Ann. Soc. belge Méd. trop.
1987, 67, 129-136

MODULATION OF LYMPHOKINE PRODUCTION DURING EXPERIMENTAL *TRYPANOSOMA BRUCEI* INFECTION IN INBRED MOUSE STRAINS

BY

M. SILEGHEM[1], A.M. MAKUMYAVIRI[2], D. LE RAY[2], R. HAMERS[1] & P. DE BAETSELIER[1]

[1]*Vrije Universiteit Brussel, Instituut voor Moleculaire Biologie, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium*
[2]*Prins Leopold Instituut voor Tropische Geneeskunde, Laboratorium voor Protozoölogie, Nationalestraat 155, B-2000 Antwerpen, Belgium*

Summary — Experimental *Trypanosoma brucei* AnTAR 1 infection induces a severe depression of interleukin 2 production, but does not inhibit the production of other T cell derived lymphokines such as macrophage activating factors. This selective suppression of interleukin 2 production was found in susceptible C3H/He mice as well as in relatively resistant CBA/Ca mice and hence is not related to the differential resistance of the tested inbred mouse strains.

KEYWORDS: *Trypanosoma brucei*; Interleukin 2; Macrophage Activating Lymphokines; Trypanotolerance

Introduction

African trypanosomes are notorious potent modulators of the host's immune system. Suppression of immune responses to parasite unrelated antigens and mitogens has frequently been described during experimental trypanosome infection (reviewed in 1).

Regarding non specific immunosuppression of T cell responses, numerous recent investigations have focused on the impairment of lymphokine secretion. Inhibition of the mitogen-induced interleukin 2 (IL-2) production has been reported during experimental infections with *Leishmania donovani*(11), *Trypanosoma cruzi* (5, 13), *T. congolense* (9), and *T. brucei* (13). Hence, inhibition of the production of interleukin 2, a lymphokine which plays a central role in T cell activation and proliferation, might be a common mechanism exerted by trypanosomatids to mediate immune suppression.

In the present report, we have analysed the parasite-induced modulation of T cell lymphokine production in inbred mouse strains which differ markedly in their susceptibility to experimental *T. brucei* infection.

Materials and methods

Animals and parasites

C3H/He and CBA/Ca female mice were purchased from Bantin & Kingman Ltd, England, and were used at the age of 12 weeks. The metacyclic trypanosomes used for infection were harvested from the salivary glands of

Glossina morsitans morsitans infected with *Trypanosoma brucei* EATRO 1125 AnTAR 1 (7).

Cell cultures and bioassays

Lymph nodes and spleens were isolated from normal and trypanosome infected mice and cell suspensions were prepared in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10 % fetal calf serum (Gibco), 50 units penicillin/streptomycin, 300 μg L-glutamin and 5×10^{-5}M mercaptoethanol. Aliquots containing 4×10^6 viable cells per ml were dispersed in flat bottomed 24-well plates (Nunc, Roskilde, Denmark) with or without 5 μg concanavalin A per ml (Sigma, St Louis, Missouri). The cultures were incubated for 24 hrs at 37° C in a humidified atmosphere containing 5 % CO_2.

The IL-2 levels in the culture supernates were determined by the ability of the cell-free supernates to stimulate the IL-2 dependent proliferation of CTL-L cells. This was done by incubating aliquots of 10^4 CTL-L cells suspended in RPMI medium prepared as described above in microwell plates (Nunc) with the IL – 2 containing supernates in triplicate at a final dilution of 1:4, 1:8, 1:16 and 1:32. The proliferative responses were determined by the incorporation of (methyl-^{3}H)thymidine purchased from Amersham (Buckinghamshire, England). In order to quantitate more precisely the amount of IL-2, a stock solution of recombinant IL-2 containing 30000 LBRM units/ml, kindly provided by Dr J.P. Lecocq (Transgène, Strasbourg, France), was titrated for activity in each experiment and the number of IL-2 units in the test samples was calculated by the method of Farrar *et al.* (4).

The presence of macrophage-activating factors (MAF) in the cell-free supernates was determined by their capacity to prime the bioluminescent reactivity of a cloned macrophage hybridoma (2C11-12) as described previously (2). Briefly, 2C11-12 cells suspended in RPMI 1640 supplemented with 10% foetal calf serum, 50 units penicillin/streptomycin, 300 μg L-glutamin and 235 μg NCTC medium (Flow Laboratories, Irvine, Scotland, U.K.) were cultured in lumacuvettes (PST cuvettes, Lumac 4960, Basel, Switzerland) at a concentration of 10^4 cells in l ml of medium and were incubated for 2 days with 50 μl of the test sample at 37 ^{0} C in a humidified atmosphere containing 5% CO_2. The medium in the lumacuvettes was then removed and replaced by Hanks' balanced salt solution (Flow laboratories) containing a triggering substance, *Micrococcus lysodeikticus* (Miles Chemical Company, Slough, U.K.), opsonized with a polyclonal rabbit anti-micrococcus antiserum and complement (Behring, Marburg, W. Germany). The luminescent response was then recorded in a 6-channel biolumat apparatus LB9505 from the Berthold Company (Wildlab, FGR)

Immunofluorescence

The number of Thy 1^+ cells was assessed by standard fluorescence analysis (6). Briefly, 10^6 lymphocytes were incubated with a rat monoclonal anti Thyl.2 (described in 6), washed and then incubated with a FITC-labelled goat anti-rat Ig (Sigma). The number of fluorescent cells was determined on a FACS II (Becton Dickinson, Electronic Lab, Mountain View, California). The % of surface Thyl, measured as specific fluorescence positive cells was calculated as follows :

$$\frac{\%\text{fluor. cells } (+ \text{ anti Thyl}) - \%\text{ fluor. cells background}}{100 - \%\text{ fluor. cells background}} \times 100$$

Results

It has been well established that inbred mouse strains differ markedly in their relative susceptibility to infections with *T. brucei*. We have recently reported that C3H/He mice are highly susceptible to *T. brucei* AnTAR 1 infections, whereas CBA/Ca mice are relatively resistant (8). Following intraperitoneal administration of metacyclic AnTAR 1 trypanosomes, it was found that C3H/He mice were unable to control the first parasitaemic wave and died +/- 3 weeks after the infection, whereas CBA/Ca mice survived the first parasitaemic wave, which reaches its peak at about day 14, but succumbed to the second wave 45 days after the infection (fig. 1).

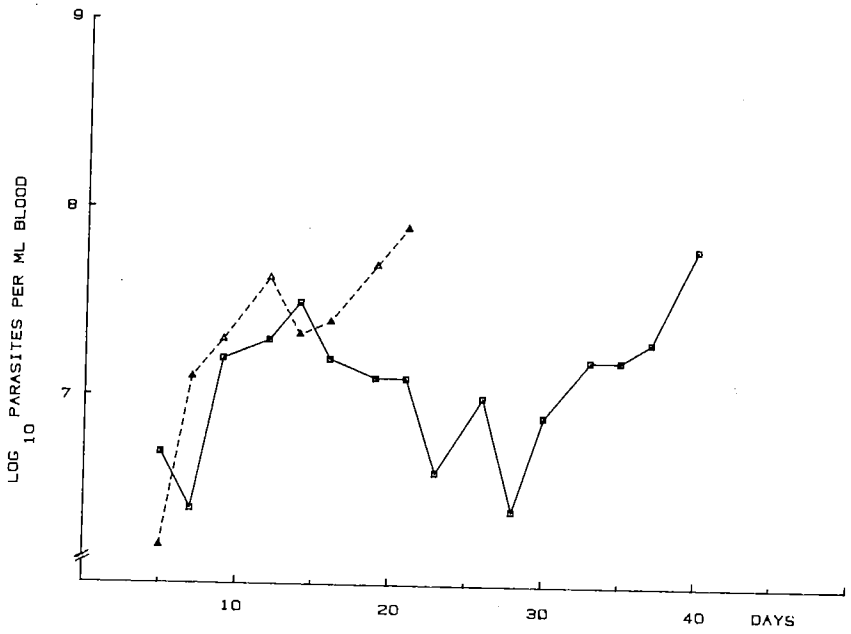

Figure 1.
Parasitaemia in C3H/He mice (△ △ △) and CBA/Ca mice (□ □ □) following intraperitoneal administration of metacyclic stage of *Trypanosoma brucei brucei* AnTAR 1

Using indirect immunofluorescence as a test system, we found that the relative number of T cells in the splenic populations decreased markedly in both strains during the first parasitaemic wave. In the lymph nodes, however, the relative number of T cells remained unaffected in the C3H/He mice and was only slightly decreased in the CBA/Ca mice. For the sake of clarity, we have reviewed these results in table 1.

Table 1.

Evaluation of the number of T cells in normal and *Trypanosoma brucei* infected mice

cells tested	% of Thy 1^+ cells[a]			
	CBA/Ca mice		C3H/He mice	
	uninfected	infected[b]	uninfected	infected
lymph node cells	68	60	73	70
spleen cells	48	10	30	10

[a] The number of Thy 1^+ cells is determined by indirect immunofluorescence followed by FACS II screening. The % of fluorescent cells is calculated as described in Materials and Methods.

[b] The cell populations derived from the infected mice are harvested during the first parasitaemic wave, at day 17 after the infection.

To test the functional status of the T cells derived from normal and infected mice, we have analysed the capacity of the T cells to produce the T-cell growth factor, interleukin 2 (IL-2) as well as other lymphokines such as macrophage-activating factors (MAF) following *in vitro* mitogenic stimulation. To this aim, lymph node cells (LNC) and spleen cells were harvested from infected mice at day 17 after the infection, and stimulated in vitro with a polyclonal T cell activator, concanavalin A (Con A). The amount of IL-2 and MAF in the culture medium was then quantitated as described in the materials and methods. At this stage of the infection, the parasite growth was already curbed in the resistant CBA/Ca, but not in the susceptible C3H/He mice. Indeed, in the CBA/Ca strain, the parasitaemia reaches its peak at day 14 whereafter it decreases. On the other hand, in the C3H/He, the parasitaemia increases continuously until death of the mice at day 21 after the infection.

As shown in table 2, we found that, after Con A activation, the production of IL-2 was twice as high in uninfected CBA/Ca mice as in uninfected C3H/He mice. This superior capacity of the CBA/Ca mice to produce IL-2 was also observed after infection. The absolute IL-2 titres were, however, very low in both strains and never exceeded 0.1 unit per ml, whereas the IL-2 titre of the uninfected populations ranged from 20 to 70 units per ml. Hence, the mitogen-induced IL-2 production is nearly totally inhibited in both strains.

Table 2.

Modulation of interleukin 2 production during *T. brucei* infections

cells tested	interleukin 2 production (units/ml)[a]			
	CBA/Ca mice		C3H/He mice	
	uninfected	infected	uninfected	infected
lymph code cells				
unstimulated	0.0	0.0	0.0	0.0
Con A stimulated	42.86	0.09	23.66	0.03
spleen cells				
unstimulated	0.0	0.0	0.0	0.0
Con A stimulated	72.9	0.07	22.91	0.01

[a] The amount of interleukin 2 in the cell free supernates is quantitated by the interleukin 2 dependent proliferation of CTL-L cells and is expressed as units / ml.

Although the supernates of mitogen-activated cell populations from infected mice were nearly totally devoid of IL-2, they still contained a high amount

of MAF. The presence of MAF was determined by the ability of these supernates to prime the bioluminescent activity of a cloned macrophage hybridoma, and is expressed as counts per minute. It is demonstrated in table 2 that the MAF production by LNC derived from infected mice was only very slightly decreased in CBA/Ca mice and even increased in the sensitive C3H/He mice. The background MAF levels in the LNC populations were not altered during infection. On the other hand, the background levels in the splenic populations were decreased. It is not yet clear whether this is related to the decreased number of T cells in these populations. However, despite the low T-cell number, the mitogen-activated splenic MAF production was not impaired, but rather increased in both strains during infection.

Discussion

Using immunofluorescence as a test system, we have recently reported that the differential resistance of CBA/Ca mice (relatively resistant) and C3H/He mice (highly susceptible) to experimental *T.brucei* AnTAR 1 infections is not attributed to a differential modulation of the T-cell number. However, such immunofluorescent staining does not reveal any information about the functional status of the T cells. Indeed, we have demonstrated before that experimental infection with bloodstream AnTat 1.1 E trypanosomes does not affect the number of the T cells in the lymph node cell populations, but results in a severe suppression of the mitogen-induced IL-2 production (12). Furthermore, this inhibition was not attributed to a total paralysis of the T-cell function since the mitogen-induced production of other T-cell derived lymphokines, such as MAF, was not suppressed during infection. Hence, an experimental *T.brucei* infection causes a selective suppression of the mitogen-induced IL-2 production.

Although such a mitogenic *in vitro* T-cell activation does not provide any information about the induction of parasite-specific cellular immunity, it reveals the functional status of the T cells harvested from infected animals. Therefore, it is most useful as a test system to probe the non-specific immunosuppressive capacities of parasites. We have therefore focused on the selective inhibition of IL-2 versus MAF production following mitogenic stimulation to probe the trypanosome-mediated modulation of T-cell responsiveness in highly susceptible (C3H/He) and relatively resistant (CBA/Ca) mice.

We found that lymph node as well as spleen cells derived from both CBA/Ca and C3H/He mice were unable to secrete IL-2 as compared to normal controls. Although it is possible that the low IL-2 titre in the splenic populations is partially caused by the three to five fold decrease of the relative number of Thy 1^+ cells during infection (8), such a modulation of the T-cell number cannot account for the LNC unresponsiveness. Indeed, the relative number of lymph node Thy 1^+ cells was not markedly altered during the first parasitaemic wave in either strain. Despite their inability to produce the T-cell growth factor, both LNC and splenic populations from CBA/Ca as well as from C3H/He had retained their capacity to produce macrophage-activating lymphokines.

Our results are therefore in accordance with other investigations, indicating that IL-2 production is suppressed during *Trypanosoma congolense* infections in both resistant and susceptible mice (9). Our results are, however, in disagreement with the results of Murray *et al.* (10) showing that spleen cells derived from susceptible, but not those derived from resistant mice manifest a suppressed MAF production during experimental *Leishmania donovani* infection. This discrepancy might be attributed to the different assay system (microbicidality versus chemiluminescent responses) or to the difference in parasite species.

Although during the infection we did not observe a suppression of MAF production in either strains, we have demonstrated in table 2 that the amount of MAF production from spleen cells derived from the relatively resistant CBA/Ca mice was twice as high as that from spleen cells derived from the susceptible C3H/He mice. However, it is not yet clear whether these observations are related to the previously described finding that the serum levels of interferon γ, a potent macrophage stimulator, are increased in resistant, but not in susceptible mice during experimental *T. rhodesiense* infections (3). Indeed, the high in vitro production of MAF was observed only following mitogenic T-cell stimulation, and hence was not attributed to a constitutive parasite-induced interferon production. Furthermore, the higher production of MAF by the resistant strain was restricted to spleen cell populations, and was not found in mitogen-stimulated lymph node cell populations.

Table 3

Modulation of the production of macrophage activating lymphokines during experimental *T. brucei* infections

cells tested	MAF production (cpm × 10^{-3})[a]			
	CBA/Ca mice		C3H/He mice	
	uninfected	infected	uninfected	infected
lymph code cells				
unstimulated	3,470	2,770	2,560	3,110
Con A stimulated	25,480	17,200	18,510	20,030
spleen cells				
unstimulated	3,560	2,730	7,870	3,180
Con A stimulated	25,840	45,440	15,010	20,890

[a] The presence of MAF in the cell free supernates is quantitated by their ability to prime the bioluminescent reactivity of a cloned macrophage hybridoma (2C11-12). The light emission, measured as counts per minute, is measured each minute during 30 min following the addition of a macrophage trigger (opsonized micrococci). The results are further expressed as the integrated value of this graph

2C11-12 cells incubated without a cell derived supernate manifest a background light emission of 3350 × 10^3 cpm.

In conclusion, experimental *Trypanosoma brucei* AnTAR1 infection induces a severe suppression of interleukin 2 production, but does not inhibit MAF production. This selective suppression of IL-2 production is, however, not related to the differential resistance of the tested inbred mouse strains.

Acknowledgements - *This work was supported by the ILRAD/Belgium Research Centers Agreement for Collaborative Research, by contract nr.3.0057.84 of the Fonds voor Geneeskundig Wetenschappelijk Onderzoek, and by EEC/STD contract TSD-A-118-B (MR) M. Sileghem and P De Baetselier are fellows of the NFWO. We would like to thank E. Vercauteren and M. Gober for technical assistance.*

Modulatie van lymfokine produktie tijdens experimentele *Trypanosoma brucei* infectie bij ingeteelde muizestammen.

Samemenvatting — Experimentele *Trypanosoma brucei* AnTARI infecties veroorzaken een suppressie van de produktie van interleukine 2, maar hebben geen invloed op de produktie van andere T cel lymfokines zoals makrofaag activerende faktoren. Deze selectieve inhibitie van interleukine 2 produktie wordt zowel bij de gevoelige C3H/He als bij de relatief resistente CBA/Ca stam waargenomen. Dit toont aan dat er geen verband bestaat tussen de besproken selectieve suppressie van interleukine 2 produktie en de relatieve gevoeligheid van de geteste ingeteelde muizestammen.

Modulation de la production de lymphokines durant l'infection expérimentale par *Trypanosoma brucei* chez des souches consanguines de souris.

Resumé — L'infection expérimentale de la souris par *Trypanosoma brucei* AnTAR1 entraîne une diminution considérable de la production d'interleukine 2. La production par la cellule T d'autres lymphokines n'est pas touchée. Cette dépression sélective de la synthèse d'interleukine 2 se retrouve tant chez les souris susceptibles C3H/He que chez les souris relativement résistantes CBA/Ca et ne peut donc pas être mise en rapport avec la susceptibilité relative aux trypanosomes des souches consanguines de souris étudiées.

Received for publication on January 21, 1987.

REFERENCES

1. Bancroft G F , Askonas B A: Regulation of antibody production in protozoal infections. Clinics in Immunology and Allergy, 1982, **2**, 511-540.
2. De Baetselier P, Brys L, Mussche L, Remels L, Vercauteren E, Schram E: Generation and analytical applications of luminescent macrophage cell lines. C.R.C. Presse, 1986, *in press*.
3. DeGee A L, Sonnenfeld G, Mansfield J M: Genetics of resistance to the African Trypanosomes. V. Qualitative and quantitative differences in interferon production among susceptible and resistant mouse strains. The Journal of Immunology, 1985, **134,** 2723-2726.
4. Farrar J J, Mizel S B, Fuller-Farrar J, Farrar W L, Hilfiker M L : Macrophage independent activation of helper T cells. I. Production of interleukin 2. The Journal of Immunology, 1980, **125,** 793'798.
5. Harrel-Bellan A, Joskowicz M, Fradelizi D, Eisen H: Modification of T cell proliferation and interleukin 2 production in mice infected with *Trypanosoma cruzi*. Proceedings of ther National Academy of Sciences, 1983, **80,**3466-3469.
6. Ledbetter J A, Herzenberg L A : Xenogeneic antibodies to mouse differentiation antigens. Immunological Reviews, 1979, **47,**63.
7. Makumyaviri A M, Demey F, Claes Y, Verhulst A, Le Ray D: Caractérisation de la capacité vectorielle de *Glossina morsitans morsitans* (Diptera Glossinidae) vis-à-vis de *Trypanosoma brucei brucei* EATRO 1125 (AnTARI). Ann. Soc. belge Méd. trop., 1984, **64,** 365-372.
8. Makumyaviri A M, Sileghem M, Le Ray D, Hamers R, De Baetselier P: Système lymphocytaire et résistance relative de la souris consanguine à *Trypanosoma brucei brucei. Submitted.*
9. Mitchell L A, Pearson T W, Gauldie J: Interleukin-1 and interleukin-2 production in resistant and susceptible mice infected with *Trypanosoma congolense*. Immunology, 1986, **57,** 291-296.
10. Murray H W, Masur H, Keithly J S: Cell-mediated immune response in experimental visceral leishmaniasis. I. Correlation between resistance to *Leishmania donovani* and lymphokine generating capacity. The Journal of Immunology 1982,**129,** 344-350.
11. Reiner N E, Finke J H: Interleukin 2 deficiency in murine leishmaniasis *donovani* and its relationship to depressed spleen cell responses to phytohaemaglutinin. The Journal of Immunology, 1983, **131,** 1487-1491.
12. Sileghem M, Hamers R, De Baetselier P: Active suppression of interleukin 2 secretion in mice infected with *Trypanosoma brucei*. Parasite Immunology, 1986, **8,** 641-649.
13. Tarleton R L, Kuhn R E: Restoration of in vitro immune responses of spleen cells from mice infected with *Trypanosoma cruzi* by supernatants containing interleukin 2. The Journal of Immunology, 1984,**133,** 1570-1575.