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Non-specific suppression of antigen-induced lymphocyte blastogenesis in *Onchocerca volvulus* infection in man

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SUMMARY

Lymphocyte blastogenic responses to *O. volvulus* antigen (Oncho Ag), SKSD, and the mitogen PHA were tested in three groups of persons: light to moderately infected persons (INF); previously exposed but uninfected persons (EXP) and normal controls (NC). The exposed group showed significant responsiveness to Oncho Ag (Δct/min = 6,002 ± 1,375), while the infected (Δct/min = 943 ± 418) and normal control (Δct/min = 428 ± 418) groups did not. The mean blastogenic response to SKSD were EXP, 8,644 ± 5,249; NC 6,039 ± 2,880; INF, 2,619 ± 1,012. The reduced reactivity in the INF group to Oncho Ag showed a significant correlation with reactivity to SKSD ($P < 0{\cdot}05$). To elucidate the mechanism of hyporesponsiveness in the infected group rigorous adherent cell depletion, by adherence to plastic followed by a nylon wool column, was utilized. When 20% plastic adherent cells were added back to the T cells prepared in this fashion, the mean blastogenic response to SKSD was significantly augmented ($P < 0{\cdot}01$). In contrast, the responsiveness to Oncho Ag was not significantly altered. The addition of indomethacin (1 μg/ml) or autologous plasma had no significant effect on reactivity to either SKSD or Oncho Ag. There were no significant differences in the mean reactivity of the three groups to PHA-M (Δct/min EXP 78,514 ± 12,564; INF 62,393 ± 14,447; NC 61,423 ± 4,465). These results suggest that *O. volvulus* infection is associated with decreased lymphocyte reactivity to both parasite related and unrelated antigens, and imply that the mechanism for the two types of hyporesponsiveness may be distinct. While a weakly adherent suppressor cell may account for non-specific hyporesponsiveness, the mechanism of parasite specific decreased reactivity remains unknown.

INTRODUCTION

Onchocerciasis is one of the major filarial infections of man, and a leading cause of blindness. The major disease manifestations are thought to relate, directly or indirectly, to the immune response which the host mounts against the parasite (Henson, Mackenzie & Spector, 1979). Although infection with *Onchocerca volvulus* can be associated with severe disability, the majority of individuals with light infection have little symptomatology. This suggests that the parasite may possess some mechanism which permits avoidance of the host immune response, but such mechanisms remain to be elucidated.

Defects in cellular immunity have been described in the lymphatic filariases *Wuchereria bancrofti* (Ottesen, Weller & Heck, 1977) and *Brugia malayi* (Piessens *et al.*, 1980a, 1982) in man. In

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both cases, the defect in lymphocyte blastogenesis was specific for filarial antigens, with preservation of reactivity to unrelated antigens and to mitogens. Antigen specific adherent suppressor cells and suppressor T lymphocytes have been implicated in the lack of responsiveness seen in *B. malayi* infection (Piessens *et al.*, 1980b, 1982). In *O. volvulus* infection, diminished reactivity to parasite derived antigens with preservation of PHA reactivity has been reported (Bryceson, 1976; Ngu, 1978). However, other reports, based on delayed skin test reactivity, indicate that the depressed cell-mediated immunity may not be restricted to parasite related antigens (Buck *et al.*, 1969; Prost, Nebout & Rougemont, 1979).

In the present study, we evaluated antigen- and mitogen-induced lymphocyte blastogenesis in *O. volvulus* infected persons. The results show that persons with *O. volvulus* infection have decreased cellular responsiveness to both onchoceral and to unrelated antigens, and suggest that a nylon wool adherent suppressor cell may account for part of the diminshed lymphocyte reactivity.

MATERIALS AND METHODS

Study subjects. Infected Liberians, age 17–60 (mean 32 ± 14) were chosen based on light to moderate intensities of infection, and the absence of physical signs to suggest malnutrition or wasting. Skin biopsies were obtained from the shoulders, hips and posterior calves using a 1·5 mm Stortz sclerectomy punch instrument (Stortz Instrument Co., St Louis, Missouri, USA). The specimens were incubated in flat bottomed 96 well microtitre plates (Costar, Cambridge, Massachusetts, USA) in 0·9% saline at ambient temperature for 3 h to permit the microfilariae to emerge from the biopsy specimens, and the number of microfilariae was determined by direct count with an inverted microscope. Using this technique, infected persons had a range of 2–43 microfilariae per skin snip. Normal controls were five volunteers (mean age 30 ± 6) living in Liberia or the United States with no exposure to the insect vector of *O. volvulus* infection. The exposed group consisted of three Liberians from the endemic area (mean age 38 ± 19) who were free of active infection as determined by skin biopsy.

Crude onchocercal antigen preparation. Nodules (onchocercomata) removed from infected individuals in Liberia were placed in 6% dimethylsulphoxide (Fisher, Fair Lawn, New Jersey, USA) for cryopreservation, and were frozen and stored in liquid nitrogen. Nodules were thawed, teased and incubated in minimal essential media (MEM) (K.C. Biologicals, Lenexa, Kansas, USA) with 100 u/ml penicillin and 100 μg/ml streptomycin (P/S) (MA Bioproducts, Kensington, Maryland, USA) to allow emergence of microfilariae. The remaining nodule material was subsequently incubated in 0·9% saline at room temperature in order to permit any residual dimethylsulphoxide to diffuse out, and a homogenous paste was produced by fragmentation and grinding on ice in a Ten-Broeck tissue grinder. This preparation was then centrifuged at 4°C at 20,000 **g** for 90 min. The final supernatant was filtered through a 0·2 μm sterilizing filter and the protein concentration was determined by the Petersen modification of the Lowry method (Petersen, 1977).

Lymphocyte blastogenesis. Cell separations were begun within 60 min of obtaining the samples of blood. Peripheral blood mononuclear cells (PMBC) were prepared by Ficoll-Hypaque (Ellner, 1978) sedimentation of heparinized (20 u/ml) blood. Adherent cells were collected by incubation in plastic Petri dishes (Optilux, Falcon Plastics, Oxnard, California, USA) in RPMI 1640 (K.C. Biologicals)–P/S with 5% fetal calf serum (FCS) for 1 h at 37°C in 5% CO_2. After removal of non-adherent cells by gentle washing with RPMI 1640 at 37°C four times adherent cells were dislodged by gentle scraping with a rubber policeman. Adherent cells prepared in this fashion have been shown to be greater than 80% esterase positive (Ellner, 1978). Non-adherent cells were further depleted of non-T cells by incubation on an acid washed nylon wool column. Cell counts were performed with a haemocytometer chamber, and differential counts were based on Wright's stained preparations. Using these morphological criteria, the T cell enriched population consisted of $96{\cdot}0 \pm 1{\cdot}2\%$ lymphocytes. This population has been shown to contain 70–90% sheep erythrocyte rosetting and $< 1\%$ non-specific esterase positive cells (Ellner, 1978).

Cells were adjusted so that each plate well contained 0·1 ml of a suspension of $1{\cdot}5 \times 10^6$/ml. Cells were cultured in RPMI 1640, P/S, 2mM L-glutamine (Flow Laboratories, McLean, Virginia, USA)

with 10% heat-inactivated (56°C for 30 min) pooled normal human serum (PHS) or heat-inactivated autologous human serum (AHS). For reconstituted cultures, adherent and T cell enriched populations were combined in a 1:4 ration to yield a final concentration of $1{\cdot}5 \times 10^6$/ml. One hundred microlitre aliquots of cell suspension were placed in flat bottomed microtitre wells for mitogen stimulation, or in round bottomed microtitre wells (Cooke Industries, Arlington, Virginia, USA) for antigen stimulation. Mitogen, PHA (PHA-M, DIFCO, Detroit, Michigan, USA) was added in 10 μl amount for a final concentration of 1:100, 1:500 and 1:5,000. Streptokinase–streptodonase (SK-SD) (Lederle, Valley Stream, New York, USA) was used in a 1:100 final dilution (100 u streptokinase/ml). Onchocercal antigen was used in concentrations of 1, 10, and 100 μg/ml. Indomethacin (Sigma, St Louis) was dissolved in 95% ethanol at 10 mg/ml, then diluted to a final concentration of 1·0 μg/ml in the microtitre wells. All variables were tested in quadruplicate. Plates were incubated at 37°C in a humidified CO_2 incubator. Following 2 day culture for mitogen stimulation and 4 day culture for antigen stimulation 1 μCi ^{3}H-thymidine (specific activity 5·0 Ci/mmole) (Amersham, Arlington Hts., Illinois, USA) was added to each well. Eighteen hours later, the cells were harvested onto filter paper using a mini-MASH harvester (MA Bioproducts, Kensington, Maryland, USA). The filter paper was dried, the individual discs placed in vials in liquid scintillant, and ^{3}H activity determined in a liquid scintillation counter. Results are expressed as Δct/min (experimental minus control).

RESULTS

Blastogenic responses of whole peripheral blood mononuclear cell populations (PBMC)

To establish that crude *O. volvulus* nodule material (Oncho antigen) was antigenic in a lymphocyte blastogenesis assay, PBMC from persons previously exposed to *O. volvulus* but free of evidence of infection (EXP) were tested. Mean Δct/min for this group was 6,002 ± 1,375 (Table 1). In contrast, normal controls (NC) failed to show significant responsiveness (mean Δct/min 428 ± 221). The response of the group with proven active infection (INF) with *O. volvulus* (943 ± 418) did not differ significantly from the normal control group, but was significantly less than the EXP group ($P < 0{\cdot}005$, unpaired *t*-test).

Mean blastogenic response to SKSD was lower in the INF group (2,619 ± 1,012) than in either the EXP (8,644 ± 5,249) or NC (6,039 ± 2,880) groups, but these differences were not significant. However, the SKSD response was markedly diminished (Δct/min) < 2,000) in four of the eight in the INF group and in no one in the EXP or NC groups. Of interest was the finding that the depressed reactivity to *O. volvulus* antigen appeared to parallel the diminished reactivity to SKSD (Table 2). Indeed, a statistically significant correlation between reactivity to these two antigens was found ($P < 0{\cdot}05$, Spearman rank).

Table 1. Lymphocyte blastogenic response to *O. volvulus* antigen, SKSD, and PHA in PBMC from infected, previously exposed and normal subjects

	O. volvulus antigen (100 μg/ml)	SKSD (100 u SK/ml)	PHA‡
Infected (8†)	943 ± 418*	2,619 ± 1,012	62,393 ± 13,514
Exposed (3)	6,002 ± 1,375	8,644 ± 5,249	78,514 ± 12,564
Normal controls (5)	428 ± 221	6,039 ± 2,880	61,423 ± 4,465

* Mean ± s.e.(mean).

† Number of persons studied.

‡ Maximal responsiveness to any of the three concentrations.

Table 2. Lymphocyte blastogenesis in PBMC from *O. volvulus* infected persons in response to crude *O. volvulus* worm antigen and to SKSD*

Subject†	Oncho	SKSD
1	0	0
2	76	0
3	286	1,786
4	416	2,436
5	543	616
6	584	6,199
7	2,631	7,711
8	3,012	2,204

* Expressed as Δct/min.
† Arranged in order of increasing Δct/min to oncho antigen.

Effect of adherent cell depletion on PBMC responses

Depletion of adherent cells by plastic followed by a nylon wool column, with subsequent reconstitution with 20% of the plastic adherent PBMC (mϕ) from the first step, resulted in significant augmentation of the blastogenic response to SKSD in the eight infected persons (Fig. 1) ($P < 0{\cdot}01$, paired *t*-test). In only one of these individuals did the purified non-adherent population, without mϕ reconstitution, show significantly increased reactivity compared to whole PBMC (5,887 *vs* 1,786 ct/min, $P < 0{\cdot}001$, unpaired *t*-test). In contrast to the SKSD response, adherent cell depletion, with or without mϕ reconstitution, had no significant effect on the response to Oncho antigen (Fig. 2). Only one subject showed an increase of > 1,000 ct/min and this augmentation was not statistically significant.

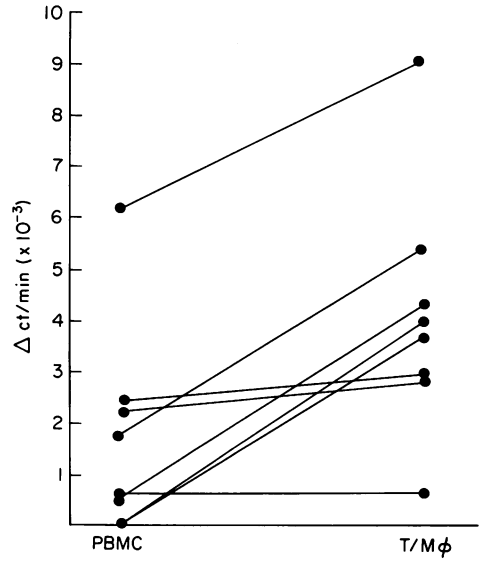

Fig. 1. SKD-induced lymphocyte blastogenesis in PBMC and T cell mϕ populations from *O. volvulus* infected subjects.

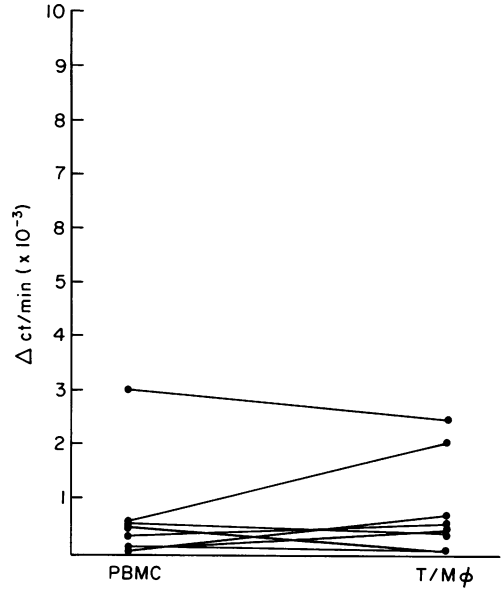

Fig. 2. *O. volvulus* antigen-induced lymphocyte blastogenesis in PBMC and T cell mφ populations from *O. volvulus* infected subjects.

Effect of indomethacin and of autologous plasma

Indomethacin treatment of PBMC populations had no significant effect on the SKSD or Oncho Ag response in the infected groups (mean SKSD Δct/min 1,721 ± 774, with indomethacin 3,164 ± 1,925; mean Oncho Ag response 629 ± 376, with indomethacin 1,125 ± 430). Similarly, the use of autologous plasma, instead of normal pooled human plasma, resulted in no significant change in reactivity to either SKSD or Oncho Ag.

Phytohaemagglutinin (PHA) reactivity

There were no significant differences in the mean reactivity of the three groups to PHA (Δct/min INF 62,393 ± 14,447; EXP 78,514 ± 12,564; NC = 61,423 ± 4,465). However, three persons in the infected group showed PHA responses which were below the lower limit of the 95% confidence level established by the normal control group (43,919 ct/min) (Table 1). The two lowest of these showed normal PHA reactivity (both greater than 50,000 ct/min) when adherent cells were removed with or without mφ reconstitution.

DISCUSSION

Onchocerciasis has been reported to be associated with defects in cellular immunity. In particular, a diminished prevalence of PPD reactivity in *O. volvulus* infected persons, and an increased prevalence of lepromatous leprosy have suggested a cellular defect which is not parasite specific (Buck *et al.*, 1969; Prost *et al.*, 1979). Further, diminished delayed hypersensitivity skin test results to *O. volvulus* antigen were found in persons with moderate levels of infection as compared to those with very light infection (Bartlett *et al.*, 1978). Decreased lymphocyte blastogenesis and migration inhibition factor production with intact mitogen reactivity have also been reported (Bryceson, 1976; Ngu, 1978). In the present report, we show that *O. volvulus* infected persons have diminished lymphocyte blastogenic responses to both parasite antigen and to an unrelated antigen, streptokinase–streptodornase. In contrast, PHA responsiveness was normal in six of eight subjects studied.

Lymphocyte blastogenesis induced by SKSD was significantly increased by removal of a nylon wool adherent population of cells and reconstitution with monocyte enriched plastic adherent cells. The identity of the relevant cell, adherent to nylon wool but not to plastic, remains to be established, but a weakly adherent T cell or B cell subpopulation seems most likely. In this regard, the report of an inhibitory weakly adherent T cell from patients with osteogenic sarcoma (Yu *et al.*, 1977) may be relevant. In addition, a recent report using a mouse system has demonstrated antigen non-specific suppression of T cells by a B cell population in response to antigen–antibody complexes (Miyama-Inaba *et al.*, 1982). This is particularly relevant in view of the frequent occurrence of increased levels of circulating immune complexes in onchocerciasis (Lambert *et al.*, 1978; Paganelli, Ngu & Levinsky, 1980; Steward *et al.*, 1982). Finally, nylon wool columns deplete basophils which may be immunosuppressive by virtue of their production of histamine as has been demonstrated using cells from subjects with schistosomiasis (Hofstetter, Fasan & Ottesen, 1982). It is important to note that, in contrast to the SKSD response, adherent cell depletion had no effect on *O. volvulus* antigen responsiveness. This suggests that there may be more than one distinct cellular mechanism which leads to lymphocyte unresponsiveness in *O. volvulus* infection.

These findings contrast sharply with other recent studies in the lymphatic filariases caused by *Wuchereria bancrofti* (Ottesen *et al.*, 1977) and *Brugia malayi* (Piessens *et al.*, 1980, 1982). Studies of lymphocyte reactivity in bancroftian filariasis did show that individuals who were exposed but not infected demonstrated brisk reactivity to worm antigen, while those with microfilaremia had markedly reduced or negligible responsiveness (Ottesen *et al.*, 1977). On the other hand, responses to SKSD or PPD were normal, suggesting parasite antigen specific unresponsiveness. Similarly, in *B. malayi* infection, parasite antigen specific unresponsiveness was found in persons with patent microfilaraemia (Piessens *et al.*, 1980a). Subsequent studies indicated that the diminished responsiveness in microfilaraemic individuals was due in part to an adherent suppressor cell, and that sera from infected individuals were also suppressive (Piessens *et al.*, 1980b). Moreover, antigen specific T suppressor cells, reactive with OKT8 monoclonal antibody are increased in such patients and appear to have a role in depressed blastogenic responses (Piessens *et al.*, 1982). In contrast, in the present study we found that individuals with patent infection demonstrate hyporesponsiveness to both parasite derived and to an unrelated antigen, SKSD. Although a non-specific decrease in lymphocyte blastogenic response has not been previously reported in human filariasis, these findings are consistent with results of skin test surveys which show depressed PPD responsiveness in *O. volvulus* infection (Buck *et al.*, 1969; Rougemont *et al.*, 1977). Of interest in this regard is the finding of non-specific suppression of *in vitro* lymphocyte proliferation by high concentrations of *B. malayi* microfilarial antigen (Piessens *et al.*, 1982). Finally, we found no evidence of an effect of autologous plasma as opposed to normal plasma, nor of an effect of indomethacin which has been reported to inhibit suppressor cell activity in other systems (Goodwin, Bankhurst & Messner, 1977). However, a larger group of infected persons with a wide range of intensities of infection must be studied before a role for a soluble factor or an indomethacin sensitive suppressor mechanism can be excluded.

One of the paradoxes in onchocerciasis is the ability of microfilariae to migrate through tissues with no apparent host response. This is despite the presence of antibodies directed against microfilariae which are capable of mediating attachment and killing by host granulocytes *in vitro* (Mackenzie, 1980; Greene, Taylor & Aikawa, 1981). It is possible that the depressed cellular reactivity contributes to this lack of host response, perhaps as a result of a lack of granulocyte regulatory factors which normally are produced by mononuclear cells, particularly T lymphocytes.

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