-2R expression. A second, prostaglandin-independent suppressive mechanism was simultaneously generated which blocked the expression of IL-2R on both CD4$^+$ and CD8$^+$ targets but which did not alter the amount of IL-2 secreted. This second mechanism was, again, mediated by cells that co-purify with the Mϕ fraction (Mac1$^+$, Thy-1$^-$, fibronectin receptor$^+$, nylon wool-adherent and plastic-adherent).[11,12] Although prostaglandins have been clearly identified as the effector molecules mediating down-regulation of IL-2 production, the molecules responsible for the decrease of the IL-2R expression remain unknown.

Due to the heterogeneity of Mϕ populations derived from infected mice, it was extremely difficult to attempt the characterization of the molecular mechanisms implicated in the observed immunosuppression. In the present report, we describe the use of a cloned Mϕ hybridoma cell line to mimic the parasite-induced immunosuppression *in vitro*.

A cloned Mϕ hybridoma (2C11-12), generated in our laboratory,[13] was allowed to adhere to a plastic surface and subsequently opsonized with antibody-coated long slender *T. brucei* trypomastigotes at a Mϕ: parasite ratio of 1:100. The culture was then repeatedly washed until no free parasites or immune complexes could be observed by microscopy. The pulsed Mϕ were released by vigorous pipetting, irradiated at 900 rads to stop their proliferation, and then co-cultured with freshly isolated lymph node cells at a final ratio of 1:20. This concentration roughly corresponded with the amount of Mϕ found in the lymph node cell populations. Finally, the cultures were stimulated with concanavalin A (Con A). All the cultures

Correspondence: Dr P. De Baetselier, Unit of Cellular Immunology, Institute of Molecular Biology-V.U.B., Paardenstraat 65, 1640 Sint Genesius Rode, Belgium.

Table 1. Suppression of T-cell proliferation by parasite-pulsed 2C11-12 cells

	T-cell proliferation‡ Δ c.p.m. × 10^{-3}	IL-2 secretion§ U/ml	IL-2R expression¶ mean fluorescence	
			$CD4^+$ cells	$CD8^+$ cells
LNC*	184	8·9	85	101
LNC+2C11-12-*T. brucei*†	73	9·2	19	26
LNC+2C11-12-SRBC†	216	ND	ND	ND

*Lymph node cells (LNC) isolated from normal F_1(C57Bl/6 × BALB/c) mice were cultured at a concentration of 2×10^6 cells/ml and were stimulated with Con A.

† The cells were co-cultured with 2C11-12 hybridoma cells which were first opsonized with *T. brucei* trypomastigotes (2C11-12-*T. brucei*) or sheep red blood cells (2C11-12-SRBC).

‡ The proliferation of the cells was measured by incorporation of [^{3}H]thymidine and is expressed as counts per minute per 200 μl aliquot (4×10^5) cells. The shown data are Δ values (c.p.m. sample − c.p.m. background).

§ The amount of IL-2 in the cell-free supernatants was measured by titration on CTL-L2 cells. In contrast to most CTL-L2 clones, the clone used was unsensitive to IL-4 but responded normally to IL-2.[11] The results are expressed as half-maximal IL-2 units per ml. The limit of detection of the assay is 1 U/ml. Background values were always below the detection limit.

¶ The expression of IL-2R on $CD8^+$ and $CD4^+$ T-cell subsets was measured by double immunofluorescence. The 7D4 monoclonal antibody (rat anti-mouse IL-2 receptor α chain; ATCC, Rockville, MD) was purified by affinity chromatography and labelled with fluorescein isothiocyanate (FITC). The cells were stained directly with the 7D4 antibody and then incubated with phycoerythrin-conjugated anti-L3T4 antibody (Becton-Dickinson, Mountain View, CA) or with a biotin-conjugated anti-Ly-2 antibody plus phycoerythrin-conjugated streptavidin (Becton-Dickinson). The fluorescent activity was analysed on FACStar (Becton-Dickinson), a gate was set on the phycoerythrin-positive cells and the gated cells were then analysed for FITC activity. The results were analysed as the mean fluorescent activity and expressed as arbitrary FITC units. The shown data are the delta values (FITC units sample–FITC units background).
ND, not done.

Table 2. Addition of excess parasites *in vitro* inhibits both IL-2 secretion and IL-2R expression

	T-cell proliferation Δ c.p.m. × 10^{-3}	IL-2 secretion U/ml	IL-2R expression mean fluorescence
LNC*	272	8·6	162
LNC + 10^7 *T. brucei*/ml	275	9·7	158
LNC + 10^8 *T. brucei*/ml	1	0·1	12

*Lymph node cells (LNC) from normal mice were cultured at a concentration of 2×10^6/ml and stimulated with Con A. Purified living parasites are added at different concentrations. The T-cell proliferation, IL-2 secretion and IL-2R expression were measured as described in Table 1 except that the IL-2R expression ws analysed on the whole T-cell population and not separately on $CD4^+$ and $CD8^+$ fractions.

were supplemented with indomethacin (10 μg/ml) and catalase (10,000 units/ml) to avoid prostaglandin and hydrogen peroxide-mediated suppression, respectively.

It was found that the 2C11-12 hybridoma pulsed with *T. brucei* blocked the Con A-induced T-cell proliferation whereas Mϕ pulsed with sheep red blood cells were not inhibitory (Table 1). Antibody-coated parasites alone added in suspension at an equivalent concentration were not suppressive (data not shown). It thus appeared that 2C11-12 cells acquired a suppressive potential following *in vitro* interactions with opsonized living parasites. Although it was tempting to use such cells as a model for the infection-associated impairment of T-cell unresponsiveness, it was important to consider the possibility that the observed suppression was not simply due to an intoxication of the cultures or to an irrelevant Con A unresponsiveness. Indeed, using T-cell proliferation as a test system, it was impossible to discriminate between a suppression, relevant to the infection-associated pathology, or an irrelevant paralysis or intoxication. However, the previously described capacity of Mϕ from infected mice to affect differentially IL-2 production and IL-2R expression provided us a way to evaluate the functional relevance of the *in vitro* generated suppressive Mϕ.

We found that the *T. brucei*-pulsed 2C11-12 cells failed to block the production of IL-2 in the presence of a prostaglandin synthesis inhibitor (indomethacin) but still suppressed the expression of IL-2R on both $CD4^+$ and $CD8^+$ targets (Table 1). This behaviour resembled the suppressive characteristics of $M\phi$ from infected mice and ruled out the possibility of a paralysis/intoxication since high levels of IL-2 secretion were maintained.

In vitro suppression of T-cell proliferation could also be achieved by adding high numbers of living parasites to the cultures as shown in Table 2. It seems, however, that this system is not as adequate as the parasite-pulsed 2C11-12 cells to mimic the infection-associated suppression. Indeed, when very high numbers of trypanosomes were added, the secretion of IL-2 secretion was blocked absolutely, even in the presence of indomethacin. The mechanisms by which suppression is achieved by adding an excess parasites *in vitro* are thus not necessarily identical to the mechanisms causing suppression during infection.

On the basis of these results, we conclude that the parasite-induced immunosuppression can be mimicked perfectly *in vitro* using *T. brucei*-pulsed 2C11-12 cells. Addition of an excess of living parasites, on the other hand, can cause a depression which is different from the suppression occurring during infection.

Besides the development of an *in vitro* simulation model, our data also stress, once more, the importance of parasite—$M\phi$ interactions in the generation of immunosuppression. It has been shown previously that peritoneal $M\phi$ which were allowed to phagocytose irradiated *T. brucei in vivo* for 1–2 hr were able to induce suppression of antigen-specific B-cell responses following transfer to naive recipients.[14] Our present data thus confirm the key role of the $M\phi$ as the primary targets of immunosuppression and also show that the whole process can be mimicked entirely *in vitro* by using the differential suppression of IL-2 and IL-2R expression as an indicator to probe the functional relevance of the observed inhibition.

In vitro models have also been developed to study the immunosuppressive potential of related parasites such as *T. cruzi*. The addition of living trypomastigotes to human peripheral blood mononuclear cells results in a decreased T-cell proliferation subsequent to mitogenic stimulation.[15] Interestingly, it has been shown recently that the expression of IL-2R is markedly suppressed in such models, whereas the IL-2 secretion is not affected.[16,17] It would thus appear that the mechanisms of immunosuppression in the *in vitro T. brucei* model are similar to the mechanisms of suppression in the *in vitro T. cruzi* model, the major difference being the fact that the *T. brucei* trypomastigotes must be pulsed on a $M\phi$ to initiate suppression.

In conclusion, the suppression of T-cell proliferation associated with *T. brucei* infection in mice can be simulated *in vitro* using the 2C11-12 $M\phi$ hybridoma pulsed with *T. brucei* trypomastigotes.

ACKNOWLEDGMENTS

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and from the F.G.W.O. [No. 3.0072.88). Maarten Sileghem and Patrick De Baetselier are fellows of the N.F.W.O. (National Fund for Scientific Research, Belgium).

REFERENCES

1. Corsini A.C., Clayton C., Askonas B.A. & Ogilvie B.M. (1977) Suppressor cells and loss of B-cell potential in mice infected with *Trypanosoma brucei*. *Clin. exp. Immunol.* **29,** 122.
2. Wellhausen S.R. & Mansfield J.M. (1980) Characteristics of the splenic suppressor cell-target cell interaction in experimental African trypanosomiasis. *Cell. Immunol.* **54,** 414.
3. Yamamoto K., Onodera M., Kato K., Kakinuma M., Kimura T. & Richards F.F. (1985) Involvement of suppressor cells induced with membrane fractions of trypanosomes in immunosuppression of trypanosomiasis. *Par. Immunol.* **7,** 95.
4. Jayawardena A.N., Waksman B.H. & Eardly D.D. (1978) Activation of distinct helper and suppressor T cells in experimental Trypanosomiasis. *J. Immunol.* **121,** 622.
5. Pearson T.W., Roelants G.E., Poinder, M., Lundin, L.B. & Mayor-Withey K.S. (1979) Immune depression in trypanosome-infected mice. III. Suppressor cells. *Eur. J. Immunol.* **9,** 200.
6. Grosskinsky C.M., Ezekowitz R.A.B., Berton G., Gordon S. & Aksonas B.A. (1983) Macrophage activation in murine African trypanosomiasis. *Infect. Immun.* **39,** 1080.
7. Fierer J., Salmon J.A. & Askonas B.A. (1984) African trypanosomiasis alters prostaglandin production by murine peritoneal macrophages. *Clin. exp. Immunol.* **58,** 548.
8. Askonas B.A. (1985) Macrophages as mediators of immunosuppression in murine African trypanosomiasis. *Curr. Top. Microbiol. Immunol.* **117,** 119.
9. Sileghem M., Hamers R. & De Baetselier P. (1986) Active suppression of interleukin 2 secretion in mice infected with *Trypanosoma brucei* AnTat 1.1E. *Par. Immunol.* **8,** 541.
10. Sileghem M., Hamers R. & De Baetselier P. (1987) Experimental *Trypanosoma brucei* infections selectively suppress both interleukin 2 production and interleukin 2 receptor expression. *Eur. J. Immunol.* **17,** 1417.
11. Sileghem M., Darji A., Remels L., Hamers R. & De Baetselier P. (1989) Different mechanisms account for the suppression of interleukin 2 production and the suppression of interleukin 2 receptor expression in *Trypanosoma brucei*-infected mice. *Eur. J. Immunol.* **19,** 119.
12. Sileghem M., Darji A., Hamers R., Van de Winkel M. & De Baetselier P. (1989) Dual role of macrophages in the suppression of interleukin 2 production and interleukin 2 receptor expression in trypanosome-infected mice. *Eur. J. Immunol.* **19,** 829.
13. De Baetselier P. & Schram E. (1986) Luminescent bioassays based on macrophage cell lines. *Meth. Enzymol.* **133,** 507.
14. Grosskinsky C.M. & Askonas B.A. (1981) Macrophages as primary target cells and mediators of immune dysfunction in African trypansomiasis. *Infect. Immun.* **33,** 1498.
15. Maleckar J.R. & Kierzenbaum F. (1983) Inhibition of mitogen-induced proliferation of mouse T and B lymphocytes by bloodstream forms of *Trypanosoma cruzi*. *J. Immunol* **130,** 908.
16. Beltz L.A., Sztein M.B. & Kierzenbaum F. (1988) Novel mechanism for *Trypanosoma cruzi*-induced suppression of human lymphocytes. Inhibition of IL-2 receptor expression. *J. Immunol.* **141,** 289.
17. Kierzenbaum F., Sztein M.B. & Beltz L.A. (1989) Decreased human IL-2 receptor expression due to a protozoan pathogen. *Immunol. Today,* **10,** 129.