Human macrophage-mediated cytotoxicity of *Schistosoma mansoni*

Functional and structural features of the effector cells

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Summary

Human monocytes were purified from peripheral blood and cultured *in vitro* on hydrophobic membranes. Such cells developed into mature tissue-type macrophages after approximately 1 week in culture. During this maturation period the macrophages developed a potent cytotoxic mechanism whereby they could kill the schistosomula of *Schistosoma mansoni* in standard *in vitro* cytotoxicity assays. Cytological and ultrastructural studies of the cells grown *in vitro* indicated that macrophages developed many of the classical histological and ultrastructural features of 'activated' cells with ruffled plasma membranes and significant increases in rough endoplasmic reticulum and Golgi vesicles. Effective cytotoxicity depended upon contact of the effector cells and their parasite target. Further, experiments using metabolic inhibitors indicated that cytotoxicity was dependent upon protein synthesis. Initial results point to the macrophage factor being distinct from some of the better-characterised macrophage secretory products such as tumour necrosis factor, proteases and products of oxygen metabolism.

Key words: monocyte, macrophage, cytotoxicity, *Schistosoma mansoni*, in *vitro*.

Introduction

Macrophages grown *in vitro* can kill a range of targets including tumour cells, microorganisms and tissue parasites (James et al. 1982). In some instances, cytotoxic destruction of the targets by the macrophages can occur in the absence of specific antibody to that target. The molecular and ultrastructural basis of such cytotoxicity is being intensively investigated. To date many secretory factors have been identified including pre-eminently proteases, tumour necrosis factor (TNF), and products of oxygen metabolism (Adams and Nathan, 1983; Drysdale et al. 1988).

The majority of the work has involved the use of mouse and rat peritoneal exudate cells or macrophage cell lines. However, attention has also focussed on the cytotoxic and functional potential of human macrophages (Musson, 1983; Jungi and Hafner, 1986; Kreipe et al. 1988). The relatively immature monocyte has little or no cytotoxicity towards a range of tumour targets. However, as the cells grow into recognisable tissue macrophages many of their secretory functions increase in magnitude, and in parallel with this there are large increases in cellular cytotoxicity of tumour targets (Hammerström, 1979; Kinehart et al. 1979; Andreesen et al. 1983, 1988).

Murine macrophages, in a manner analogous to tumour cytotoxicity, are also able to inflict damage upon the multicellular parasite *Schistosoma mansoni* and cells that have been activated by interferon-gamma (IFN) kill *in vitro* the schistosomula of this important human parasite (Bout et al. 1981; James et al. 1984b). Further research has implicated the macrophage as an important potential effector cell in the expression of immunity in successfully vaccinated mice (James et al. 1984a). The mechanism of cytotoxicity in the mouse involves a macrophage secretory product that seems able to damage the internal organs of the susceptible schistosomula (McLaren and James, 1985; James and Giaven, 1987).

In contrast, human monocytes have demonstrated minimal ability to destroy schistosomula *in vitro* (Ellner and Mahmoud, 1979). Work in our laboratories has confirmed that freshly isolated human monocytes possess little or no anti-schistosomula activity, but that this may be increased by prior stimulation of the cells with IFN. More interestingly, unstimulated monocytes (with no added IFN) could be grown *in vitro* into mature macrophages that expressed very high levels of cytotoxicity against the parasite (Cottrell et al. 1989). Thus in the maturation of monocyte into macrophage the cell acquired a potent schistosomulcidal mechanism, which was fully expressed between 5 and 7 days in culture.

The present work therefore was undertaken to define more precisely the nature of cytotoxicity in our developing macrophages. As a starting point we have looked for
cytotoxic mechanisms already described for the in vitro macrophage destruction of tumour targets (James et al. 1982). Additionally, we considered such an approach may provide us with new insights into the changing interactions of the maturing effector cells with defined mediators of macrophage activation.

Materials and methods

The methods used in the present study have already been described in detail elsewhere, so only a brief outline will be given here (Cottrell et al. 1989).

Monocyte purification and culture

Samples of 100 ml of blood were obtained from healthy donors. These samples were then deproteinized and mixed with 12 ml of dextran (6% dextran 500, Pharmacia; in Hanks' balanced salt solution, HBSS), and 12 ml of 2.7% EDTA in HBSS. After 30 min the leucocyte-enriched supernatant was removed, and 7 ml samples were spun over 3 ml of Nycodenz (Nycomed, UK) for 15 min at 600 £ at 20°C (Boyum, 1983). Monocytes were harvested from the interface, above the Nycodenz, and washed twice with cold HBSS. Cells were counted with a Coulter counter, and cell viability was measured using the Trypan Blue exclusion method. Cytospin preparations were stained with Giemsa and for non-specific esterase (Koski et al. 1976). The mean number of viable monocytes from 100 ml of venous blood was 21 X 106.

Cells were resuspended after two further washes with HBSS, at a concentration of 5 X 105 cells per ml in RPMI with 10% heat-inactivated, pooled AB serum. These cells were then cultured on Petriperm, hydrophobic culture dishes (W. C. Heraeus, Hanau) in 5% CO2, at 37°C (Andresen et al. 1983). Cells were harvested at set intervals by chilling the plates for 60 min at 4°C, followed by vigorous pipetting. The macrophages were washed twice with cold HBSS, counted and cell viability was assessed before use in the cytotoxicity assays.

Cytotoxicity assay

TriPLICATE 0.5 ml tubes (Eppendorf, F. R. Germany) contained 5X105 cells; and 50 live schistosomula in 0.2 ml of medium (Eagle's Minimal Essential Medium; 20 mM-Hepes, supplemented with 100 units ml-1 of penicillin and 100 µg ml-1 of streptomycin, plus 10% heat-inactivated foetal calf serum; Flow). Inhibitors dissolved in the same medium were added to give the appropriate final concentration in a total volume of 0.3 ml per tube. The cells and schistosomula (with or without inhibitors) were co-cultured for a period of 48 h at 37°C in an atmosphere containing 5% CO2. Control cultures were set up separately, either in plain medium or with inhibitor so that the effects of inhibitors on effector cells and schistosomula could be evaluated (see Results and Table 2, below).

Reagents

Human recombinant interferon-gamma (IFN); human recombinant tumour necrosis factor (TNF); and a mouse monoclonal antibody to human TNF were all kind gifts from Dr G. R. Adolf (Ernst Boehringer-Institut für Arzneimittel Forschung, Vienna, Austria). The inhibitors used in the study are listed in Table 1. These were obtained from Sigma (UK). Separate experiments showed that at the concentrations used in the inhibitor experiments there was no significant toxic effect of any of the inhibitors directly on the schistosomula (Table 2). In some experiments macrophages were stimulated with a mixture of calcium ionophore (A23187, 1 µM) and Escherichia coli LPS (0128: B12; 1 ng ml-1), both dissolved in normal medium (Sigma).

Schistosoma mansoni: life cycle and schistosomula preparation

Details of the parasite and its routine laboratory maintenance have been given elsewhere (Veith and Butterworth, 1983). Schistosomula were prepared by allowing infective cercariae to penetrate isolated rat skin in vitro, according to the method of Clegg and Smithers (1972).

Electron microscopy

Pellets of monocytes or macrophages were fixed in 2.5% glutaraldehyde, post-fixed in osmium tetroxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 301 electron microscope.

Table 1. The metabolic inhibitors used in the present study

<table>
<thead>
<tr>
<th>Inhibitor of:</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen burst</td>
<td></td>
</tr>
<tr>
<td>Catalase (bovine liver) (a,b,c)</td>
<td>2 X 10-2 to 2 X 10-3 units ml-1</td>
</tr>
<tr>
<td>Cytochrome c (type III) (b)</td>
<td>1 mg ml-1</td>
</tr>
<tr>
<td>Sodium azide (d)</td>
<td>1 mM</td>
</tr>
<tr>
<td>Chlorpromazine (b)</td>
<td>100 µM</td>
</tr>
<tr>
<td>Dimethyl sulphoxide (e)</td>
<td>150 mM</td>
</tr>
<tr>
<td>Lysosome function</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone (b)</td>
<td>100 µg ml-1</td>
</tr>
<tr>
<td>Trypan Blue (b)</td>
<td>1 mg ml-1</td>
</tr>
<tr>
<td>Aurothioglucone (b)</td>
<td>1 mg ml-1</td>
</tr>
<tr>
<td>Ammonium chloride (f)</td>
<td>10 µM</td>
</tr>
<tr>
<td>Chloroprene (f)</td>
<td>1 µM-100 µM</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td></td>
</tr>
<tr>
<td>Cycloheximide (f)</td>
<td>1-10 µg ml-1</td>
</tr>
<tr>
<td>Actinomycin D (f)</td>
<td>1-10 µg ml-1</td>
</tr>
<tr>
<td>Proteases</td>
<td></td>
</tr>
<tr>
<td>Tosyllysylchloromethylketone (f)</td>
<td>100 µM</td>
</tr>
<tr>
<td>Alpha-1 anti-trypsin (f)</td>
<td>100-500 µg ml-1</td>
</tr>
<tr>
<td>Microtubules</td>
<td></td>
</tr>
<tr>
<td>Colchicine (f)</td>
<td>1 µM</td>
</tr>
<tr>
<td>Iron-binding proteins</td>
<td></td>
</tr>
<tr>
<td>Ferrous sulphate (g)</td>
<td>50-100 µM</td>
</tr>
</tbody>
</table>

Also shown are references to the relevant papers (in parenthesis).

(a) Wozencraft et al. (1984); (b) Malkin et al. (1987); (c) Adams and Nathan (1983); (d) Kazura et al. (1981); (e) Rao et al. (1988); (f) James and Glaven (1987); (f) Drapier and Hibbs (1986).

Table 2. The effects of metabolic inhibitors on the macrophage cytotoxicity of schistosomula in vitro

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>No. exps</th>
<th>Percentage cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cells</td>
</tr>
<tr>
<td>Normal medium</td>
<td>8</td>
<td>94±2</td>
</tr>
<tr>
<td>Actinomycin D:</td>
<td>1 µg ml-1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10 µg ml-1</td>
<td>3</td>
</tr>
<tr>
<td>Cycloheximide:</td>
<td>1 µg ml-1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10 µg ml-1</td>
<td>5</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>2</td>
<td>44±12*</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>3</td>
<td>83±5</td>
</tr>
</tbody>
</table>

The results are expressed as the mean percentage of dead parasites plus or minus the standard error of the mean.

*p<0.05; **p<0.001.
glutaraldehyde in 0.09M-cacodylate buffer, pH 7.2, containing 3 mM-calcium chloride, for 30 min at 20°C. The fixative was then removed and replaced with 0.1M-cacodylate buffer, pH 7.2, with 3 mM-calcium chloride. The fixed pellets of cells were stored at 4°C before further processing. The pellets were post-fixed in 1% osmium tetroxide in 0.1M-cacodylate buffer, pH 7.2, containing 3 mM-calcium chloride, for 1 h at room temperature, dehydrated in ethanol and embedded in Araldite. Thin sections (50–80 nm) were stained with lead citrate and examined in a Philip's 201C microscope operating at 60 kV.

**Statistics**
In the cytotoxicity experiments parasite attrition was scored by counting the numbers of dead schistosomula (x), and the numbers of live schistosomula (y). In the tables and graphs, data are presented as percentage cytotoxicity:

\[
\frac{x}{x+y} \times 100.
\]

In these experiments simple Student's t-tests were performed on the log-transformed cytotoxicity data.

**Results**

**Onset of anti-schistosomula activity**
Purified human monocytes were grown in vitro for 7 days. On specified days cells were harvested and tested against live schistosomula in the standard cytotoxicity test. The results, which are the pooled data from 14 individual experiments, showed that freshly isolated monocytes (day 0) killed less than 10% of the schistosomula targets. This did not achieve a significant difference from the control level of kill with no cells in the culture (9%). However, from day 2 onwards there was a marked increase in cytotoxicity of the parasites, so that by day 7 of culture the macrophages could kill 100% of the schistosomula (Fig. 1). Cells 5–7 days old were therefore very effective cytotoxic macrophages and these were subsequently used in all the experiments.

Initial experiments were also undertaken to assess the minimal numbers of macrophages required to kill each schistosomulum. At a ratio of 10³ effectors: target (log 4), cytotoxicity was 100%. Even when this was reduced to a log ratio of 3.5, cytotoxicity exceeded 90% (Fig. 2).

**Monocyte–macrophage morphology**
Examination of thin sections in the electron microscope showed that the freshly isolated cells have the characteristic ultrastructure of human blood monocytes, with smooth outlines, large indented nuclei, a limited Golgi region, small rounded mitochondria and only a few cisternae of the rough endoplasmic reticulum (Fig. 3). After incubation in vitro for as little as 2 days the monocytes developed into typical macrophages, with many lamellipodia and surface blebs (Fig. 4). The mitochondria were elongated and in some regions the rough endoplasmic reticulum enlarged to form extensive arrays of cisternae (Fig. 5). The Golgi region was also greatly enlarged and contained arrays of smooth cisternae and a variety of vesicles and granules (Fig. 6). Thus the cells have all the morphological characteristics of the mature tissue macrophage.

The findings of light microscopy confirmed this view of the in vitro development of the monocytes into mature histiocytes. The cells enlarged and, over a period of 7 days displayed increasing amounts of granular cytoplasm. Also there were conspicuous ruffled plasma membranes; and enlarged complex nuclei, including multinucleate forms in some instances (Fig. 7).

**Effects of metabolic inhibitors upon macrophage cytotoxicity**
Groups of inhibitors were used with known effects on cytotoxic macrophages, as listed in Materials and methods (Table 1). Using cells that were 5–7 days old, an average of 94% schistosomula were killed in the in vitro tests. However, both inhibitors of protein synthesis, namely cycloheximide and actinomycin D, caused marked reductions in the level of parasite attrition. Actinomycin D was particularly effective in this regard, reducing cytotoxicity to a minimum of 13% (Table 2). Cell viability in the highest concentrations of actinomycin D and cycloheximide was 72% and 76%, respectively, compared to 81% viability of control cells cultured in medium alone.
Fig. 3. A freshly isolated monocyte has a large, indented nucleus (n), small rounded mitochondria (m), a few cisternae of rough endoplasmic reticulum (er) and a small Golgi region (G). Electron micrograph. Bar, 1 μm.

Fig. 4. After incubation for 2 days in vitro a monocyte has developed into the typical macrophage with surface lamellipodia. n, nucleus. Electron micrograph. Bar, 1 μm.

The only other inhibitor that successfully reduced cytotoxicity was Trypan Blue, which caused a reduction to 44%. Cell viability after 48 h was 83%. Although another inhibitor of lysosomal function, ammonium chloride, reduced cytotoxicity to 83% this did not achieve statistical significance. Three other inhibitors of lysosomes, namely chloroquine, aurothioglucose and hydrocortisone, had no effects on macrophage killing.
None of the oxygen-burst scavengers had any effects upon cytotoxicity including cytochrome c, catalase, azide, chlorpromazine and dimethyl sulphoxide (DMSO) (all tested in two separate experiments). Two substances that interfere with trypsin function, tosyllysylchloromethylketone (TLCK) and alpha-1 anti-trypsin had no effect on the levels of macrophage killing. Colchicine, which inhibits microtubule polymerisation, similarly had no inhibitory action on the macrophages. Finally, excess iron in the form of FeSO₄ had no effect, indicating that the effector molecule did not bind iron.

**The effects of TNF**

TNF has a well-documented cytotoxic action on a number of tumour and microbial targets (Beutler and Cerami, 1988). We investigated therefore the ability of pure rTNF (recombinant TNF) to kill schistosomula *in vitro* over 48 h. Further, TNF has been implicated as yet another macrophage stimulator, so TNF was added to cultures of macrophages that were 1 day old. Such cells have minimal cytotoxic effects, but do have the potential to increase following stimulation with IFN, for example (Cottrell et al. 1989). In two experiments the background cytotoxicity of schistosomula over 48 h (in medium only) was 6±3%. No concentration of TNF (2×10⁴, 2×10⁵ or 2×10⁶ units ml⁻¹) either on its own or acting with additional cells caused a significant increase in percentage cytotoxicity. For instance, at the highest TNF concentration there was 8±5% cytotoxicity with cells, and 14±5% without cells.

In further experiments we added mouse monoclonal antibody to human rTNF to the cultures. A total of 10⁴ neutralising units were added to each tube containing the

Fig. 5. The cytoplasm of a mature macrophage contains elongated mitochondria (m) and numerous cisternae of the endoplasmic reticulum (er). n, nucleus; l, lipid droplets. Electron micrograph. Bar, 1 µm.
macrophages and schistosomula. In three experiments, macrophages (5 or 7 days old) killed 88±8% of schistosomula. The addition of the monoclonal antibody to the cultures had no effects on macrophage killing of the parasites, the mean cytotoxicity being 86±8%. Without cells cytotoxicity was just 2%.

Arginine and effects of arginase

Arginase has been shown to be cytotoxic for tumours and has been implicated as a potential cytotoxic factor for schistosomes (Olds et al. 1980). Also arginine, in some experimental tumoricidal systems, is a prerequisite for the synthesis by the macrophage of cytotoxic factor that inhibits tumour mitochondrial respiration (Hibbs et al. 1987). To look at the effects of both these substances we constructed the following experiment. Five-day-old macrophages were used against schistosomula in either the conventional complete MEM medium, medium depleted of all amino acids, or medium without amino acids but with additional arginine. We also tested the effects of arginase (5 units ml$^{-1}$) in complete medium. The results of two experiments are summarised in Table 3. They showed that no additional amino acids were required in the medium for the expression of cytotoxicity, and that schistosomula could survive in the depleted media if macrophages were absent. Further, arginase on its own had no cytotoxic effects on the schistosomula.

Table 3. The effects of eliminating amino acids (−AA) from the medium, and the presence of arginase on the macrophage cytotoxicity

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>MEM**</th>
<th>MEM−AA</th>
<th>MEM−AA+arginine</th>
<th>MEM+arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>No cells</td>
<td>13±1</td>
<td>14±8</td>
<td>15±2</td>
<td>16±3</td>
</tr>
</tbody>
</table>

The results are expressed as the mean percentage cytotoxicity plus or minus the standard deviation.
**MEM is complete medium with all amino acids (AA).

showed that no additional amino acids were required in the medium for the expression of cytotoxicity, and that schistosomula could survive in the depleted media if macrophages were absent. Further, arginase on its own had no cytotoxic effects on the schistosomula.
Fig. 7. Giemsa-stained cytospin preparations of freshly isolated monocytes (A); 2-day-old macrophages (B); and 7-day-old mature macrophages (C and D). All preparations at the same magnification, photographed under oil immersion. Note the enlargement of the cells with the increasing complexity of the cell nuclei; the development of abundant granular cytoplasm; and in (D) the 'ruffled' plasma membrane. Also note the presence of lipid droplets within the cytoplasm of the cells. X1000.

The requirement for macrophage-parasite contact
To see whether physical contact of effector and target was a necessary element in the successful killing of the parasite, we utilised the standard Millipore chambers (0.45 μm pore size) to separate the components of our cytotoxic assay. Thus 2×10^6 macrophages were cultured in the chambers with 100 schistosomula; or cells were cultured alone with schistosomula in the 12 mm diameter well that housed the Millipore chamber. All combinations of cells and parasites were cultured in a total volume of 0.5 ml of medium. The results showed that separation of the effector cells and the parasites by Millipore filter reduced cytotoxicity to 11±4% compared to 100% when macrophages had uninterrupted access to the schistosomula (no filter).

Activity of macrophage supernatants
To investigate the possibility that macrophages were capable of secreting factors toxic for schistosomula we looked at the anti-schistosomula activity of media conditioned by our 7-day-old cells. Initial results indicated, however, that unstimulated cells produced little or no activity in the supernatants. We attempted therefore to increase the secretory potential of the macrophages by standard regimes of stimulation involving IFN followed by E. coli LPS and calcium ionophore (Zacharchuk et al. 1983). Accordingly, 7-day-old cells were cultured overnight with IFN (10^4 units ml⁻¹), followed by washing and a second 30-min stimulation with a combination of LPS and ionophore. The cells were washed once again, and then cultured in MEM for 2 h at a concentration of...
10^6 cells ml⁻¹. Supernatants from these macrophages were then tested against schistosomula over 48 h. In normal, unconditioned medium there was a background kill of 13±2%. The results showed that stimulation of the cells with IFN alone did not cause a statistically significant increase in the cytotoxic activity of the supernatants (19±2%). However, macrophages treated with LPS and ionophore did show statistically significant increases in the secretion of anti-schistosomula factor(s) into the conditioned medium. Further, this seemed to be independent of whether the cells were given an initial exposure to the IFN (cytotoxicity 31±5%); or not (37±4%), before stimulation with LPS and ionophore (both results significant; P<0.05).

**Discussion**

Macrophages are able to destroy a range of microorganisms, and tumour cell targets (Andrew et al. 1985; Drysdale et al. 1988). Crucial to an analysis of cytotoxicity is an understanding of the mechanisms of macrophage activation and maturation (Adams and Hamilton, 1984). The better documented mechanisms of tumour cell killing include a range of macrophage secretory products such as cytotoxic proteases (Adams and Nathan, 1983); TNF (Matthews, 1981); lysosomal enzymes (Leoni et al. 1985); oxygen metabolites (Nathan et al. 1983); and arginase (Currie and Basham, 1978).

Macrophage-mediated destruction of the schistosomula bears many similarities to tumour cytotoxicity in *in vitro* systems that utilise mouse peritoneal exudate cells (James et al. 1982; McLaren and James, 1985). However, the role of the human monocyte and tissue macrophage is less clear. We present evidence here to show that the acquisition of cytotoxicity by monocytes is dependent upon a maturational event. A parallel developmental change is seen when monocytes and *in vitro* cultured human macrophages are tested against tumour cell targets. Monocytes are relatively ineffectual, but levels of tumour cytotoxicity increase as the cells develop into typical tissue macrophages over periods of 7 to 14 days (Hammerstrøm, 1979; Rinhart et al. 1979; Andreesen et al. 1983). As the cells develop and increase their protein content there are significant increases in lysosomal enzymes (Musson, 1983); whilst other functions such as the generation of oxygen metabolites are down-regulated (Nakagawara et al. 1981). The relative contribution of these cytotoxic mechanisms will probably therefore be a function of the developmental status and degree of activation of the human macrophage (Andreesen et al. 1988; Kreipe et al. 1988).

Light and electron microscopy of the growing monocyte confirms the typical development into a tissue-type, mature macrophage. There is a characteristic large increase of rough endoplasmic reticulum, and associated Golgi apparatus, indicating a high level of protein synthesis, and potential secretory activity (Carr, 1980). In this context, it is an interesting finding that inhibitors of protein synthesis are the most effective agents in the abrogation of the macrophage cytotoxicity. Similar results in mouse macrophages have also been obtained by other workers (James and Glaven, 1987). Thus, the results from our metabolic and morphological studies implicate active protein synthesis as one component necessary for effective macrophage killing of the parasite *in vitro*.

Stimulated macrophages treated with agents known to disrupt membranes, release significant cytotoxic activity into the supernatant. This may be the consequence of low levels of secretion in 'unstimulated' macrophages, or it might indicate that the effector molecules are membrane-bound. Certainly, for the unstimulated macrophage (i.e. not treated with ionophore and LPS) intimate contact with the schistosomula targets is a necessary prerequisite for cytotoxicity. Separation of effector cells and targets by a Millipore filter, which allows for the passage of medium but not cells, completely abolishes effective parasite killing. We may speculate that such contact promotes locally high concentrations of secreted molecules, or even promotes the interchange of membrane-bound material from the effector cells to the parasite surface.

We present evidence here that some of the macrophage secretory products outlined above are probably not important in the destruction of the schistosomula. For instance, it is unlikely that TNF is the effector molecule, as monoclonal antibody to rTNF is unable to block the normal macrophage killing; and rTNF has no effect on the *in vitro* cultured schistosomula. Similarly, the inhibition experiments using oxygen radical scavengers indicate that the products of oxygen-burst metabolism are unlikely candidates for the effector molecules in our system. This result reinforces earlier work in the mouse that shows that non-oxidative metabolic pathways are operational in the macrophage destruction of the parasite (James and Glaven, 1987; Malkin et al. 1987). Of the remaining non-oxidative mechanisms, we find that trypsin is another unlikely candidate, as two agents known to block its activity have no effect in our cytotoxicity assays.

Ultrastructural studies of the interaction of mouse macrophages and schistosomula show that damage is directed at mitochondria and smooth muscle (McLaren and James, 1985). It is intriguing therefore to find that activated mouse macrophages may kill tumour targets by inhibiting mitochondrial respiration (Kilbourn et al. 1984). More recent experimental evidence has clearly shown that this mechanism of tumour cell damage is dependent upon the metabolism of arginine in the macrophages (Hibbs et al. 1987). Interesting as these recent studies are, there is no evidence from our system that cytotoxicity is the result of an arginine-dependent metabolic pathway.

Further work is being carried out in our laboratory to define the mechanism of cytotoxicity of the schistosomula. In this context, the use of defined populations of inactive monocytes and maturing cytotoxic macrophages is proving useful.

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