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Interaction of African trypanosomes with the immune system

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African trypanosomes cause disease in man and domestic animals. The parasites have the ability to escape immune control by two means: by antigenic variation of the surface glycoprotein coat so that waves of variant parasites arise and by inducing a general immunosuppression affecting immune responses to the parasite as well as to parasite-unrelated antigens.

The cellular basis of the immune dysfunction will be discussed in relation to a mouse model system – it is the result of proliferative stimuli to T- or B-cells which then become refractory to selection by antigen and normal control signals. Recent experiments have focused on macrophages as important direct target cells for parasite action. We have obtained no evidence for a parasite derived mitogen acting directly on B- or T-cells. *In vitro* cell proliferation is associated with accessory cells and relates only to T-cells. During infection, macrophages become activated with changes in receptor expression and mediator release, so that there is, for example, spontaneous IL-1 release (with a role in T- and possibly in B-cell proliferation) and several-fold increases in PGE_2 secretion, with its immunosuppressive activities.

We also find parasitaemia-associated release of α–β and γ interferon by various cells which in turn influences immune function. The active parasite component is associated with parasite membranes, but its nature has not been further defined. We proposed that the macrophage changes provide a general pathway causing immune dysfunction associated with many infections, be they parasitic or caused by other invading organisms.

African trypanosomes escape immune control

African trypanosomes, like all other successful parasites, must escape the immune defences of the host to survive. This is achieved by two mechanisms: first, individual parasites can vary their surface glycoprotein coat, so that the host is confronted with a continuous progression of distinct antigenic variants. (Cross 1975; Vickerman 1978). Secondly, infection with African trypanosomes results in a profound and generalized suppression of immunological function which extends to parasite-related and unrelated antigens. Immunosuppression is a common feature of many infections, whether of parasitic, viral or bacterial origin. It is particularly severe in African trypanosomiasis and in view of the antigenic variation this has serious implications for the outcome of the disease (see review Bancroft & Askonas 1982).

Trypanosome infections of man or cattle leave the host more susceptible to secondary infections and these are often the cause of death. Infected hosts also show impaired responses to vaccination. Patients with *Trypanosoma gambiense* infection have depressed antibody responses to all the vaccines tested and low skin reactivity on challenge with PPD, *Candida* or streptococcal antigens (see Greenwood 1974*a*). In cattle infected with *T. congolense*, IgG antibody responses to *Brucella abortus* vaccine are suppressed, while IgM antibody inhibition varies with the virulence of the infection. Cattle infected with *T. vivax* but free of parasitaemia showed normal responses to the vaccine (Rurangirwa *et al.* 1983). In another series of similarly infected cattle,

vaccination against bovine pleuropneumonia led only to slightly depressed antibody responses. However 50 % of these animals were not immune against infection with bovine pleuropneumonia while control animals were completely protected (Ilemobade *et al.* 1982).

Thus immunosuppression is an important feature of this infection in man and animals. Furthermore, in view of the problems that antigenic variation presents to vaccinating against the disease, a deeper understanding of the changes in lymphoid function during trypanosomiasis is an important goal. African trypanosomes can also infect mice and parasite clones can be isolated that vary from chronic to acute, thus reflecting patterns of parasitaemia seen in the field. Much recent work has therefore used mouse models of infection in the analysis of the cellular basis of the immune dysfunction induced by trypanosomes

Murine trypanosomiasis results in multiple changes in immune function and this topic has been extensively reviewed in recent years (e.g. Mansfield, 1981; Roelants & Pinder 1983; Bancroft & Askonas 1982, 1984). In brief, infection causes a proliferative stimulus to lymphoid cells, null cells and macrophages in the lymphoid tissue to which the parasites home and a general immunosuppression. In more chronic infections a short-lived enhancement of antibody formation precedes immune dysfunction. The function of every T- or B-cell subpopulation examined is affected, with the possible exception of cells responsible for some delayed skin reactions. While serum Ig levels are elevated during infection, antibody formation to specific antigens declines rapidly, and IgG antibody production is affected earlier than IgM (Hudson *et al.* 1976).

During the course of infection we see generation of T-suppressor cells that show no antigen specificity (Jayawardena *et al.* 1978; Corsini *et al.* 1977) and of suppressive macrophages or adherent spleen cells depleted of T-cells (Wellhausen & Mansfield 1979; Pearson *et al.* 1979). Undoubtedy, splenomegaly and destruction of lymphoid architecture also contribute to the immune dysfunction.

The extent of immunosuppression correlates with the virulence of the parasite clone examined. Clones of *T. b. brucei* varying in virulence all suppress IgG antibody but differ in their intrinsic capacity to depress IgM antibody formation to red blood cells *in vivo* (Sacks *et al.* 1980). These studies have made use of the observation that lethally irradiated parasites administered to mice mimic the immunosuppressive effects of an infection. The suppressive activity was associated with a crude parasite membrane fraction which could be isolated from different clones of trypanosomes.

In addition, antibody responses to the parasite are similarly impaired (Sacks & Askonas 1980; Inverso & Mansfield 1983). Immunological control of parasite waves relies on variant specific IgM antibodies (see review Mansfield 1981; Bancroft & Askonas 1984). Induction of IgG antibody directed against the parasite is more susceptible to suppression by the infection than IgM antibody; with our clone NIM7 causing a chronic infection (Sacks & Askonas 1980), IgG antibody production is wholly suppressed by day 28 of infection by which time the first three waves of parasitaemia have been controlled. However, IgM antibody can still be induced and further waves of variant parasites can be controlled, until death ensues (days 80–100). This pattern parallels the findings in acute or chronic infections of cattle as mentioned above. Interestingly, Black *et al.* (1982) found that not all blood forms of the parasite induce antibodies – and that the stumpy degenerate forms are more effective in that respect. This would relate parasite virulence with monomorphic rather than pleomorphic parasite forms.

Genetics of trypanosome infection

It was hoped that comparing the course of parasitaemia in different mouse strains might highlight factors influencing susceptibility and resistance to African trypanosomes. However, resistance to *T. b. brucei* or *T. congolense* was only relative. The most resistant mouse strain (C57B1/6) had a lower first wave parasitaemia than susceptible strains, but death was only delayed by several days (e.g. Clayton 1978; Murray *et al.* 1983; Roelants & Pinder 1983). Resistance was not H-2 linked and showed dominant inheritance. To see whether macrophages were involved in this relative resistance, Grosskinsky examined the course of infection in Biozzi mice (Biozzi *et al.* 1979) originally selected as high (H) or low (L) responders for erythrocyte (RBC) agglutinins or anti-*Salmonella* responses (SAL). No significant difference in H or L mice was observed, although SAL-H had higher parasite agglutinin levels and a 100-fold lower first wave parasitaemia than SAL-L, mice from both strains had the same survival time (Grosskinsky 1981).

Adjuvants and the course of infection

A number of experiments have attempted to influence the course of trypanosome infection by administration of adjuvants and immunostimulants. The cell wall components of mycobacteria, trehalose dimycolate and muramyl dipeptide are known to activate macrophages and when given simultaneously (but *not* individually) increase host resistance by lowering the first peak of parasitaemia by 10- to 100-fold and increasing the survival time (Grosskinsky 1981). Similar changes in infections with *T. congolense* or *T. b. brucei* can also be obtained with *Corynebacterium parvum*, BCG, or *Bordetella pertussis* (e.g. Murray & Morrison 1979). In more recent experiments, administration of interferon (IFN) α/β or poly I:C, impaired the development of the first parasitaemia wave with clones of *T. b. brucei*. Unlike the case of *T. congolense* described by Herman & Baron (1971), this regimen had no effect on the survival time of *T. b. brucei* infected animals. (G. J. Bancroft, unpublished observation). Such results suggest that preactivated macrophages may reduce the parasite load during the first wave of parasitaemia. However, in general, procedures that serve to activate non-specifically macrophages and the immune system, only marginally influence survival times and essentially do not affect the fatal outcome of the infection (see also Whitelaw *et al.* 1983 for *T. congolense*).

NK cell levels

There is considerable interest in the role of natural killer (NK) cells in defence against infectious agents, including the pathogenic protozoa. To assess the importance of NK cells in African trypanosomiasis, the course of infection was examined in mice homozygous for the beige gene, which have impaired NK cell activity (Roder & Duwe 1979). Beige mice infected with a chronic clone of *T. b. brucei* developed comparable parasitaemias and had an increased survival time (by 20 %) when compared with their NK cell competent heterozygote litter-mates. This suggests that NK cells are not involved in controlling the course of infection (Grosskinsky 1981). Nevertheless, like most other immune functions, NK cell activity of the host is severely disturbed during infection with *T. b. brucei*. In contrast with previous reports with *T. musculi* Albright *et al.* 1983), NK activity in the spleens of *T. b. brucei* infected mice was not significantly altered during the first week of infection (figure 1). However, after the first peak of parasitaemia,

NK activity expressed as total cytotoxic units per spleen was reduced to below 20% of uninfected controls. Thus, at the time when most other immune functions are impaired, the levels of measurable NK cell activity are also reduced. This was observed at the time of greatest changes in splenic architecture and cellularity and cytotoxicity could not be restored by administration of IFN α–β or the IFN-inducer poly I:C (data not shown). As described for other immune functions, this was not an irreversible event since NK cell activity and its responsiveness to IFN returned to normal levels after curing the infection with trypanocidal drugs (G. J. Bancroft, unpublished observations.)

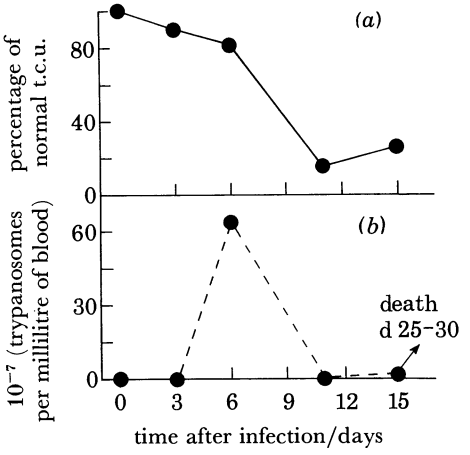

FIGURE 1. Impaired NK cell activity during the course of infection with *T. b. brucei* clone NIM2.

Female (CBA × C57) F1 mice were injected with 400 *T. b. brucei* clone NIM2 i.p. (Sacks *et al.* 1980) and assayed for NK cell activity against ^{51}Cr-labelled YAC target cells according to the method of Dawkins & Shellam (1979).

Cytotoxicity is expressed as a percentage of normal total cytotoxic units (t.c.u.) where t.c.u. is cytotoxic activity per 10^6 spleen cells (c.u.) multiplied by spleen cell yield per 10^6. The activity of uninfected control mice in the same assay was taken as 100%.

C.u. of uninfected controls: 54.8; t.c.u. of uninfected controls 3562. Spontaneous ^{51}Cr release: 4%.

The description of the immune changes by several groups was straightforward and it became clear that the immune dysfunction could not be assigned to a single event. What is more difficult in the complex immune system is to pinpoint the parasite action in molecular terms at the level of individual cell types and this has not yet been resolved. Many questions remain to be answered. What is the nature of the parasite product or products that cause such havoc in the immune system? Which cell types are directly affected by the parasite? Are there one or more direct target cells for parasite action that then affect lymphoid cell function? Although Ig production increases, why is specific antibody induction so profoundly inhibited? Are the immunosuppressive events specific for trypanosomiasis or are similar pathways involved in immune depression in other infections?

ABSENCE OF DIRECT INTERACTION BETWEEN PARASITE AND T OR B CELLS

The most striking symptoms of sleeping sickness have been splenomegaly, increased serum Ig levels and anaemia. These observations prompted the suggestion that trypanosomes contain a B-cell-mitogen (Greenwood 1974*b*). However, we have not been able to obtain any evidence

for such a mitogen. Soluble extracts of parasites or purified surface glycoproteins *per se* do not stimulate mouse or human lymphoid cells *in vitro* or show any *in vivo* effects. There has been one report of proliferative stimulation of mouse spleen cells *in vitro* by a parasite autolysate, but we have not been able to reproduce this (see review, Bancroft & Askonas 1984). While irradiated dead parasites injected *in vivo* cause splenomegaly and immunosuppression, all the parasite activity appears associated with a crude parasite membrane fraction. In contrast to our results, Diffley (1983) reports spleen changes after intravenous injection of purified surface glycoprotein. Mitogenic effects by membrane fractions *in vitro* were restricted to T-cells over a six- to seven-day period. This stimulation did not require the presence of B-cells, but in both human peripheral blood cells (Selkirk *et al.* 1983) or mouse spleen cells (S. R. Beer, unpublished results), T-cell proliferation required the presence of monocytes or accessory cells. Thus evidence pointed to the role of intermediary cells rather than a direct T- or B-cell mitogen. *In vivo*, B-cells clearly are stimulated in lymphoid tissues and in part this may be a consequence of B-cell growth or maturation factors released from activated T-cells.

Interaction of trypanosomes and macrophages

The control of waves of parasitaemia is associated with the production of variant specific antibodies and clearance of trypanosomes from the blood by phagocytic cells in the liver and other organs (e.g. MacAskill *et al.* 1980). The ingestion and degradation of trypanosomes by macrophages only occurs in the presence of anti-parasite antibodies. As mentioned above, IgM antibodies are sufficient for phagocytosis, presumably acting via complement receptors on the macrophage surface. During the course of infection with *T. b. brucei*, macrophages show all the characteristics of activation (Grosskinsky *et al.* 1983) as similarly observed following B.C.G. infection (Ezekowitz *et al.* 1981). Within four days of infection with *T. b. brucei* clone NIM 6, the phenotype of peritoneal macrophages starts to change, and by day 9 (after control of the first parasitaemia wave) there is a considerable reduction in the cell surface expression of the mannose, Fc and complement receptors, and the F4/80 marker. Concomitantly, surface Ia expression increases and the release of H_2O_2 and O_2^- as of plasminogen activator is greatly enhanced. The increase in Ia antigen expression with *T. b. brucei* is in contrast to the reduction of Ia reported for macrophages from *T. rhodesiense* infected mice (Bagasra *et al.* 1981). Macrophage numbers are increased in the spleen and peritoneal cavity, and these changes are likely to involve recruitment of macrophages as well as changes in resident cells. During the prepatent period before the next wave of parasitaemia, there is partial recovery of normal properties, except that Ia expression and plasminogen activator release remain elevated.

Activation of macrophages is frequently dependent on T-cell derived mediators such as interferon γ, but in *T. b. brucei* infections similar (although somewhat delayed) changes occur in athymic nude mice as in normal litter-mates, affecting also B-cell function (Clayton *et al.* 1979). The experiments suggest T-cell-independent pathways of macrophage activation. On the other hand, the generation of some functional T-cells during infection of nude mice cannot be excluded. We have not detected T-cell function in athymic nude mice during the course of semi-acute *T. b. brucei* infections, but Langhorne *et al.* (1983) do report T-cell activity in nude mice infected with a chronic strain of *T. rhodesiense*. Furthermore, in T-cell competent mice, the production of interferon γ during the course of infection (Bancroft *et al.* 1983) may contribute to the observed macrophage activation.

Since macrophages play a central role in immune regulation (reviewed by Beller & Unanue 1982) it is important to consider the possible relation of changes in macrophage function to the modulation of immune responses by trypanosomes. Grosskinsky & Askonas (1981) have shown that macrophages harvested from infected mice, or after ingesting opsonized trypanosomes, can enhance or suppress T-cell-dependent antibody responses of normal mice *in vivo*. Macrophages therefore appear to be at least one of the key target cells for the action of trypanosomes and can mediate immune dysfunction. However, the question then arises as to which of the altered macrophage properties are important for this.

Infection with *T. b. brucei* induced profound changes in the release of interleukin-1 (IL-1) and prostaglandins, two factors known to regulate the immune system. Infection with *T. b. brucei* clone NIM 9 which leads to death within six to seven days enhances IL-1 release by day 5 and increases the responsiveness of macrophages to further stimulation *in vitro* by LPS (table 1). During a less acute infection, spontaneous IL-1 secretion reaches maximum levels following the first wave of parasitaemia even in the absence of *Escherichia coli* lipopolysaccharide (LPS) (Bancroft & Vessey 1984). At the height of parasitaemia with clone NIM 6, PGE_2 release increases sixfold while prostacyclin remains constant (as measured by $PGF_{i\alpha}$) and thus the prostacyclin/PGE_2 ratio is reversed to favour PGE_2 production. Late in infection, no significant amount of either prostaglandin is secreted and this hyporesponsive state is similar to that seen with thioglycollate induced peritoneal cells (Fierer *et al.* 1984).

TABLE 1. INFECTION WITH *T. B. BRUCEI* ENHANCES INTERLEUKIN-1 SECRETION BY PERITONEAL MACROPHAGES

peritoneal macrophage donor	LPS stimulation *in vitro*	IL-1 assay: [^{3}H]TdR incorporated $\times 10^{-3}$ counts per minute $\pm$s.e.	parasitaemia: trypanosomes per millilitre of blood $\times 10^6$
day 0 (uninfected)	−	6.5±2	0
	+	10±1	
NIM9 – day 3	−	11±2.4	< 1
	+	11±1	
NIM9 – day 5	−	25±2.7	200
	+	138±13	

Female (CBA × BALB/c) F1 mice were infected with 1000 *T. b. brucei* clone NIM9 parasites i.p. (Sacks *et al.* 1980). Peritoneal exudate cells were plated in Costar 24 well tissue culture plates at 2×10^6 cells per 0.5 ml per well in RPMI–10% foetal calf serum–5×10^{-6} M indomethacin.

Adherent cell monolayers were incubated with or without LPS (20 μg ml^{-1}) for 24 h. IL-1 activity in the supernatants (at four dilutions), was then assayed using syngeneic thymocytes plus 2 μg ml^{-1} PHA for three days (as modified from Gery *et al.* 1977). Results given are at 1:2 dilution of the supernatants.

Thus alterations in the production of immunoregulatory factors such as prostaglandins, interleukin-1 and interferons (Bancroft *et al.* 1983) are a clear feature of trypanosome infection. However, it is still not possible to define the precise role of these changes in the immunomodulation induced by the parasite. This work is hampered by the complex interactions between the mediators and the limited availability of pure factor preparations.

Discussion and conclusions

The many cell interactions in the immune network and the multiple changes in immune function during the course of trypanosomiasis present a complex pattern. The main features are polyclonal proliferation of lymphoid T- and B-cells, and other cells in lymphoid tissues. The extent of the disruption is influenced by several factors, including the virulence of the parasite strain, the host genotype, as well as the type of antigen used and the lymphoid organ examined. The mechanism by which T- or B-cells become refractory to selection by antigen is not clear, while maturation of B-cells into production of non-specific Ig molecules proceeds. Originally, this was attributed to clonal exhaustion, but the very rapid suppression in acute infections would exclude this possibility. An explanation may lie in the observation that B- and T-cells at different phases of activation or antigen stimulation are unable to collaborate (Calderon & Thomas 1980) and may vary in the release of amplifying or suppressive mediators. Polyclonal activation of T- and B-cells is by no means limited to trypanosomiasis but also accompanies other infections such as malaria and Chagas' disease. No directly acting trypanosome mitogen has been defined so far, although isolation of mitogenic substances appears to be a generally difficult exercise with many protozoa (see review, Bancroft & Askonas 1982).

We have described many changes in macrophage function during the course of infection. Thus, the spontaneous IL-1 release by macrophages during trypanosomiasis may well play a role in the activation of T-cells, and possibly also B-cells. However, T-cells in the spleen begin to divide so rapidly after infection (Corsini *et al.* 1977) that an additional pathway may need to be invoked. A present difficulty lies in obtaining pure macrophage populations from spleen, the first site at which our clones of *T. b. brucei* replicate in mice. B-cells may also obtain signals to proliferate via activated T-cells. Certainly *in vitro*, T-cell activation in the presence of accessory cells plus parasite fractions precedes any activation of B cells (Selkirk *et al.* 1983; S. R. Beer, unpublished results).

We can conclude that macrophages are at least one key target cell for trypanosome action. The interaction between parasites, antibodies and macrophages and the resulting changes in phenotype and mediator release by macrophages are likely to contribute to the immune dysfunction and would also provide a common pathway for the mediation of immunosuppression in many different infections.

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Discussion

W. E. Ormerod (*London School of Hygiene and Tropical Medicine, Kepple Street, London WC1E 7HT, U.K.*). Dr Askonas interprets the fall in the peak parasitaemia as being due to antibody; I would like to suggest that this fall is a feature of the life cycle of *Trypanosoma brucei* and is not primarily due to antibody. As I pointed out earlier in the meeting the pleomorphic nature of *T. brucei* tends to be ignored, thus a rising parasitaemia is characterized by slender forms which, after a period of division, becomes senile and stumpy; it is at this point that the parasitaemia falls. Immunodepression may prolong the peak of parasitaemia so that succeeding peaks will tend to overlap, as in the studies of Luckins (1972) and Balber (1972); nevertheless stumpy trypomastigotes still succeeded slender. In my experiments (Ormerod *et al.* 1974), in which the strain of *T. brucei rhodesiense* was the same as that used by Luckins but at an earlier passage, there was virtually no overlap, even after immunodepression, of the first and second peaks: the stumpy trypomastigotes, with an attenuated immune response, were larger, stumpier and even more sluggish than in the control infections; they had mostly disappeared from the blood before the new wave of slender forms had entered it. I suggest that the role of antibody is not an essential one in decreasing the parasitaemia, but merely assists in removing parasites which are moribund, having reached the end of their normal life span.

References

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B. A. Askonas. You have raised this point on previous occasions with me. You are dealing with a very special strain of *T. rhodesiense* and it is not possible to generalize your conclusions. In our laboratory, all the *T. brucei* clones and strains that we have studied overwhelm immunosuppressed mice very rapidly. I am also wondering how strongly your mice were immunosuppressed. We find that even small amounts of IgM antibodies are effective in clearing parasites and the IgM antibody production is the last one to be inhibited by immunosuppressive treatment.

M. J. Turner (*M.R.C. Biochemical Parasitology Unit, Molteno Institute, Cambridge*). It would of course be extremely interesting to identify the immunosuppressive agent in trypanosomes, and towards this end it would be useful if some specificity in expression could be demonstrated, to allow comparative biochemical analyses to be performed. Is it known whether immunosuppression can be generated by membranes prepared from procyclic trypomastigotes? Do the immunosuppressive properties survive cyclical transmission?

B. A. Askonas. This question raises an interesting point. I am afraid I cannot answer it because so far we have only studied the blood forms of *T. brucei*. The problem for this type of work would be the low yield of parasites at other stages of development and particularly in the fly.