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Interleukin-1 and interleukin-2 production in resistant and susceptible inbred mice infected with *Trypanosoma congolense*

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SUMMARY

In vitro production of interleukin-1 (IL-1) by LPS-stimulated adherent peritoneal exudate and spleen cells and alveolar macrophages, and interleukin-2 (IL-2) by concanavalin A-stimulated splenocytes were measured in resistant (C57BL/6J) and susceptible (A/J) inbred mice during the early stages of subacute infections with the African trypanosome, *Trypanosoma congolense*. Production of IL-1 was severely depressed in both mouse strains as early as 24 hr after intraperitoneal injection of bloodstream trypanosomes. Similarly, in both mouse strains, an early decline in IL-2 activity was observed, followed by partial recovery then depression to subnormal levels. These changes in measurable IL-1 and IL-2 activity in infected mice concurred with progressive depression in the spleen cell proliferative response to the mitogen concanavalin A.

INTRODUCTION

Animals infected with African trypanosomes show profound changes in their immune systems, one of which is a generalized immunodepression that affects both humoral and cellular immune responses (reviewed by Mansfield, 1981). Many of the investigations concerning immunodepression in trypanosomiasis have evaluated immunological changes that occur relatively late in infection, i.e. after the establishment of bloodstream parasitaemias. It is not surprising that profound immunological changes would occur as a result of the antigenic load imposed on the host immune system by large numbers of trypanosomes which occur in successive waves of antigenically distinct parasites. There have been few, if any, investigations of the early host–parasite interactions that may lead to perturbations of the immune system. Immunological responses during the initial host–parasite interaction may critically influence susceptibility to trypanosomiasis and may determine the outcome of the infection.

Interleukin-1 (IL-1) and interleukin-2 (IL-2), soluble mediators released from activated macrophages (Gery & Waksman, 1972) and T lymphocytes (Farrar *et al.*, 1982), respectively, are essential elements of lymphokine cascade involved in the initiation of immune and inflammatory responses (Oppenheim & Gery, 1982; Mizel, 1982; Weigent, Stanton & Johnson, 1983). Alterations in the levels of either of these lymphokines during the early stages of African trypanosomiasis could have a significant effect on the outcome of the infection.

In the experiments described herein, *in vitro* production of interleukin-1 and interleukin-2 were measured in inbred mice exemplifying the extremes of resistance (C57BL/6J) or susceptibility (A/J) to infections with a relatively avirulent clone of the African trypanosome *Trypanosoma congolense* (Morrison *et al.*, 1978). The results of these investigations showed that the *in vitro* production of IL-1 by adherent cells obtained from the spleen, lung and peritoneal cavity was severely depressed by *T. congolense* infection in both A/J and C57BL/6J mice as early as 24 hr following intraperitoneal injection of trypanosomes. An early decline in IL-2 activity, which concurred with reductions in IL-1 activity, was also observed in both strains of mice.

Abbreviations: AM, alveolar macrophages; Con A, concanavalin A; DEAE, diethylaminoethyl; FCS, fetal calf serum; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid; [^{3}H]TdR, methyl tritiated thymidine; IL-1, interleukin-1; IL-2, interleukin-2; i.p., intraperitoneal; [^{125}I]UdR, iododeoxyuridine; LPS, lipopolysaccharide; MNC, mononuclear cells; PEC, peritoneal exudate cells; PSG, phosphate-buffered saline-glucose; SPLC, spleen cells.

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MATERIALS AND METHODS

Animals

Female A/J and C57BL/6J mice aged 8–10 weeks, and female C3H/HeJ mice aged 6 weeks, were obtained from the Jackson Laboratory, Bar Harbor, ME. Female or male Long Evans rats were bred from stock purchased from Charles River (Canada) Ltd, St Constant, Quebec.

Trypanosomes and infections

Trypanosoma congolense ILRAD 588, a triply cloned parasite

originally derived from an infected bovid (Morrison *et al.*, 1978), was obtained from Dr W. I. Morrison of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya. Cryostabilates were prepared from trypanosomes isolated from the blood of cyclophosphamide-immunosuppressed (Smith, Levine & Mansfield, 1982) rats by DEAE-cellulose chromatography (Lanham & Godfrey, 1970). Stabilates were thawed rapidly at 37° just before use, and trypanosomes were diluted in sterile phosphate-buffered saline-glucose (PSG; Lanham & Godfrey, 1970). Mice were injected intraperitoneally (i.p.) with $2{\cdot}0$–$2{\cdot}5 \times 10^5$ trypanosomes in a volume of 0·1 ml.

Interleukin-1 determinations

Interleukin-1-containing supernatants were generated in the following way. Mice were killed at 1, 2, 3, 7 and 9–10 days after i.p. injection of *T. congolense*. Normal (sham-injected with 0·1 ml of PSG, pH 8·0) mice were included as controls. Cell populations from five mice of each strain were pooled at each time. Peritoneal exudate cells (PEC), obtained by lavaging the peritoneal cavity with ice-cold sterile 0·34 M sucrose, and alveolar macrophages (AM) obtained by intratracheal lavage, were washed twice in serum-free RPMI-1640 medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol and 1 mM HEPES buffer by centrifugation for 7 min at 500 *g* at 4° and were adjusted to 1×10^6 cells/ml in the same medium. Spleen cell (SPLC) suspensions were prepared and washed in the above medium, adjusted to $1{\cdot}5 \times 10^7$ cells/ml and passed through nylon wool columns at room temperature. Non-adherent cells were collected, washed twice in serum-free medium, and adjusted to 3×10^6 cells/ml in the same medium. PEC, A, or SPLC were pipetted in 1-ml aliquots into the wells of 24-well culture plates and incubated at 37° (10% CO_2 in air) for 2 hr. At the end of this period, non-adherent cells were removed and the wells were washed three times with 1 ml of serum-free medium. One ml of serum-free medium containing 10 μg/ml of indomethacin (Sigma Chemical C., St Louis, MO) was pipetted into each well. Half the wells in each group also received LPS (*E. coli* 055:B5 lipopolysaccharide, Sigma) at a final concentration of 10 μg/ml. The cells were incubated for 24 hr at 37° in 10% CO_2 in air. Aliquots of medium (without cells) containing indomethacin ± LPS were also included to serve as negative controls in the IL-1 assay described below. Supernatants were removed and pooled for each treatment, then centrifuged, filter-sterilized and stored at −20° until tested.

Interleukin-1 (IL-1) was measured in culture supernatants from adherent PEC, AM or SPLC using a modification of the costimulation assay of Mizel (1980) which measures IL-1 augmentation of the proliferation of thymocytes (from 6–10-week-old female C3H/HeJ mice adjusted to 5×10^6 cells/ml in RPMI-1640 medium containing antibiotics, 2-mercaptoethanol, HEPES buffer and 10% preselected, heat-inactivated fetal calf serum) stimulated with suboptimal doses of mitogen (Con A, Difco, Detroit, MI, added at a final concentration of 1 μg/ml). The cells were incubated in a total volume of 0·2 ml in 96-well culture plates at 37° in 10% CO_2 in air for 72 hr. During the last 18 hr, the cells were pulsed with [^{125}I]deoxyuridine ([^{125}I]UdR, Edmonton Radiopharmacy Centre, Edmonton, Alberta, 0·1 μCi per well). The cells were harvested onto glass fibre filters and counted for [^{125}I] radioactivity. Results are expressed as the net c.p.m. incorporated above the negative (medium ± LPS) control.

Interleukin-2 determination

Interleukin-2 (IL-2)-containing culture supernatants were prepared in the following way. Three to five mice of each strain were killed at 0·5, 1, 2, 3, 7 and 9–10 days after i.p. injection of *T. congolense*. Three to five normal (sham-injected) mice of each strain were included as controls. Spleen cell (SPLC) suspensions were prepared in RPMI-1640 medium containing antibiotics, 2-mercaptoethanol, HEPES buffer (20 mM) and heat-inactivated FCS (10%) and washed twice by centrifugation, then adjusted to 2×10^6 cells/ml in the same medium. Five ml of SPLC suspension were incubated horizontally in 25-ml culture flasks for 24 hr at (37° in 10% CO_2 in air) in the presence or absence of Con A at a final concentration of 5 μg/ml. Aliquots of medium ± Con A without cells were also incubated to serve as negative controls in the IL-2 assay (below). At the end of the incubation, cell-free supernatants were prepared and stored at −20° until tested for IL-2 activity.

Amounts of IL-2 activity present in SPLC culture supernatants from normal and *T. congolense*-infected A/J and C57BL/6J mice were determined by their ability to support the growth of the IL-2-dependent cytotoxic T-cell line, MTL 2·8·1 (Bleackley, Havele & Paetkau, 1982). MTL 2·8·1 cells and EL-4S2 thymoma cells (used as an IL-2 source) were obtained from Dr D. Kilburn, Department of Microbiology, University of British Columbia, Vancouver, BC. Details for maintaining these cell lines have been described elsewhere (Mitchell, 1984). The medium used in the assay was the same as described above; however, phenymethylsulphonyl fluoride was added at a final concentration of 25 μg/ml. All supernatants were tested in triplicate. MTL cells (washed and adjusted to 5×10^3 cells/0·1 ml in the above medium) were mixed with 0·1 ml of culture supernatants in 96-well microculture plates. Supernatants containing optimum amounts of IL-2 (from EL-4 thymoma cultures) served as a positive control, while negative control wells received only culture medium ± Con A. The plates were incubated for 24 hr at 37° in 10% CO_2 in air, and pulsed for a further 24 hr with a labelling mixture consisting of [^{125}I] UdR (0·1 μCi/well) in medium containing 5 μg/ml fluorodeoxyuridine and 0·1 mM deoxyinosine (both from Sigma). Supernatants were replaced with 200 μl of PBS containing 20 mM EDTA to dissociate the adherent MTL cells from the wells. The cells were harvested onto glass fibre filters and counted for radioactivity. Results are expressed as the growth stimulation index (GSI):

$$\text{GSI} = \frac{\text{c.p.m. incorporated with test supernatant}}{\text{c.p.m. incorporated with medium}}.$$

Mitogen stimulation assays

SPLC from normal or trypanosome-infected mice were prepared in RPMI-1640 medium containing antibiotics, 2-mercaptoethanol, 10 mM HEPES buffer and 10% preselected, heat-inactivated FCS, dispensed into the wells of 96-well culture plates at 2×10^5 cells/0·2 ml and stimulated with Con A at final concentrations of 8, 4, 2, 1, 0·5 and 0·25 μg/ml. Zero control wells received only medium. All assays were done in triplicate. The cells were incubated at 37° in 10% CO_2 in air for a total of 72 hr and pulsed during the last 5 hr with 1·0 μCi per well of methyl [^{3}H]thymidine ([^{3}H]TdR, Amersham Radiochemical Corporation, Oakville, Ontario). Cells were harvested onto glass fibre filters and counted for radioactivity. Results are expressed as the stimulation index (SI):

$$SI = \frac{\text{c.p.m. incorporated with mitogen}}{\text{c.p.m. incorporated with medium alone}}.$$

RESULTS

Interleukin-1 determinations

Alveolar cells and peritoneal exudate adherent cells from normal and trypanosome-infected mice had the morphology of macrophages, and >95% were positive for non-specific esterase I and took up the supravital dye Neutral Red (0·5% in PBS, pH 7·4). The splenic adherent cell population consisted of some cells with typical macrophage morphology and staining characteristics; however, the majority were round lymphoid-like cells.

Interleukin-1 activity was generated by incubating alveolar macrophages (AM) and adherent peritoneal exudate (PEC) and spleen cells (SPLC) from normal and trypanosome-infected mice in medium containing indomethacin to inhibit prostaglandin synthesis by macrophages, with or without LPS (to stimulate the synthesis and release of IL-1). Results were expressed as the net c.p.m. above those observed with the respective medium controls (± LPS), as some additional stimulation was always observed when thymocytes were incubated with LPS. This was not due to the presence of interleukin-2, as all supernatants from adherent cells obtained from normal and infected mice showed no IL-2 activity in the MTL cell assay.

Supernatants obtained from LPS-stimulated PEC of normal mice of either strain had IL-1 activity comparable to the positive control (Fig. 1), whereas without LPS stimulation little or no IL-1 activity was observed (data not shown). During the first 3 days after injection of *T. congolense*, LPS-induced IL-1 activity became profoundly depressed in both A/J and C57BL/6J mice. By Day 7 of infection, no IL-1 activity was found in supernatants from either mouse strain. In fact, the c.p.m. incorporated into thymocytes was less than that observed in either of the medium controls, suggesting that suppressor activity might be present.

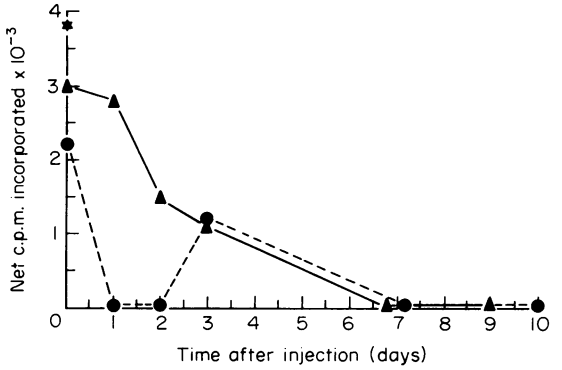

Figure 1. IL-1 activity in supernatants from LPS-stimulated adherent peritoneal exudate cells of normal and *T. congolense*-infected A/J and C57BL/6J mice. Mice were infected with 2·5 × 10^5 *T. congolense* ILRAD 588 parasites. Normal mice (Time 0) received 0·1 ml of PSG, pH 8·0. Levels of IL-1 were measured by adding dilutions of test supernatants to C3H/HeJ thymocytes stimulated with 1 μg/ml of Con A. Only the values measured in 1/2 dilutions of supernatants are shown in the graph and are expressed as the net [^{125}I]UdR c.p.m. incorporated into thymocytes above that observed with the negative control. C.p.m. incorporated into thymocytes incubated with positive control (known IL-1 containing PEC supernatant) are indicated by (★); A/J (▲); C57BL/6J (●).

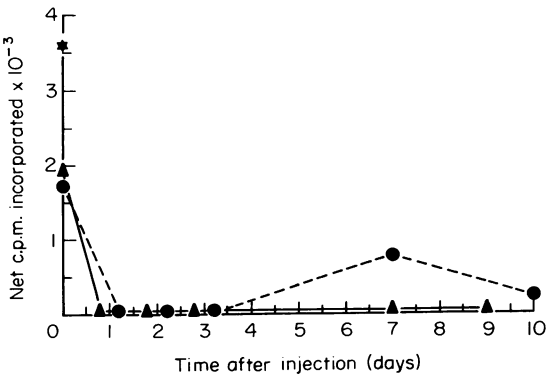

Figure 2. IL-1 activity in LPS-stimulated adherent spleen cell supernatants from normal and *T. congolense*-infected A/J and C57BL/6J mice. Supernatants from LPS-stimulated adherent spleen cells from normal (Time 0) mice and mice infected with 2·5 × 10^5 *T. congolense* were tested as described in the legend to Fig. 1. Results obtained with 1/2 dilutions of supernatant are shown and are expressed as the net c.p.m. incorporated into thymocytes above the medium control. A/J (▲); C57BL/6J (●); positive control (★).

Similar observations were made when SPLC supernatants were tested for IL-1 activity (Fig. 2). A similar amount of IL-1 activity was present in normal A/J and C57BL/6J supernatants. However, within 24 hr after trypanosome injection, no IL-1 activity was found in either mouse strain. By Day 7 of infection, some IL-1 activity was observed in SPLC supernatants from C57BL/6J mice. However, no IL-1 activity was detectable in SPLC supernatants from infected A/J mice during the period studied. As was observed with PEC supernatants, thymocyte proliferative responses were depressed below control values.

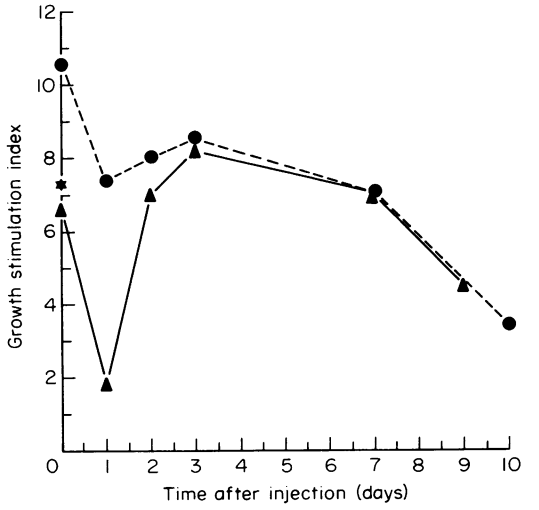

Figure 3. IL-2 activity in supernatants from Con A-stimulated spleen cells from normal and *T. congolense*-infected A/J and C57BL/6J mice. Supernatants from Con A-stimulated spleen cells of five normal and five trypanosome (2·5 × 10^5 *T. congolense*)-infected A/J (▲) and C57BL/6J (●) mice were tested at 1/2 dilutions for their ability to support the growth of IL-2-dependent MTL 2·8·1 cells. Results are expressed as the growth stimulation index (see 'Materials and Methods'). The symbol (★) indicates the growth stimulation index achieved with the positive control (EL-4 supernatant at 0·5% final concentration).

Table 1. Endogenous proliferative activity of spleen cells in *T. congolense*-infected mice

Time after injection (days)	C.p.m.* incorporated	
	A/J	C57BL/6J
0	360±43	398±29
0·5	215±25	360±54
1	499±39	489±27
2	326±30	383±35
3	297±86	206±50
8	669±208	ND
9	ND†	388±75

* [³H]TdR incorporated into unstimulated spleen cells determined in three mice of each strain at the times indicated after i.p. injection of $2{\cdot}5 \times 10^5$ *T. congolense* ILRAD 588 organisms. Results are expressed as the mean ± standard error.

† ND, not determined.

In a second set of experiments (data not shown) IL-1 activity was measured in supernatants from LPS-stimulated adherent PEC, SPLC and AM obtained from A/J and C57BL mice at 0, 0·5, 1, 2, 3 and 7 days after i.p. injection of $2{\cdot}5 \times 10^5$ viable *T. congolense*. Cells of three mice from each strain were pooled at each time and treated as described above. Adherent cell (all populations) supernatants obtained at 12–24 hr post-injection contained IL-1 activity comparable to normal (sham-injected) controls. However, on Days 2, 3 and 7 post-injection, no IL-1 activity was found. Again, there were no marked differences between mouse strains. There was no evidence of suppressor activity as was observed in the first set of IL-1 measurements.

Interleukin-2 determinations

Interleukin-2 (IL-2) activity was measured in supernatants from bulk cultures of spleen cells taken from normal and *T. congolense*-infected mice in two separate experiments.

In the experiment shown in Fig. 3, supernatants from Con A-stimulated pooled spleen cells were tested for IL-2 activity in

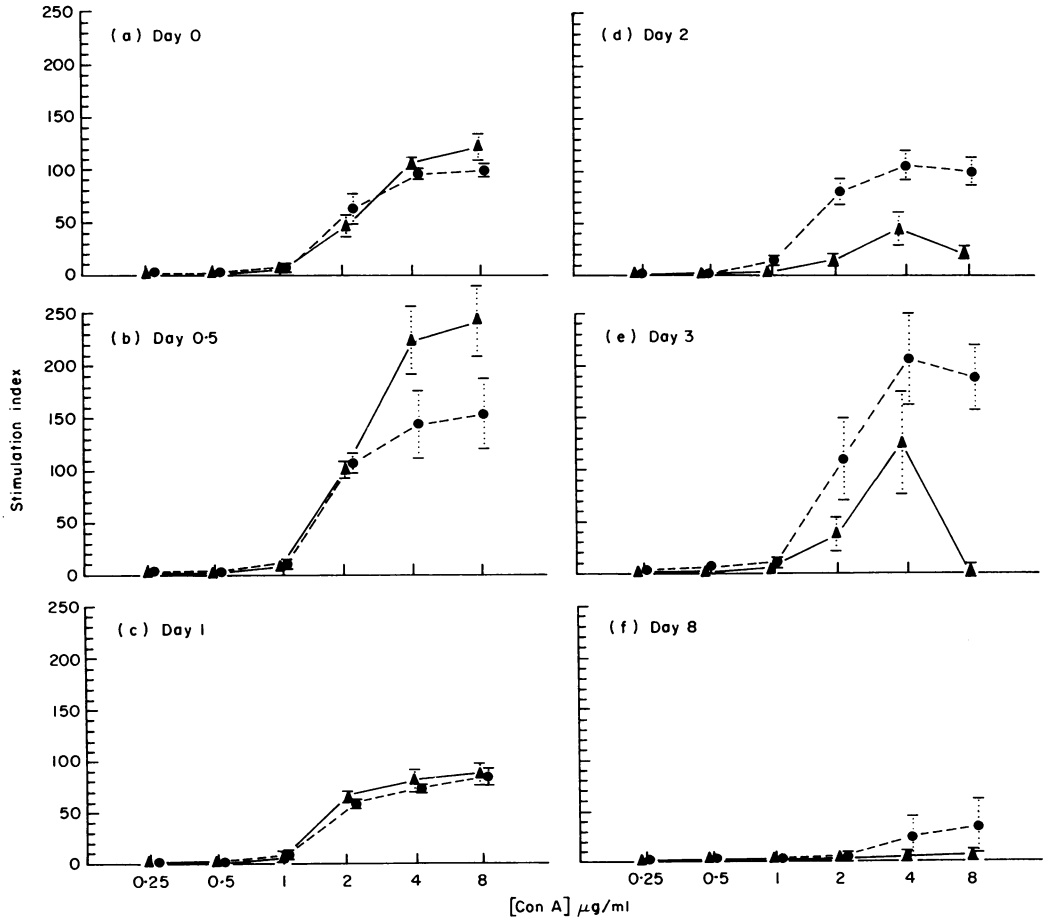

Figure 4. Con A-induced proliferative responses of spleen cells from normal and *T. congolense*-infected A/J and C57BL/6J mice. Proliferative responses and dose-response kinetics of spleen cells to Con A were tested in normal (Day 0) and infected A/J (▲) and C57BL/6J (●) mice at various times after i.p. injection of $2{\cdot}5 \times 10^5$ *T. congolense* ILRAD 588 parasites. Results are expressed as the mean ± standard error of determinations on three mice of each strain.

the MTL cell assay. Those from normal C57BL mouse splenocytes contained more IL-2 activity than those from normal A/J mice. However, 24 hr after trypanosome injection, IL-2 activity was reduced in both mouse strains. It is noteworthy that IL-1 activity in the adherent cell population of these spleens was also depressed (or absent) at this time. By Days 9–10 of infection, IL-2 production was notably depressed in both A/J and C57BL/6J mice.

Similar results were obtained in another experiment (data not shown) in which SPLC supernatants from three normal or three infected mice of each strain at 0·5, 1, 2, 3, 8 and 9 days after trypanosome injection were tested, with the exception that a moderate increase in IL-2 activity was observed in C57BL/6J mice during the first 24 hr of infection, followed by a rapid decline. IL-2 activity in supernatants from infected A/J mice did not increase until 48 hr after trypanosome injection, and then only transiently. In both strains of mice, IL-2 activity was depressed to subnormal levels after Day 3. In both experiments, no IL-2 activity was observed in the absence of Con A stimulation.

Onset of immune depression in *T. congolense*-infected A/J and C57BL/6J mice

In a separate set of experiments, SPLC were prepared from C57BL/6J and A/J mice at various intervals after the injection of $2{\cdot}5 \times 10^5$ *T. congolense* and tested for endogenous (unstimulated) proliferative activity ([^{3}H]TdR uptake) and responsiveness to Con A.

Endogenous proliferative activity was increased in A/J mouse spleens by Day 8 of infection. However, little or no change was observed in C57BL/6J spleens (Table 1).

When SPLC were stimulated with Con A (Fig. 4), a transient enhancement of proliferation in both mouse strains was observed 12 hr after trypanosome injection. However, depressed proliferative responses and/or altered dose response kinetics were observed in both A/J and C57BL mice after Day 1.

DISCUSSION

Although it is difficult to make judgements about differences in interleukin levels between resistant and susceptible mice at various times after infection, it is clear from these investigations that *T. congolense* infection resulted in a decline in the production of IL-1 and IL-2 and depression of proliferative responses in the spleen before parasites were detectable in the peripheral blood.'

Decreased production of IL-1 by adherent peritoneal exudate cells, spleen cells and alveolar macrophages was observed as early as 24–48 hr following intraperitoneal injection of bloodstream trypanosomes. These results contrast with studies involving inbred mice infected with *Mycobacterium lepraemurium* (Hoffenbach, Lagrange & Bach, 1984), *Trypanosoma cruzi* (Harel-Bellan *et al.*, 1983) and *Plasmodium* spp. (Lelchuk, Rose & Playfair, 1984), in which IL-1 production was found to be normal or enhanced during infection.

The observed decrease in IL-1 was temporally reflected in the decreased production of IL-2 by Con A-stimulated spleen cells from *T. congolense*-infected mice. Similar early and progressive declines in IL-2 production have been observed inbred mice infected with the microbial agents described above. Generalized immunodepression is often observed in infections involving these microorganisms. However, the results of the former investigations suggested that immune depression was due to a failure of T lymphocytes to produce IL-2, rather than to a failure in IL-1 synthesis by accessory cells or the inability of T cells to respond to IL-2. The results of the experiments reported here suggest that the immunodepression associated with *T. congolense* infection may be at the level of the macrophage, i.e. a failure in IL-1 production which, in turn, leads to a reduction in IL-2 synthesis.

Our experiments also showed that IL-2 levels were progressively depressed in both resistant (C57BL/6J) and susceptible (A/J) mice as the infection approached the first parasitaemic peak. However, during the first few days after trypanosome injection, the amounts of IL-2 measured in bulk spleen cell cultures were variable. This may have been due to absorption of IL-2 by IL-2-receptor-bearing blast cells present during the initial culture period, or early acting transient IL-2 inhibitors that have been described in other systems (Andrus and Lafferty, 1981). Certainly, numerous blast cells were seen in spleens obtained from mice during the early infection period. Also, increased endogenous proliferative activity (in the spleens of A/J mice) was indicated by the increased [^{3}H]TdR incorporation in these cells in the absence of added mitogen.

Con A-induced spleen cell proliferative responses progressively decreased in both A/J and C57BL/6J with time after the injection of *T. congolense*, so that by Days 7–10 of infection severe depression was observed in both mouse strains. This correlated with severe reductions in IL-1 and IL-2 levels in the spleen at this time and, therefore, may have been due to a deficit in interleukin generation, as has been suggested for leishmanial infections in mice (Reiner & Finke, 1983).

The immunodepression observed in the present study may reflect the activation of immunoregulatory mechanisms to counter trypanosome-induced polyclonal activation of lymphocytes (Mansfield, 1981; Morrison & Murray, 1978). Thoman & Weigle (1984) have described early acting antigen non-specific suppressor cells activated by IL-2. Presumably, these cells act by absorbing IL-2 and may function in the down-regulation of the immune system by preventing excessive amounts of IL-2 from expanding irrelevant lymphocyte clones. Antigen non-specific suppressor cells have been described in mice infected with African trypanosomes (Eardley & Jayawardena 1977; Mansfield & Wallace 1974; Pearson *et al.*, 1979; Grosskinsky & Askonas, 1981). As it has been observed that immune depression association with African trypanosomiasis is an active phenomenon associated with living trypanosomes (Roelants *et al.*, 1979), products secreted by trypanosomes (Albright & Albright, 1981; Tizard *et al.*, 1978) or parasite structural components (Clayton *et al.*, 1979) have also been associated with immunodepression in trypanosomiasis. Therefore, it is possible that trypanosomes may directly inhibit the production of interleukins leading to a similar degree of immunodepression in the two strains of mice, as we observed in our study. However, the outcome is undoubtedly different in A/J and C57BL/6J mice. One possible explanation lies in the observation that C57BL/6J mice infected with *T. congolense* ILRAD 588 showed a more rapid and early infiltration of mononuclear cells (MNC) into their spleens and livers, as opposed to A/J mice in which the MNC response was of a lesser magnitude and occurred much later (L.A. Mitchell and T. W. Pearson, unpublished results).

Thus, resistant mice may be able to reconstitute the interleukin cascade through the recruitment of new accessory cells and lymphocytes from the bone marrow, and hence, make an effective anti-trypanosome immune response.

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