

# Generation of cytolytic T cells in individuals infected by *Mycobacterium tuberculosis* and vaccinated with BCG

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## Abstract

**Background** Macrophage activation by cytokines provides only a partial explanation of antimycobacterial immunity in man. Because cytolytic T lymphocytes have been shown to contribute to immunity in animal models of intracellular infection, the generation of mycobacterial antigen specific cytotoxic T cells was examined in the peripheral blood of patients with tuberculosis.

**Methods** Subjects comprised 36 patients with active tuberculosis (18 newly diagnosed) and 32 healthy volunteers, of whom 25 had had BCG vaccination and seven were Mantoux negative. The ability of purified protein derivative (PPD) stimulated peripheral blood lymphocytes to lyse autologous, mycobacterial antigen bearing macrophages was examined by using a chromium 51 release assay.

**Results** PPD stimulated lymphocytes from normal, Mantoux positive, BCG vaccinated subjects produced high levels of PPD specific cytotoxicity, whereas lymphocytes from unvaccinated, uninfected subjects caused little or no cytolysis. The generation of cytolytic T lymphocytes by patients with tuberculosis was related to their clinical state. Those with cavitating pulmonary disease or lymph node tuberculosis generated PPD specific lymphocytes with cytotoxic ability similar to that of those from Mantoux positive control subjects, whereas lymphocytes from patients with non-cavitating pulmonary infiltrates showed poor antigen specific cytolysis. After seven days of stimulation with PPD *in vitro*, lymphoblasts contained both CD4<sup>+</sup> and CD8<sup>+</sup> cells. Mycobacterial antigen specific cytolysis was restricted to the CD4<sup>+</sup> cell population and was blocked by monoclonal antibodies directed against major histocompatibility class II (MHC) antigens.

**Conclusion** CD4<sup>+</sup> cytolytic T cells can lyse autologous macrophages presenting mycobacterial antigen and were found in patients with cavitating pulmonary tuberculosis or tuberculous lymphadenitis and in normal, Mantoux positive control subjects. The ability to generate these T cell responses seems to be a marker for response to mycobacteria and

may contribute to tissue damage in tuberculosis. These responses do not provide protective immunity against *Mycobacterium tuberculosis* but may help in disease localisation.

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Tuberculosis remains a major global health problem, especially, though not exclusively, in developing countries. The global estimate of the number of people with tuberculosis is approximately 50-60 million,<sup>1</sup> with eight to 10 million new cases each year.<sup>2</sup> Although mortality figures have been falling steadily over the past three decades, tuberculosis is recognised as a complication of T cell deficiency states, in particular of HIV infection.<sup>3</sup> Patients with AIDS are a hundred times more likely to contract tuberculosis than people without this disease.<sup>4</sup> This situation is of obvious importance in countries where tuberculosis and AIDS are already prevalent.

The causative agent, *Mycobacterium tuberculosis*, is a facultative intracellular pathogen that can replicate within human monocytes and macrophages. Protective immunity to tuberculosis depends on the interaction of T lymphocytes and mononuclear phagocytes,<sup>5</sup> while hypersensitivity to mycobacterial antigens (also mediated by T lymphocytes) contributes to the typical caseous tissue destruction. Protective immunity and hypersensitivity are clearly related but the detailed cellular mechanisms have not been fully explained. As a result of the work of Mackaness<sup>6</sup> it has been generally accepted that activation of macrophage antimycobacterial mechanisms by T cell secreted lymphokines such as gamma interferon is the cornerstone of protective immunity. Mycobacterial growth in mice is readily inhibited by lymphokine activated macrophages.<sup>7,8</sup> Activation of human macrophages with a variety of recombinant lymphokines, however, achieves modest inhibition of mycobacterial growth at best and is unlikely to fully explain antimycobacterial immunity.<sup>9-12</sup>

The generation of cytolytic T cells in response to intracellular bacterial pathogens, including mycobacteria, has been shown in animals.<sup>13</sup> Mycobacterial antigen specific cytolytic T cells are able to lyse autologous antigen bearing macrophages in murine tuberculosis. Cytolytic T cells have also been shown to inhibit the intracellular growth of *M tuberculosis* in a manner independent of interferon.<sup>14</sup>

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Furthermore, selective depletion of CD8<sup>+</sup> cytotoxic T cells increases the susceptibility of mice to infection with virulent *M tuberculosis*.<sup>15</sup> In man, mycobacterial antigen specific cytolytic T cells have been generated from the peripheral blood of BCG vaccinated subjects,<sup>16,17</sup> from patients with leprosy,<sup>18,19</sup> and from those with tuberculosis.<sup>20</sup> There has been speculation, therefore, about the function of cytolytic T cells in the immune response to tuberculosis. We have investigated the cytolytic activity of purified protein derivative (PPD) stimulated lymphocytes from patients and normal control subjects.

## Methods

### PATIENTS AND CONTROL SUBJECTS

Thirty six patients with bacteriologically or histopathologically proved tuberculosis were recruited from East Birmingham and Dudley Road Hospitals, Birmingham. The clinical details were documented and radiographs reviewed by one individual (ADP). Eighteen patients had newly diagnosed tuberculosis and had received less than two weeks' treatment when tested; the remaining 18 patients had received treatment for more than two weeks. Seventeen patients had localised cavitating pulmonary tuberculosis, eight had extra-thoracic lymph node tuberculosis, six had pulmonary disease with subapical diffuse pulmonary involvement and little or no evidence of localised cavitation (diffuse, non-cavitating tuberculosis), and three had miliary tuberculosis. One patient had abdominal tuberculosis and one had pleural disease. One patient who presented initially with diffuse pneumonic disease but later developed diffuse lymphadenopathy was tested on both occasions. Five of the patients had lymphadenopathy and five developed an abscess that required needle aspiration or surgical drainage. No patient had known risk factors for HIV infection or any other disease associated with immunosuppression. Three patients had diabetes (one insulin dependent, two non-insulin dependent).

Thirty two healthy volunteers acted as control subjects. Twenty five had previously received BCG vaccination and were known to be Mantoux positive; they were not retested for this study. The size of the tuberculin reaction was not known in most of the subjects. Seven subjects had no history of BCG vaccination or exposure to tuberculosis and were Mantoux negative.

### TISSUE CULTURE MEDIA

Complete medium (RPMI) consisted of RPMI 1640 (Gibco Biocult, Paisley) supplemented with glutamine (2 mM), penicillin (100 U/ml), and gentamicin (50 U/ml).

### SEPARATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood cells were separated from defibrinated blood by Ficoll-Hypaque centrifugation, washed three times in RPMI and resuspended at 10<sup>6</sup> mononuclear cells per ml in RPMI with 10% autologous serum.

### GENERATION OF STIMULATED T BLASTS (EFFECTOR CELLS)

Isolated peripheral blood cells were incubated in 2 ml volumes (1 × 10<sup>6</sup> cells/well) in 24 well tissue culture plates (Cell Cult) at 37°C, in 5% CO<sub>2</sub> in air for seven days. PPD (Statens Serum Institute, Denmark) at 10 µg/ml final concentration was added at the beginning of the culture period to generate antigen specific effector cells. Interleukin 2 stimulated cells were prepared as above except that 20 U/ml recombinant Interleukin 2 were added instead of PPD at the beginning of culture. Interleukin 2 was used as a non-specific T cell stimulant.

### SURFACE MARKER ANALYSIS

The surface phenotype of lymphoblast cultures stimulated by antigen for seven days was determined by a sensitive rosette assay using monoclonal antibody coated indicator red cells<sup>35</sup> or by flow cytometry by Becton-Dickinson FACScan. Data were collected using the consort 30 program and analysed with the LYSYS program. The following monoclonal antibodies were used, CD3 (OKT3 obtained from American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852), CD4 (BMA 040, Behringwerke AG, Marburg, Germany), CD8 (B941 a kind gift of Dr C Mawas, Marseilles, France), TCR-delta (T-cell Sciences, Boston, USA).

### PREPARATION OF MACROPHAGE TARGETS

Freshly isolated cells were distributed in 150 µl volumes (1.5 × 10<sup>5</sup> cells/well) in 96 well round bottom microtitre tissue culture plates (Flow). We and others have previously shown that approximately 10% of the added mononuclear cells adhered as monocytes and this number was used to compute effector:target ratios in cytotoxicity assays.<sup>20,21</sup> After six days' incubation, the non-adherent cells were washed off and the adherent cells incubated with antigen (PPD 25 µg/ml or streptokinase/streptodornase (SK/SD) 250 U/ml as control antigen and labelled with chromium-51 (2 µCi/well, CJS-1, Amersham International) overnight in a final volume of 100 µl. After 16–24 hours incubation the plates were washed three times with warm RPMI containing 5% heat inactivated, group A positive human serum after which the wells were replenished with 50 µl of RPMI containing 10% human serum.

### CYTOTOXICITY ASSAY

This method has been described in detail elsewhere.<sup>18,20</sup> Briefly, effector cells stimulated with antigen for seven days were harvested from the 24 well plates, washed once, and resuspended in RPMI supplemented with 10% human serum at 3 × 10<sup>6</sup>/ml and 7.5 × 10<sup>5</sup>/ml. One hundred µl of these suspensions were added to target macrophages in triplicate to give effector:target ratios of approximately 20:1 and 5:1. Triplicate wells with 100 µl of medium were used to determine spontaneous isotope release.

Some patients with tuberculosis were so profoundly lymphopenic that very low numbers of effector and target cells were available.

For these patients only one effector:target cell ratio was tested for each antigen combination.

After 18 hours' incubation the supernatant was aspirated from individual wells into counting tubes. One hundred  $\mu\text{l}$  of 1% Triton X-100 (Sigma) were added to each cell pellet and incubated for 45 minutes at 60°C to lyse the remaining adherent cells. The entire volume was aspirated subsequently and transferred to further counting tubes. Activity of both the supernatant and pellet was assessed by measurement of radioactive decay with a gamma counter (LKB).

The percentage isotope release for each well was calculated by using the formula:

$$\% \text{ Isotope release} = \frac{\text{cpm supernatant}}{\text{cpm supernatant} + \text{cpm pellet}}$$

The level of cytotoxicity in each well was determined as follows:

$$\text{Cytotoxicity} = \frac{\% \text{ isotope release in test well} - \% \text{ isotope release in spontaneous release well.}}$$

Results are presented as the mean (SD) of triplicate estimates of the percentage  $^{51}\text{Cr}$  release. The SD between triplicates was not normally greater than 5%. Spontaneous release in these assays was usually no greater than 20% of the total uptake of isotope.

Cytolysis of macrophages which had not been incubated with antigen was termed non-specific cytotoxicity. Cytolysis of macrophages incubated with PPD was termed antigen specific cytotoxicity.

#### MONOCLONAL ANTIBODY BLOCKING

The MHC restriction of PPD stimulated cells was determined by using antibodies directed against MHC class I (W6/32, A, B, C specific W6/32, SeraLab, UK) and class II (BU26, DP, DQ, DR specific, Dr M Goodall, Department of Immunology, Birmingham University) antigens. The antibodies were preincubated with target macrophages for 30 minutes before the addition of the effector cells and were present throughout the assay.

#### LYMPHOCYTE PROLIFERATION ASSAYS

Lymphocytes were isolated as described previously and added in triplicate to U bottom microtitre plates at a concentration of  $1 \times 10^5$  well.<sup>21,22</sup> Antigen was added at the concentrations indicated previously and the plates were incubated for six days before the addition of tritiated thymidine (Amersham, UK) at 0.15  $\mu\text{Ci}/\text{well}$  for 16 hours. Cells were harvested using an automatic cell harvester (Skatron, Finland) by suction through glass fibre paper and radioactivity was assessed using a beta counter (LKB Rackbeta 2).

The stimulatory capacity of the antigen is expressed as a lymphocyte proliferation index and is calculated as follows:

$$\frac{\text{mean dpm in cultures with antigen} - \text{mean dpm in cultures without antigen}}{\text{mean dpm cultures without antigen}}$$

#### DEPLETION OF T CELL SUBSETS

Effector cells stimulated with PPD for seven days were generated from Mantoux positive control subjects as described above. These were used to obtain effector cells which were depleted of CD4<sup>+</sup> or CD8<sup>+</sup> lymphocytes. This depletion was performed by incubation with mouse monoclonal CD4 or CD8 antibodies (described in the surface marker section above) before a second incubation with goat anti-mouse immunoglobulin coated magnetic beads (Dynabeads, Dynal, Trondheim, Norway). For comparison, effector cells were used without any depletion procedure ("unmanipulated effector cells") or after sham depletion by incubation with anti-mouse immunoglobulin coated Dynabeads alone.

#### MAGNETIC SEPARATION AND CYTOTOXICITY ASSAY

Suspensions of effector cells and beads at a 20:1 bead target cell ratio were incubated for 90 minutes at 4°C in a glass test tube. Suspensions were then adjusted to 2 ml with phosphate buffered saline and placed next to a cobalt-samarium magnet (Dynal, MPC-1) for two to three minutes. Dynabeads, including those bound to cells, adhered to the inside wall of the tube adjacent to the magnet. The unbound cell suspension was removed by careful aspiration into another tube. Depleted cells were pelleted by centrifugation for five minutes at 700 *g*, washed once with RPMI containing 10% autologous serum, and resuspended to  $3 \times 10^6/\text{ml}$  in RPMI.

The phenotype of the various populations was assessed using antibody coated sheep erythrocytes, before and after the depletion procedure, to ensure that CD4<sup>+</sup> or CD8<sup>+</sup> cells had been adequately removed. The above procedure normally resulted in >90% reduction of either CD4 or CD8 cells. The lysis mediated by unmanipulated, sham depleted, CD4 or CD8 depleted populations was tested using the cytotoxicity assay detailed above.

#### STATISTICAL ANALYSIS

The non-parametric Wilcoxon test was used for statistical analysis throughout. Measurements of statistical significance refer to comparisons made using an effector:target ratio of 20:1 unless otherwise indicated. Other tests used are quoted in the relevant results sections. Where ranges are quoted they correspond to maximum and minimum values.

## Results

#### GENERATION OF CYTOLYTIC T CELLS BY CONTROL SUBJECTS AND PATIENTS WITH TUBERCULOSIS

Peripheral blood lymphocytes from the 32 normal donors and 36 patients with tuberculosis, when stimulated in vitro with 10  $\mu\text{g}/\text{ml}$  PPD for seven days, lysed autologous macrophage targets in a dose dependent manner (figure 1 and table 1; individual values available from the author on request). There was a wide variation in the ability of lymphocytes to generate lymphoblasts capable of killing autologous macrophages in both groups. Macro-

Table 1 Cytolysis of autologous macrophages produced by purified protein derivative (PPD) stimulated effector cells from patients with tuberculosis or normal control subjects (median (range))

Subject details	Target antigen/E:T ratio					
	No Ag		SK/SD		PPD	
	20:1	5:1	20:1	5:1	20:1	5:1
Diffuse non-cavitating/miliary (patient Nos 1-9)	10 (2-28)	3 (0-12)	20 (6-30)	8 (3-11)	27 (14-41)	13 (5-39)
Cavitating pulmonary/lymph node (patient Nos 10-36)	18 (5-39)	8 (0-36)	20 (10-40)	9 (0-30)	46 (35-62)	36 (19-47)
Normal controls (Mantoux negative, n = 8)	12 (0-15)	4 (0-14)	13 (7-13)	4 (3-7)	22 (15-46)	10 (4-24)
Normal controls (Mantoux positive, n = 25)	17 (5-49)	8 (1-34)	19 (5-49)	8 (4-36)	49 (28-60)	33 (18-52)

E:T refers to the effector:target ratio. SK = streptokinase, SD = streptodornase.

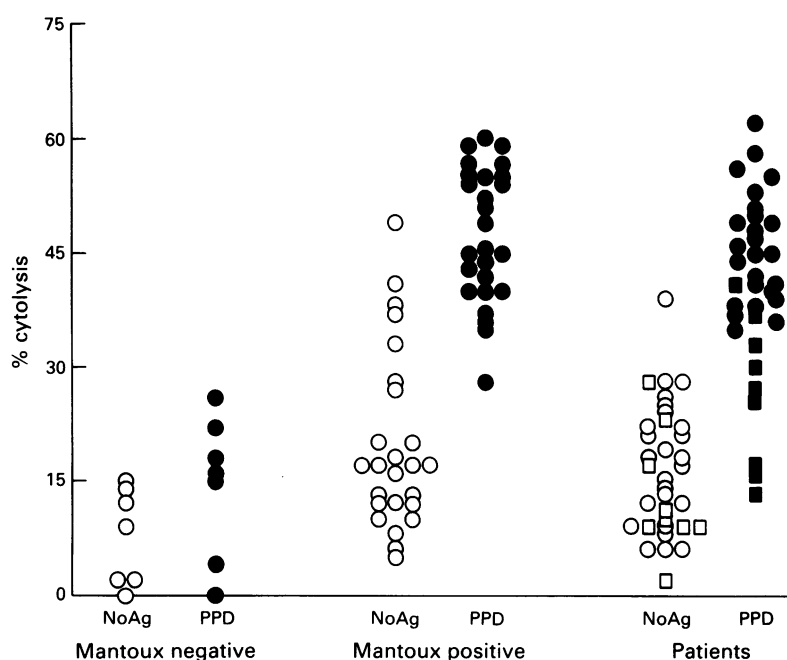


Figure 1 Percentage cytolysis of antigen unpulsed (open symbols) or purified protein derivative (PPD) pulsed (closed symbols) macrophages by PPD stimulated effector cells from Mantoux positive or Mantoux negative control subjects. Patients with diffuse non-cavitating or miliary tuberculosis are shown as squares. The effector:target cell ratio was 20:1.

phages pulsed with PPD were killed to a greater extent than those not pulsed with PPD (unpulsed) or macrophages pulsed with the control antigen streptokinase/streptodornase ( $p < 0.001$  for all comparisons) in both groups.

PPD stimulated lymphocytes from normal BCG vaccinated subjects showed greater antigen specific cytolytic capacity than those from normal unvaccinated individuals ( $p < 0.001$ ). PPD stimulated lymphocytes from patients with tuberculosis were less able to lyse PPD pulsed macrophages than those from normal BCG vaccinated subjects ( $p < 0.03$ ). When patients were stratified according to clinical features, those with cavitating pulmonary disease and extrathoracic lymphadenopathy tended to generate higher amounts of antigen specific cytotoxicity than those with non-cavitary pulmonary disease ( $p < 0.001$ ). The cytolytic activity of PPD stimulated lymphocytes from patients with cavitary disease did not differ significantly from those of BCG vaccinated control subjects ( $p = 0.67$ ).

Normal subjects and patients showed similar levels of non-specific lysis (lysis of antigen unpulsed macrophages), which ranged from 10 to 90% of the total lysis in both groups. Lysis of macrophages pulsed with control antigen (streptokinase/streptodornase) did not differ from that seen with unpulsed macrophages in either group. No relation was found between the ability to generate antigen specific or non-specific cytotoxic T cells and age, sex, or race.

Interleukin 2 stimulated cells from Mantoux positive control subjects were only poorly cytotoxic to autologous antigen pulsed and unpulsed macrophages (figure 2), even though there was a good proliferative response to this cytokine (lymphocyte proliferation index  $> 80$ ).

Proliferative responses to mycobacterial antigen were measured in 22 normal subjects and in 11 patients. There was a weak positive correlation between lymphocyte proliferation and cytolysis of antigen pulsed macrophages ( $r = 0.37$ ,  $p > 0.04$ ) (figure 3).

#### LYMPHOCYTE SURFACE MARKER ANALYSIS

The surface phenotype of cells stimulated by PPD for seven days was characterised in parallel with cytotoxicity assays in 12 Mantoux positive control subjects and nine patients (table 2). The lymphoblast population in all groups consisted predominantly of  $CD3^+$  cells, most of which were  $CD4^+$  (table 2). Variable expression of HNK1 and CD11b (markers for natural killer cells) was found (data not shown). Only a minority of cells expressed the gamma/delta T cell receptor. There was no significant difference in the phenotype of cells from patients and control subjects and no statistical correlation between the  $CD4:CD8$  ratio in the PPD stimulated cells and the level of specific or non-specific cytotoxicity.

Surface marker analysis of unstimulated lymphocytes from tuberculous pleural effusions from two patients also showed a predominance of  $CD3^+$  and  $CD4^+$  cells and low numbers of gamma/delta cells.

#### EVIDENCE THAT MYCOBACTERIAL ANTIGEN SPECIFIC LYSIS OF MACROPHAGES IS MHC CLASS II RESTRICTED

Antibodies specific for class II MHC antigens inhibited antigen specific lysis in both patients and normal control subjects (table 3). The mean inhibition observed at an antibody dilu-

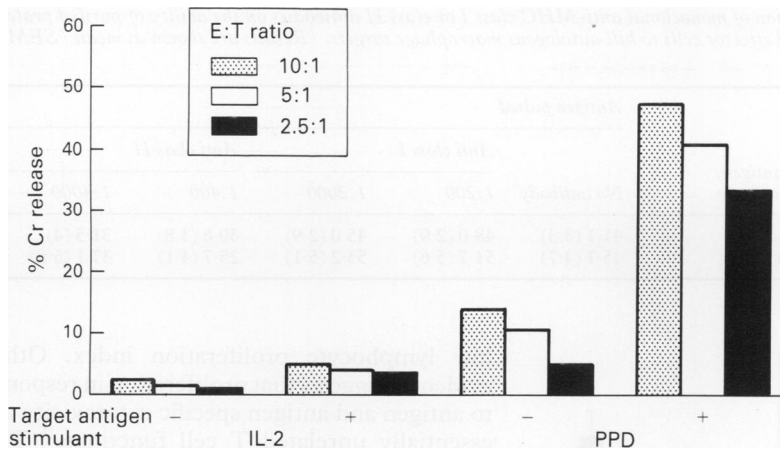


Figure 2 The lysis of autologous macrophages by peripheral blood lymphocytes stimulated in vitro with recombinant interleukin 2 (IL-2) (20 U) or with purified protein derivative (PPD) for seven days. Data pooled from two experiments using normal donors. E:T = effector:target ratio.

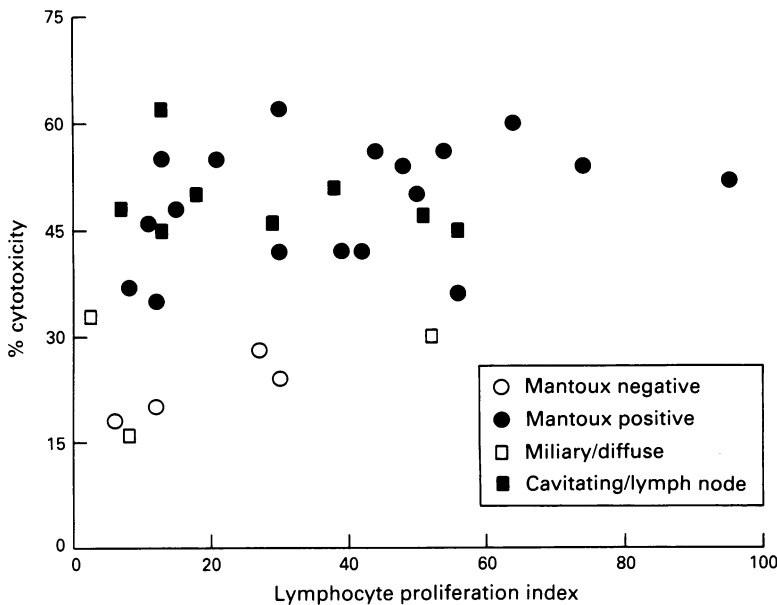


Figure 3 Relation of lymphocyte proliferation to purified protein derivative (PPD) (10 µg/ml) and the ability of these effector cells to kill PPD pulsed, autologous macrophage targets. Lymphocyte proliferation index is plotted against cytotoxicity of PPD pulsed targets at an effector:target ratio of 20:1.

tion of 1:400 was 45% ( $p < 0.01$ ) in patients and 65% ( $p < 0.001$ ) in control subjects. In contrast, no significant blocking or a modest enhancement of cytolysis was observed when antibodies directed against class I determinants were used.

T CELL SUBSET DEPLETION

PPD stimulated lymphocytes were depleted of CD4<sup>+</sup> or CD8<sup>+</sup> cells (as described in methods) and the depleted cell fraction was resuspended to give the final effector:target ratios indicated. Data pooled from four experiments are shown in figure 4. These experiments used cells from normal donors since only a limited number of peripheral blood cells could be obtained from patients. Effector cells which were treated with magnetic beads alone (without antibody treatment beforehand) were capable of lysing antigen pulsed or unpulsed target cells to the same extent as the unmanipulated fraction; thus, use of magnetic beads alone did not itself effect cytolysis.

Depletion of CD4<sup>+</sup> cells from PPD stimulated lymphocytes resulted in a decrease in antigen specific lysis of macrophages compared with unmanipulated or sham depleted fraction ( $p < 0.001$  in each case). The residual killing of antigen pulsed targets by CD4 depleted cells was equivalent to that of unpulsed targets ( $p = 0.940$ ; figure 4).

The effector cell fraction, which was depleted of CD8 bearing cells (and was therefore enriched for CD4<sup>+</sup> cells), showed an enhanced ability to kill mycobacterial antigen bearing macrophage targets ( $p < 0.02$ ).

Discussion

We have shown that the generation of sensitised CD4<sup>+</sup> T cells can lyse autologous macrophages presenting mycobacterial antigen, and is thus an integral part of the immune response to both BCG vaccination and infection with *M tuberculosis*. This extends previous observations by ourselves and others.<sup>16 17 20</sup> Although the patients with tuberculosis had significantly less ability to generate these cytolytic T cells than did BCG vaccinated subjects, the difference was a result of the relatively poor

Table 2 Surface marker analysis of purified protein derivative (PPD) stimulated lymphoblasts and unstimulated pleural fluid populations: individual patient data and control group data

Subject details	Surface marker (% of cells)			
	CD3	CD4	CD8	γ/δ TCR
Normal controls (median (range)) (n = 12)	82 (68-98)	48 (19-80)	49 (27-77)	5 (2-13)
Cavitating pulmonary/lymph node	90	69	14	6
Cavitating pulmonary/lymph node	88	82	7	3
Cavitating pulmonary/lymph node	92	82	17	2
Cavitating pulmonary/lymph node	77	46	26	nd
Cavitating pulmonary/lymph node	95	31	40	nd
Diffuse non-cavitating/miliary	86	38	50	nd
Diffuse non-cavitating/miliary	72	50	30	nd
Pleural fluid	48	53	18	2
Pleural fluid	93	69	19	3
Patient summary (median (range))	88 (48-95)	53 (31-82)	19 (7-50)	3 (2-6)

TCR = T cell receptor, nd = not determined.

Table 3 Effects of the addition of monoclonal anti-MHC class I or class II antibodies on the ability of purified protein derivative (PPD) stimulated effector cells to kill autologous macrophage targets. (Results are shown as mean (SEM) lysis values)

Subjects	No antigen, no antibody	Antigen pulsed				
		No antibody	Anti class I		Anti class II	
			1:200	1:2000	1:400	1:4000
Patients (n = 7)	17.4 (3.3)	41.1 (3.3)	48.0 (2.9)	45.0 (2.9)	30.6 (3.8)	31.5 (4)
Controls (n = 7)	15.0 (2.8)	45.7 (4.7)	51.7 (5.6)	53.2 (5.1)	25.7 (4.1)	37.1 (5.8)

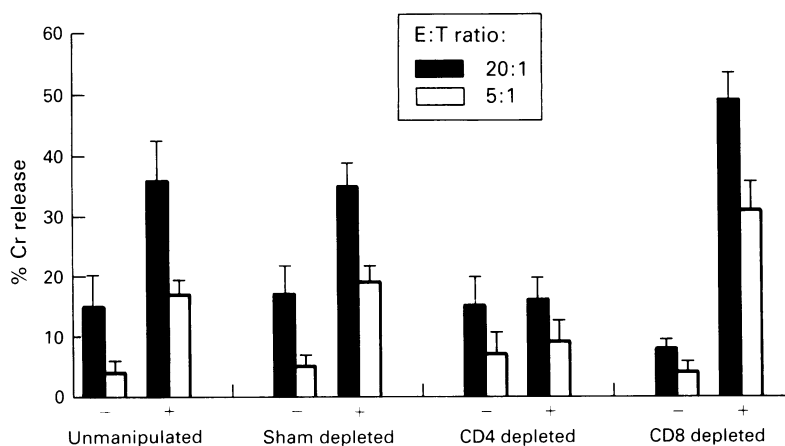


Figure 4 The effect of depleting CD4<sup>+</sup> or CD8<sup>+</sup> lymphoblasts respectively, on the ability of (PPD) stimulated effector cells to kill autologous macrophage targets. Mean (SEM) percentage lysis of PPD pulsed (+) or unpulsed (-) macrophages from four experiments, using Mantoux positive control subjects are shown. The effector populations were: PPD stimulated lymphoblasts used without further manipulation (unmanipulated); sham depleted by incubation with magnetic beads alone; CD4<sup>+</sup> cell depleted, or CD8<sup>+</sup> cell depleted.

cytolytic activity of PPD stimulated lymphocytes from patients with non-cavitary pulmonary disease. In contrast, patients with cavitary disease and those with extrathoracic lymph node disease had an ability to generate cytolytic T cells similar to that of BCG vaccinated subjects. Although only a small number of patients were studied, those with miliary disease seemed to have only a moderate capacity to generate cytolytic T cells.

Approximately 20% of patients with extensive pulmonary tuberculosis and a greater proportion of those with miliary disease are anergic as shown by absent cutaneous tuberculin hypersensitivity and poor in vitro lymphocyte proliferative responses to mycobacterial antigen.<sup>23,24</sup> Most of the patients with poor cytolytic T cell activity described here also showed relatively poor in vitro lymphocyte proliferation (and absent cutaneous hypersensitivity), and were thus anergic. There was, however, only a poor correlation between cytolytic T cell activity and lymphocyte proliferation overall. This finding contrasts with that of Kaleab *et al.*,<sup>18</sup> who found a close correlation between cytolytic T cell activity and lymphocyte proliferation in patients with leprosy and healthy control subjects. Whole BCG was used to stimulate effector cells in their study, however, while we used PPD. Furthermore, we observed that mononuclear cell populations from several of our patients showed spontaneous proliferation in the absence of antigen and this can give a falsely

low lymphocyte proliferation index. Other evidence suggests that proliferation in response to antigen and antigen specific cytotoxicity are essentially unrelated T cell functions.<sup>25</sup> The data presented here show that Interleukin 2 stimulated T cells, which show good proliferative responses, are poorly cytolytic.

We have confirmed previous data<sup>18,20,21</sup> showing that the cytolytic activity of PPD stimulated lymphocyte populations has both antigen specific and antigen non-specific components. We have also shown that in addition to the considerable variation in total cytolytic T cell activity between individuals, the relative contribution of antigen specific and antigen non-specific components to the total cytotoxicity varies considerably between individuals, whether patients or control subjects.

From previous studies of BCG vaccinated subjects and patients with leprosy and tuberculosis there is good evidence that CD4<sup>+</sup> cells restricted by MHC class II antigens are responsible for the cytotoxicity of antigen presenting macrophages.<sup>16-18,20,21</sup> This view is supported by our finding that depletion of CD4<sup>+</sup> but not CD8<sup>+</sup> cells removed the antigen specific component of cytotoxicity. Cytotoxicity of antigen unpulsed macrophages was not significantly affected by CD4<sup>+</sup>/CD8<sup>+</sup> depletion. Antigen non-specific cytotoxicity, however, is inhibited by coculture of macrophages with K562 cells as competing target cells, suggesting that cells with natural killer like activity are responsible.<sup>20</sup> An alternative suggestion is that gamma/delta T cell receptor bearing lymphocytes may be responsible for antigen non-specific cytotoxicity.<sup>26</sup> Gamma/delta T cells show broad cytolytic activity after culture with interleukin 2,<sup>27</sup> proliferate to mycobacterial antigens in both MHC restricted and unrestricted manner,<sup>28,29</sup> and have been identified in granulomatous lesions of human leprosy and leishmaniasis.<sup>30</sup> In this study, however, gamma/delta cells accounted for a small proportion only of the PPD stimulated populations and are therefore unlikely to have contributed greatly to the antigen specific or non-specific cytotoxicity shown. Furthermore, we observed that lymphocyte populations from tuberculous pleural fluid that had not been restimulated in vitro, showed low numbers only of gamma/delta cells.

There has been considerable speculation recently about the role of cytolytic T cells in the immune response to tuberculosis.<sup>26</sup> This results largely from the failure to achieve effective mycobacteriostasis with lymphokine activated human macrophages in vitro.<sup>9,31</sup> In

addition murine cytolytic T cells can inhibit mycobacterial growth independently of lymphokine activated macrophages.<sup>14</sup> Furthermore, both adoptive transfer and selective depletion experiments in mice have shown that CD8<sup>+</sup> cytolytic T cells provide protection against *M. tuberculosis* infection in vivo.<sup>15,32</sup> Mononuclear cells in tuberculous granulomata show functional heterogeneity<sup>33</sup> and tissue macrophages seem to possess significantly less antibacterial capacity than blood monocytes.<sup>34</sup> It has been suggested consequently that cytolytic T cells may have a pivotal role in antimycobacterial immunity by releasing mycobacteria from the safe haven of poorly activated tissue macrophages so that they can be phagocytosed by the more active, blood derived monocytes. Extracellular mycobacteria would also be exposed to toxic macrophage products released from dying macrophages. Some support for this hypothesis is provided by the observation that reconstitution of cellular immunity in lepromatous leprosy lesions by intralesional interleukin 2 is associated with infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> cells. There is subsequent lysis of parasitised macrophages within these lesions followed by rephagocytosis of the released bacilli by freshly migrated monocytes and a concomitant reduction in the bacterial count.<sup>19</sup>

The finding of high levels of mycobacterial-antigen specific, cytolytic T cells in certain groups of patients with tuberculosis strongly suggests that the ability to generate these cells does not equate with protective immunity. There was, however, a strong association of cytolytic activity and tissue destruction. All patients with pulmonary cavitation could generate significant amounts of cytolytic activity and high levels were also found in those with tuberculous lymphadenitis, many of whom formed cold abscesses that required open or closed drainage. It is notable that patients with pneumonic tuberculosis and miliary disease had a reduced capacity to generate mycobacterial-antigen specific, cytolytic T cells. We therefore suggest that in tuberculosis cytolytic T cells may be involved in delayed type hypersensitivity and contribute to tissue destruction.

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