The role of reactive oxygen metabolites in lymphocyte-mediated cytolysis

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Summary

To examine the possible role of reactive oxygen metabolites in lymphocyte-mediated cytolysis, the morphology of cell death following the exposure of cells to reactive oxygen metabolites in vitro was compared with the morphology of cell-mediated killing in vitro of tumour cells by natural killer (NK) cells. Ultrastructural examination of human tumour cells that were dying following incubation for 60 min with the oxygen metabolite generating systems, xanthine–xanthine oxidase or t-butylhydroperoxide, showed that cell death in both instances was exclusively by necrosis. It was unclear which oxygen metabolites were involved in killing. Cell death was not decreased by the addition of superoxide dismutase, a scavenger of the superoxide anion, to the xanthine–xanthine oxidase mixture. Although the cells were not killed by incubation with 1 mM-hydrogen peroxide, the addition of catalase, a scavenger of hydrogen peroxide, to the xanthine–xanthine oxidase mixture significantly reduced cell death. The addition of scavengers for the hydroxyl radical to either the xanthine–xanthine oxidase mixture or t-butylhydroperoxide gave inconsistent protection.

In contrast, tumour cell killing mediated by natural killer cells was by apoptosis, a morphologically distinct mode of cell death with a different basic mechanism, indicating that reactive oxygen metabolites are not directly involved in lymphocyte-mediated cytolysis.

Key words: reactive oxygen metabolites, lymphocyte-mediated cytolysis, necrosis, apoptosis.

Introduction

It has clearly been shown that reactive oxygen metabolites are involved in phagocyte-mediated antimicrobial activity (Babior, 1978; Segal, 1984) and there is considerable evidence that antibody-dependent cell-mediated cytotoxicity (ADCC) against parasites by phagocytes also depends on the generation of reactive oxygen metabolites (Klebanoff, 1980; Klebanoff et al. 1983). However, the role of reactive oxygen metabolites in lymphocyte-mediated cytolysis against tumour cells is unclear as many published findings are contradictory. Studies have shown that T-cell-mediated cytolysis does (Devlin et al. 1981; Nathan et al. 1982) or does not (MacDonald & Koch, 1977) depend on oxygen and is (Thorne et al. 1980; Thorne & Franks, 1983) or is not (Nathan et al. 1982) mediated by reactive oxygen metabolites. Similarly, it has been suggested that natural killer (NK) cell-mediated killing does (Roder et al. 1982; Babior & Parkinson, 1982; Duwe & Roder, 1984; Suthanthiran et al. 1984) or does not (Kay et al. 1983; El-Hag & Clarke, 1984; Ramstedt et al. 1984) utilize reactive oxygen metabolites. Moreover, antibody-dependent lymphocytotoxicity mediated by killer (K) cells has been shown to involve (Bowman & Shoeb, 1984) or not involve (Katz et al. 1980) reactive oxygen metabolites.

It has been recently recognized that there are two distinct morphologically recognizable types of cell death, necrosis and apoptosis (sometimes referred to as ziosis), which have different basic mechanisms (Wyllie et al. 1980; Searle et al. 1982; Kerr et al. 1984). Morphological studies of complement-mediated lysis (Goldberg & Green, 1959) and phagocyte-mediated antimicrobial or antiparasitic activity (Thorne & Blackwell, 1983; Rzepczyk & Bishop, 1984), show cell death by necrosis. However, studies
of lymphocyte-mediated cytolysis by either T cells (Don et al. 1977; Liepins et al. 1977; Sanderson & Glauert, 1977; Matter, 1979; Kerr et al. 1984), human K cells (Stacey et al. 1985) or murine NK cells (Bishop & Whiting, 1983) have shown that target cells die by apoptosis.

To examine the possible role of reactive oxygen metabolites in lymphocyte-mediated cytolysis, the morphology of cell death following the exposure of cells to reactive oxygen metabolites in vitro was compared with the morphology of NK cell-mediated killing of tumour cells. Cells were exposed to either a xanthine–xanthine oxidase mixture, t-butylhydroperoxide or hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). The xanthine–xanthine oxidase mixture generates the superoxide anion (O\textsuperscript{2–}) and to a lesser extent H\textsubscript{2}O\textsubscript{2} (Fridovich, 1970). The further reduction of enzymically produced H\textsubscript{2}O\textsubscript{2}, in the presence of ferrous or cuprous ions, results in the formation of the hydroxyl radical (‘OH) (refer to Halliwell & Gutteridge, 1984). t-butylhydroperoxide is homolytically split, in the presence of cellular haemoproteins, into its alkoxy radical and ‘OH (Cadenas & Sies, 1982). In this paper we show that the morphology of cell death following exposure to xanthine–xanthine oxidase or t-butylhydroperoxide differs from that involved in the killing of tumour cells by NK cells.

**Materials and methods**

**Culture medium**

Cells were cultured in RPMI 1640 (Commonwealth Serum Laboratories, Vic.) supplemented with 10% heat-inactivated foetal calf serum (FCS), penicillin (100 i.u. ml\textsuperscript{–1}) and streptomycin (100 μg ml\textsuperscript{–1}).

**Cell lines**

Experiments involving the addition of reactive oxygen metabolites were carried out on the human myeloid leukaemic line K562 (Lozzio & Lozzio, 1975), the human Burkitt’s lymphoma cell line BL36 (Rooney et al. 1984) and the human lymphoblastic leukaemic line HSB2 (Krishan & Raychaudhuri, 1970).

**Lymphocyte-mediated cytolysis**

Unfractionated human peripheral blood mononuclear cells were isolated from heparinized blood by isopycnic centrifugation on Ficoll/Paque (Pharmacia) as described (Boyum, 1968). Monocytes were removed by filtration through cotton wool as described (Stacey et al. 1985). The lymphocytes were resuspended in culture medium at 10\textsuperscript{5}ml\textsuperscript{–1} before being mixed with K562 or HSB2 cells in a ratio of 4:1 in culture medium, centrifuged at 250 g for 3 min to facilitate cell to cell contact and incubated at 37°C. At various times the mixtures were centrifuged at 10000 g for 1 min (in a bench microfuge) and the pellets processed for electron microscopy.

**Exposure to reactive oxygen metabolites**

Cells were exposed to either a xanthine (Sigma, USA)–xanthine oxidase (Calbiochem, Australia) mixture, t-butylhydroperoxide (Sigma) or H\textsubscript{2}O\textsubscript{2}. All reagents except xanthine were made up as required, in RPMI 1640. Xanthine was made up in 0.015 M NaOH. Cells were incubated at 37°C at 10\textsuperscript{5} ml\textsuperscript{–1} in xanthine (1 mM)–xanthine oxidase (125 munits ml\textsuperscript{–1} or 50 munits ml\textsuperscript{–1}) mixture, H\textsubscript{2}O\textsubscript{2} (1 mM or 10 mM) or t-butylhydroperoxide (1 mM) in 1 ml microfuge tubes in triplicate. Various scavengers of reactive oxygen metabolites were added to some cell cultures 10 min before the addition of xanthine–xanthine oxidase or t-butylhydroperoxide as above. Superoxide dismutase (SOD, from bovine erythrocytes, Calbiochem) was used at 350 units ml\textsuperscript{–1} as a scavenger for O\textsuperscript{2–}; catalase (from bovine liver, Calbiochem) was used at 1000 units ml\textsuperscript{–1} as a scavenger for H\textsubscript{2}O\textsubscript{2}; L-histidine (Sigma) was used at 100 mM as a scavenger for the singlet oxygen radical (‘O\textsubscript{2}); dimethylsulphoxide (DMSO), ethanol, methanol and mannitol (Sigma) were used at 100 mM, 80 mM, 300 mM and 100 mM, respectively, as scavengers for ‘OH. After incubation for 60 min, the cells were centrifuged and resuspended in cold RPMI 1640. Some cells were processed for electron microscopy. Percent-age cell death was estimated as below.

**Morphological markers of cell death**

Certain histological changes associated with cell death, classically designated as nuclear pyknosis and karyorrhexis, occur in both necrosis and apoptosis (Wyllie et al. 1980; Searle et al. 1982). Necrotic cells also show karyolysis and swelling of the cytoplasm, which eventually loses its basophilia, and cell boundaries become indistinct (Trump & Arstila, 1975; Wyllie et al. 1980; Searle et al. 1982). Apoptotic cells appear to be condensed, typically with intensely eosinophilic cytoplasm, which frequently shows surface protrusion. These protuberances separate to form roughly spherical bodies sometimes containing basophilic nuclear fragments (Wyllie et al. 1980; Searle et al. 1982). By electron microscopy, necrotic cells exhibit aggregation of chromatin and extensive nuclear and cytoplasmic swelling. Mitochondria show gross swelling and develop foeculent and sometimes granular densities. Nuclear, organelle and plasma membranes rupture, and cellular organelles disintegrate and disperse (Trump & Arstila, 1975). Typical features of apoptosis are aggregation and margination of chromatin with nuclear and cytoplasmic condensation and loss of microvilli. Generally this is followed by nuclear fragmentation and the budding off of surface protuberances to form membrane-bound bodies that contain intact cytoplasmic organelles and/or nuclear fragments (Wyllie et al. 1980; Searle et al. 1982).

**Estimation of cell death**

The percentage of cell death by necrosis was determined by the failure of necrotic cells to exclude Trypan Blue dye and was estimated by light microscopy from counts, in haemacytometer chambers, of 300 cells from each of duplicate wells from each sample. The percentage of cell death by apoptosis was estimated by electron microscopy from counts of 300 cells in each of duplicate thin sections from each
Fig. 1. HSB2 cells, following incubation in xanthine–xanthine oxidase (125 munits ml\(^{-1}\)) for 1 h, showing mitochondrial and cellular swelling, membrane rupture and dissipation of cellular contents. X5500.

sample. Where apoptotic bodies were in a compact cluster, the cluster was scored as one.

Electron microscopy
Cells were fixed in 3 % glutaraldehyde in 0.1 M-cacodylate buffer (adjusted to 300 mmol kg\(^{-1}\) H\(_2\)O real osmolality with sucrose) for 1 h at room temperature, washed, post-fixed with 1 % OsO\(_4\) for 1 h at room temperature, en bloc stained with uranyl acetate, dehydrated in ethanol and embedded in Spurr’s low viscosity embedding media (Polyscience, PA). Centrifugation was used to compact the specimen after each treatment. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 430.

Results
Ultrastructure of cell death by reactive oxygen metabolites
When K562, BL36 or HSB2 cells were incubated with a xanthine–xanthine oxidase mixture or \(\alpha\)-butylhydroperoxide, a significant proportion of the cells died by necrosis within 60 min. Cells incubated with either xanthine–xanthine oxidase or \(\alpha\)-butylhydroperoxide initially showed a decrease in the number of microvilli, marked condensation of the mitochondria and some dilation of the rough endoplasmic reticulum (ER). Typically, with further incubation there was aggregation of chromatin, gross mitochondrial swelling, increased dilation of the ER, and the cells became swollen with a resulting decrease in the density of the matrix of both the cytoplasm and nucleus and, eventually, membrane rupture (Fig. 1). Although a systematic study was not carried out, the speed of the above sequence of events, including mitochondrial swelling, varied according to the target cell line and the concentration of xanthine oxidase or \(\alpha\)-butylhydroperoxide. For example, while mitochondrial swelling always occurred sequentially to mitochondrial condensation, both condensed and swollen mitochondria could sometimes be observed in otherwise normal BL36 cells after incubation with 1 mM-\(\alpha\)-butylhydroperoxide for 1 h (Fig. 2). In some K562 cells incubated with xanthine–xanthine oxidase (125 munits ml\(^{-1}\)) for 1 h, mitochondria remained condensed although the cytoplasm was severely disrupted (Fig. 3). In some K562 cells, there was also a marked increase in the number of cytoplasmic multivesicular bodies following incubation (Fig. 4). In all cell lines, there was, finally, membrane rupture and dissipation of the cellular contents (Fig. 1). Cells incubated for 1 h with 1 mM-H\(_2\)O\(_2\) generally showed condensation of mitochondria and some dilation of the ER (not shown), although a small proportion of cells (<5 %) were observed with swollen mitochondria as described above. Incubation of cells for 1 h with 10 mM-H\(_2\)O\(_2\) resulted in rapid death by necrosis as described above (not shown). In all instances, apoptosis was rarely observed.
Percentage cell death induced by reactive oxygen metabolites

The percentage cell death by necrosis, as measured by the dye-exclusion assay, in cultures of K562 and BL36 cells following exposure for 60 min to xanthine–xanthine oxidase, t-butylhydroperoxide or H₂O₂ is shown in Table 1. There was a significant increase in necrosis by xanthine–xanthine oxidase and by t-butylhydroperoxide. The percentage necrosis in cultures incubated with 1 mM-H₂O₂ was low or not significant. Incubation of cells with 10 mM-H₂O₂ for 60 min resulted in 100% necrosis (not shown). There was no significant increase in the percentage cell death by apoptosis, as determined by counting in the electron microscope, in any of the cultures (not shown).

The percentage necrosis, as measured by the dye-exclusion assay, in cultures of K562, BL36 and HSB2 cells after incubation for 60 min with xanthine–xanthine oxidase in the presence of various scavengers of reactive oxygen metabolites is shown in Table 2. The percentage necrosis was not decreased by the addition of superoxide dismutase (SOD), a scavenger for O₂⁻, or by histidine, a scavenger for 'O₂, but was significantly decreased by the addition of catalase, a

Fig. 2. BL36 cell, following incubation with 1 mM-t-butylhydroperoxide for 1 h, showing both condensed and swollen mitochondria. ×15,000.

Fig. 3. K562 cell, following incubation in xanthine–xanthine oxidase (125 munits ml⁻¹) for 1 h, showing condensed mitochondria associated with severe cytoplasmic disruption. ×11,500.
Fig. 4. Numerous multivesicular bodies in a K562 cell, following incubation in xanthine-xanthine oxidase (125 munits ml⁻¹) for 1 h. ×21,000.

Table 1. Percentage cell death in cultures of tumour cells following incubation with xanthine-xanthine oxidase (125 munits ml⁻¹), t-butylhydroperoxide (1 mM) or H₂O₂ (1 mM)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>K562</th>
<th>BL36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine-xanthine oxidase</td>
<td>78.3 ± 12.5</td>
<td>90.2 ± 5.1</td>
</tr>
<tr>
<td>t-butylhydroperoxide</td>
<td>52.0 ± 19.9</td>
<td>31.9 ± 34.8</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>2.4 ± 3.2</td>
<td>1.2 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of four experiments.

Table 2. Effect of scavengers on percentage cell death in cultures of tumour cells incubated with xanthine-xanthine oxidase

<table>
<thead>
<tr>
<th>Cell line</th>
<th>K562</th>
<th>BL36</th>
<th>HSB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of xanthine oxidase (munits ml⁻¹)</td>
<td>125</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Scavengers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>72.8 ± 12.5</td>
<td>78.9 ± 8.7</td>
<td>67.9 ± 8.1</td>
</tr>
<tr>
<td>Catalase</td>
<td>3.1 ± 3.2*</td>
<td>6.9 ± 10.0*</td>
<td>19.5 ± 20.4**</td>
</tr>
<tr>
<td>SOD</td>
<td>74.5 ± 26.9</td>
<td>—</td>
<td>73.9 ± 12.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>—</td>
<td>64.9 ± 16.2</td>
<td>83.3 ± 9.7</td>
</tr>
<tr>
<td>Mannitol</td>
<td>—</td>
<td>70.9 ± 2.3</td>
<td>92.9 ± 6.6</td>
</tr>
<tr>
<td>DMSO</td>
<td>—</td>
<td>48.2 ± 21.9</td>
<td>54.7 ± 36.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>—</td>
<td>—</td>
<td>47.0 ± 34.9</td>
</tr>
<tr>
<td>Ethanol</td>
<td>—</td>
<td>—</td>
<td>13.1 ± 7.0*</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of three experiments. *Significantly different (P<0.05) in t-test.

Table 3. Effect of scavengers on percentage cell death in cultures of tumour cells incubated with t-butylhydroperoxide (1 mM)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>K562</th>
<th>BL36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scavengers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>53.1 ± 14.2</td>
<td>49.4 ± 32.8</td>
</tr>
<tr>
<td>Mannitol</td>
<td>19.5 ± 23.5</td>
<td>10.5 ± 1.5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>35.3 ± 18.3</td>
<td>16.6 ± 16.9</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of three experiments.

scavenger for H₂O₂. Most scavengers for 'OH that were tested, i.e. mannitol, DMSO or methanol, did not consistently decrease the percentage necrosis although DMSO and methanol markedly reduced necrosis in some experiments, resulting in the large standard deviations shown in Table 2. However, ethanol, another scavenger for 'OH, significantly reduced necrosis. There was no significant increase in necrosis in cultures incubated with scavengers alone (not shown). Similarly, the percentage cell death by necrosis in cultures of K562 and BL36 cells after incubation for 60 min with t-butylhydroperoxide could be reduced, sometimes markedly but inconsistently, by the addition of mannitol or ethanol (Table 3).

Ultrastructure of lymphocyte-mediated cytolysis

When tumour cells were incubated with peripheral blood lymphocytes, there was attachment of some
lymphocytes to some of the tumour cells. Following lymphocyte attachment, the tumour cells showed early changes associated with cell death by apoptosis, such as fragmentation of the nucleus into membrane-bound fragments, loss of microvilli and budding of the cytoplasm (Fig. 5). Once the process of apoptosis began, it appeared that the lymphocytes detached from the dying cells and the latter budded into a number of membrane-bound fragments termed apoptotic bodies (Fig. 6). Eventually apoptotic bodies underwent secondary disintegration, with mitochondrial swelling and membrane disruption (not shown).

**Discussion**

A significant percentage of cells die following incubation for 60 min with xanthine–xanthine oxidase or t-butyldihydroperoxide. Ultrastructural examination of dying cells showed that cell death in both instances was exclusively by necrosis. Cells initially showed the early reversible changes associated with cell injury, such as condensation of mitochondria, but within 60 min showed the irreversible changes associated with necrosis, such as high amplitude swelling of mitochondria and membrane rupture (Trump & Arstila, 1975).

It was unclear which oxygen metabolites were involved in killing. t-butyldihydroperoxide is homolytically split, in the presence of cellular haemoproteins, into its alkoxy radical and 'OH (Cadenas & Sies, 1982). These radicals can induce lipid peroxidation and the subsequent production of lipid oxyl and peroxyl radicals (Halliwell, 1984; Halliwell & Gutteridge, 1984). The aerobic oxidation of xanthine by xanthine oxidase generates O_2^- and to a lesser extent H_2O_2 (Fridovich, 1970). O_2^- is only poorly reactive in aqueous solution and it has been suggested that it is not reactive enough to be directly toxic in most instances (Klebanoff et al. 1983). Moreover, in the experiments described here, cell death was not decreased by the addition of SOD, a scavenger of O_2^-, to the xanthine–xanthine oxidase mixture, indicating that O_2^- was not directly involved in the killing.

It has been shown that different mammalian cells exhibit different sensitivities to H_2O_2 and that this correlates with their susceptibility to killing by activated phagocytes (Nathan et al. 1979a). The tumour cells used as targets in this study, particularly K562 and HSB2 cells, are commonly used as sensitive targets in assays of NK cell-like cytotoxicity (e.g. see Rickinson et al. 1981) yet were relatively insensitive to H_2O_2. Although the cells rapidly underwent necrosis in high concentrations of H_2O_2, they were not killed by incubation with 1 mM-H_2O_2. As the xanthine–xanthine oxidase concentrations used in the above experiments were unlikely to generate concentrations of H_2O_2 greater than 1 mM within 60 min, it appeared that in this instance H_2O_2 was not killing directly. Nevertheless, H_2O_2 appeared to mediate killing indirectly as the addition of catalase, a scavenger of

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**Fig. 5.** K562 cell, showing the morphological changes associated with cell death by apoptosis, following incubation for 3 h with human peripheral blood lymphocytes. \( \times 12,500 \).
Recent studies suggest that erythrocyte membrane damage resulting from incubation in xanthine-xanthine oxidase is due to OH (Girotti & Thomas, 1984). H$_2$O$_2$ can be reduced in the presence of ferrous or cuprous ions, resulting in the formation of 'OH (Halliwell & Gutteridge, 1984). It has been suggested that ferrous ions may be generated in the xanthine-xanthine oxidase mixture by the reduction of ferric ions by O$_2^-$ (Halliwell & Gutteridge, 1984). However, in the experiments described here, the addition of SOD to xanthine-xanthine oxidase did not prevent killing. The addition of scavengers for 'OH to the xanthine-xanthine oxidase mixture and to t-butylhydroperoxide gave inconsistent results. Of the scavengers for 'OH added to xanthine-xanthine oxidase, only ethanol significantly reduced cell death, although cell death was sometimes markedly reduced by the addition of DMSO or methanol. Similarly, the addition of mannitol or ethanol to t-butylhydroperoxide sometimes markedly reduced cell death. It has been suggested that the failure of some 'OH scavengers to prevent membrane damage induced by xanthine-xanthine oxidase is due to 'OH being produced on membranes at iron-binding sites and subsequently reacting so rapidly with target molecules that scavengers cannot compete (Girotti & Thomas, 1984).

It has been suggested that membrane damage by oxygen metabolite-generating systems is mediated in part by singlet oxygen, O$_2^*$, derived from the spontaneous dismutation of O$_2^-$ or its reduction by H$_2$O$_2$ (Lynch & Fridovich, 1978). However, there is little evidence to support this (Halliwell, 1984) and in the studies described here the addition of scavengers for O$_2^-$ and 'O$_2$ did not reduce killing.

Irrespective of the reactive oxygen metabolite involved, the killing associated with H$_2$O$_2$, xanthine-xanthine oxidase and t-butylhydroperoxide was by necrosis. However, killing mediated by NK cells was by apoptosis, a morphologically distinct mode of cell death with a different basic mechanism, indicating that reactive oxygen metabolites were unlikely to be directly involved in NK cell killing. As killing mediated by other lymphocytes (i.e. T and K cells) is also by apoptosis, reactive oxygen metabolites are unlikely to be directly involved in lymphocyte-mediated cytolyis.

Some of the confusion in the literature may be explained by the important observation that a respiratory burst can be detected in cytotoxic T-cell-mediated killing of tumour cells only in the presence of mycoplasma (Koppel et al., 1984). Recent studies suggest that lymphocytes mediate cell killing by producing other cytotoxic substances, such as lymphotoxin (refer to Ruddle, 1985) and/or pore-forming molecules (refer to Henkart, 1985; Podack, 1985), but again there are contradictions. For example, the pores induced in target cell plasma membranes by pore-forming molecules isolated from cytotoxic T cells and NK cells are said to be morphologically and biochemically related to those pores produced during complement-mediated lysis (e.g. see Dennert & Podack, 1983; Henkart et al., 1984). However, as described above, cell death resulting from lymphocyte-mediated killing and from complement-mediated lysis are morphologically quite distinct. It is unclear why lymphocytes induce apoptosis while complement

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**Fig. 6.** Apoptotic bodies containing nuclear fragments formed after incubation of K562 cells for 3 h with human peripheral blood lymphocytes. ×9000.
induces necrosis, if lymphocytes and complement produce similar lesions.

The mechanism of phagocyte-mediated killing of nucleated, non-erythroid mammalian cells is also unclear. For example, it has been suggested that the phagocyte-mediated killing of tumour cells does (Nathan et al. 1979b) or does not (Weinberg & Haney, 1983) involve reactive oxygen metabolites, while other studies have implicated the involvement of cytotoxins (Mannel et al. 1980; Matthews, 1981) and pore-forming molecules (Young et al. 1986), depending on the effector cell. Moreover, it has been shown that macrophages can kill tumour cells either in the presence or absence of antibody, the resulting target cell death having the morphological appearance of necrosis or apoptosis, respectively (Dingemans et al. 1983). It is possible that there may be a number of mechanisms that can be used by different cell types and/or in different circumstances. However, one of these mechanisms, the production of reactive oxygen metabolites, does not appear to be utilized by cytotoxic lymphocytes.

References


dependent on the presence of mycoplasma. 


