Clin. exp. Immunol. (1992) **89**, 120–125

T cell regulation of the chronic peritoneal neutrophilia during mycobacterial infections

R. APPELBERG *Centro de Citologia Experimental (Instituto Nacional de Investigação Científica) and Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal*

(Accepted for publication 19 February 1992)

SUMMARY

Intraperitoneal infection of mice with mycobacteria induces the persistent mobilization of neutrophils to the infected peritoneal cavities. The recruitment of the neutrophils was mediated by the immune system since it was enhanced by immunization and reduced in T cell-deficient nude and SCID mice. Anti-mitotic treatments with cyclophosphamide or X-rays led to a reduction in the number of mononuclear cells in the peritoneal cavity of infected mice, followed by a reduction in neutrophil numbers despite the presence of a normal circulating pool of neutrophils. The depletion of T cells with antibodies during mycobacterial i.p. infection led to a reduction in the number of neutrophils. Such a reduction was more extensive if the antibodies were administered early. Our data suggest that T cells are partially involved in the direct recruitment of neutrophils during chronic mycobacteriosis but they also play a role in the priming of other cell types for the mobilization of these phagocytes.

Keywords mycobacteria neutrophils T cells macrophages

INTRODUCTION

During infection, neutrophils are the first phagocytes to be recruited to the infectious foci. In the case of extracellular pathogens, these cells will predominate during the course of the infection. During chronic infections by intracellular parasites, monocytes will accumulate at the infectious lesion and will be the predominant type of professional phagocytes found throughout the infection [1]. Recently, we have reported that neutrophils can be detected not only during the acute initial response after the inoculation of mice by mycobacteria but also during the chronic stages of infection [2]. Such long-term accumulation of neutrophils in the infection by these intracellular parasites was shown to be dependent on the activity of the immune system, namely of T cells [3], as well as of host genetic and bacterial virulence factors [4]. Furthermore, antigen-specific T cells capable of being triggered by exposure to mycobacterial antigens to recruit high numbers of neutrophils, are present in the peritoneal cavity of intraperitoneally infected mice [5] and in the spleen of intravenously infected animals [6]. Here we further detail the participation of T cells and other cell types in the persistent recruitment of neutrophils during peritoneal infection by mycobacteria.

Correspondence: Rui Appelberg, Centro de Citologia Experimental da Universidade do Porto, Rua do Campo Alegre 823, 4100 Porto, Portugal.

MATERIALS AND METHODS

Reagents

Bacterial culture media were obtained from Difco (Detroit, MI). Tween-80 and saponin were from Sigma (St Louis, MO), anti-thymocyte serum (ATS) and normal goat serum (NGS) were purchased from Calbiochem (La Jolla, CA) and cyclophosphamide was from Asta Werke (Bielefeld, Germany). Staining of cytospin preparations was performed with the Hemacolor kit from Merck (Darmstadt, Germany).

Bacteria

Mycobacterium bovis BCG (TMCC 1011, strain Pasteur) and *M. avium* (ATCC 25291) were grown in Middlebrook 7H9 medium supplemented with 0·04% of Tween-80. Inocula were prepared from mid-log phase cultures by pelleting the bacteria, resuspending in a small volume of saline with 0·04% Tween-80, briefly sonicating the bacterial suspension and freezing at −70°C until use [7].

Animals

Female C57BL/6 mice were purchased from the Gulbenkian Institute (Oeiras, Portugal). They were housed under standard hygiene conditions and given commercial chow and acidified water *ad libitum*. Mice were used when they were 8–12 weeks old. Euthymic C57BL/6-nu/+, athymic C57BL/6-nu/nu nudes, severe combined immunodeficiency (SCID) mutants and CB-17 mice were purchased from Bommice (Ry, Denmark) and were

kept in sterile cages, given sterile food and water, and manipulated in laminar flow hoods under aseptic conditions.

Study of the inflammatory response to the i.p. infection

Mice were inoculated intraperitoneally with 2×10^6 colony-forming units (CFU) of BCG or 10^8 CFU of *M. avium* in 0·5 ml of saline–0.04% Tween-80. At different time intervals, mice were killed by ether anaesthesia and their peritoneal cavity was washed with 4 ml of PBS. An aliquot of the washout fluid was collected with a syringe fitted with a 32-G needle and the cellular concentration was determined with an automatic cell counter. Cytospin preparations were made using a Shandon II cytocentrifuge and stained with the Hemacolor staining kit. Differential cell counts were then made and the total number of the different cells present in the exudate was calculated. An aliquot from each exudate was lysed with 0·1% saponin, serially diluted in a 0·04% Tween-80 solution and plated onto Middlebrook 7H10 agar medium for bacterial viable counts. Four mice were studied per time-point and the results are expressed as the mean $\pm$ 1 s.d. Data were analysed using Student's *t*-test.

In some experiments, mice were given different treatments:

(i) One group of mice was immunized with 2×10^6 CFU of BCG Pasteur intravenously 6 months prior to the study of the i.p. infection;

(ii) Cyclophosphamide-treated mice weighed about 30 g, received a fixed dosage of a single dose of 3 mg of cyclophosphamide intravenously at different periods of time, and were then killed.

(iii) Groups of mice were irradiated with 7·5 Gy with an X-ray source prior to the study of the peritoneal cellular exudate. Bone marrow was reconstituted in some irradiated mice by an i.v. infusion of isolated syngeneic bone marrow cells. In other experiments, bone marrow was protected in one hind-limb by using a lead shield during the irradiation procedure.

(iv) ATS or NGS was administered at different times of infection (0·1–0·3 ml per dose through the lateral tail vein or by the intraperitoneal route).

Flow cytometry

Spleen cells were collected by gently mincing the spleens. After washing the cells, they were stained with one of the following FITC-conjugated antibodies: goat anti-mouse IgM (Southern Biotechnology, Birmingham, AL) or rat anti-mouse Thy1.2 (Becton Dickinson, Erembodengen, Belgium). Flow cytometric analysis of single stained cells was performed in a Coulter FACS.

RESULTS

BCG infection induces peritoneal neutrophilia

C57BL/6 mice were inoculated intraperitoneally with 2×10^6 CFU of BCG Pasteur and the peritoneal exudates were collected at different time intervals during the infection. The number of viable bacteria decreased steadily from the start of the infection, until very low numbers were detected at 60 days post-infection (Fig. 1). The decrease in bacterial numbers was due mainly to bacterial clearance rather than killing as judged from the colonization of the spleens and livers (not shown). The number of phagocytes in the peritoneal cavity of those mice is shown in Fig. 2. Macrophages predominated throughout the infection and their accumulation showed a biphasic response. In the first 2 days there was a slight decrease in the number of the resident macrophages followed by an increase in macrophage numbers, which attained a maximum during week 2 of infection. Neutrophils were seldom found in undisturbed peritoneal cavities ($\leqslant 10^5$ per cavity). The accumulation of neutrophils following the inoculation of the mycobacteria was biphasic with an acute influx in the first 8 h followed by a later and persistent influx peaking around week 2 of infection. The number of eosinophils tended to decrease after a slight increase over the resident population in the first 6 h. In the subsequent experiments we analysed the regulation of the second phase of neutrophil accumulation.

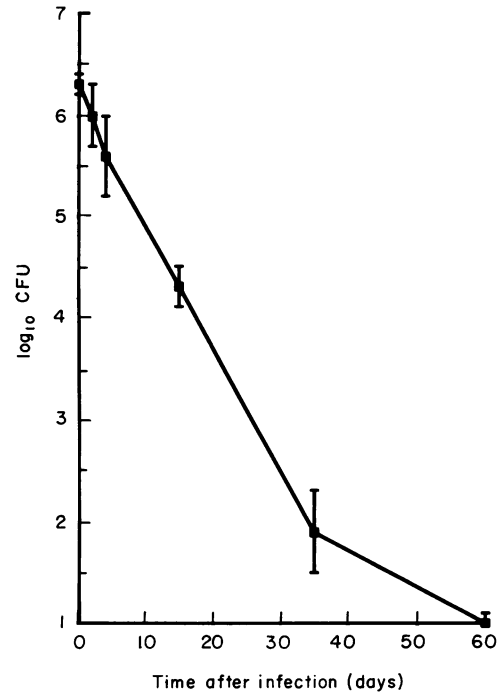

Fig. 1. Number of viable BCG in the peritoneal cavity of intraperitoneally infected mice at different time intervals after inoculation. Each time-point represents the geometric mean $\pm$ 1 s.d. of colony-forming units (CFU) from four mice.

Vaccination enhances the magnitude of neutrophil accumulation

The number of neutrophils accumulating at the peak of the neutrophilic response (day 14) to the BCG infection was compared in normal and immunized mice. As shown in Fig. 3, mice that had been immunized intravenously 6 months previously with BCG responded with a significantly ($P < 0{\cdot}01$) greater influx of neutrophils to the i.p. infection than did control mice.

Nude and SCID mice show a reduced accumulation of neutrophils

The number of neutrophils accumulating in the peritoneal cavities of BCG-infected mice was compared in euthymic and athymic mice. As shown in Table 1, control and nude mice had comparable numbers of resident macrophage and lymphoid cells and the number of neutrophils was low, showing no significant differences in those populations. The influx of neutrophils measured after 7 and 14 days of infection with BCG was reduced in nude mice compared with euthymic animals

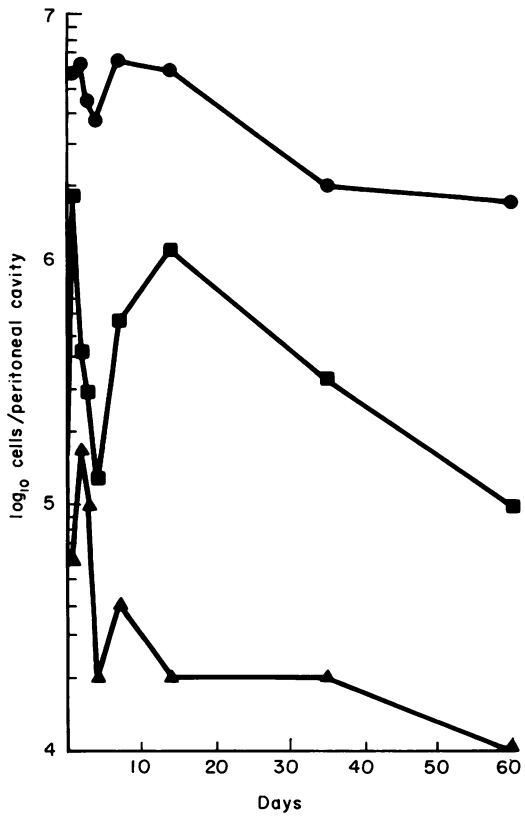

Fig. 2. Number of phagocytic cells per peritoneal cavity of C57BL/6 mice inoculated intraperitoneally with 2×10^6 colony-forming units (CFU) of *M. bovis* BCG, during 60 days of infection. The values for macrophages (●), neutrophils (■) and eosinophils (▲) represent the arithmetic mean of the cell numbers of four animals per time-point.

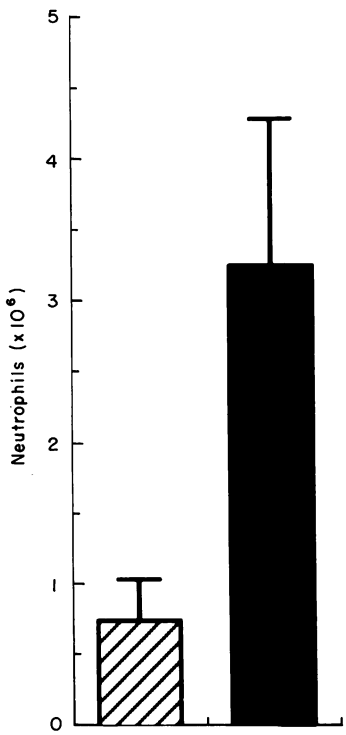

Fig. 3. Number of neutrophils per peritoneal cavity of control (■) or vaccinated C57BL/6 mice (■) 14 days after the i.p. inoculation of 2×10^6 colony-forming units (CFU) of BCG. Mice were vaccinated by i.v. inoculation of 2×10^6 CFU of BCG 6 months prior to the study. At that time they had cleared most of the vaccination inoculum. The neutrophil counts are statistically different ($P < 0.01$).

(significant results at day 14, $P < 0.01$) and the number of lymphoid cells increased more in control than in nude mice (significant results at day 14, $P < 0.01$) (Table 1). Macrophage numbers tended to be similar in both mouse strains (no statistical differences were found). These results confirmed earlier data obtained with *M. avium* infections [3]. In another set of experiments, SCID and control CB-17 mice were infected intraperitoneally with 10^8 CFU of *M. avium* and the cellular composition of the exudates was studied after 30 days of infection and compared with the cellular composition of undisturbed peritoneal cavities. As shown in Table 1, normal or infected SCID mice had very low numbers of lymphoid cells in the peritoneal cavity. The number of macrophages and lymphoid cells was significantly lower in SCID as compared with CB-17 controls ($P < 0.05$ in uninfected mice and $P < 0.01$ in infected mice). Flow cytometric analysis of the spleen cell population was performed on each individual mouse and only in one case revealed a significant number of Thy1.2$^+$ and sIgM$^+$ in a SCID mutant that was thus discarded from the study as being of a 'leaky' phenotype (results not shown). Very few or undetectable numbers of neutrophils were found in the peritoneal cavities of uninfected animals of both strains and no significant differences were found. After 30 days of infection with *M. avium*, neutrophils were found in considerable numbers in immunocompetent CB-17 mice but were detected in significantly ($P < 0.05$) lower numbers in SCID mice (Table 1).

Table 1. Number of macrophages, lymphocytes and neutrophils ($\times 10^6$) in the peritoneal cavity of uninfected mice or mice infected intraperitoneally with either 2×10^6 CFU of *M. bovis* BCG or with 10^8 CFU of *M. avium*. Mice were euthymic C57BL/6 (nu/+), athymic C57BL/6 nudes (nu/nu), CB-17, and SCID mutants of the CB-17 background (SCID)

Infection	Strain	Macrophages	Lymphocytes	Neutrophils
None	nu/nu	5·33±0·82	0·75±0·18	0·05±0·04
	nu/+	4·93±0·79	0·92±0·23	0·06±0·08
BCG, day 7	nu/nu	2·30±0·38	2·40±0·31	0·07±0·04
	nu/+	3·34±0·74	4·08±1·20	0·23±0·19
BCG, day 14	nu/nu	4·16±1·69	2·71±1·13	0·02±0·02
	nu/+	3·23±0·57	7·07±1·63	0·44±0·15
None	CB-17	2·98±0·39	2·83±1·18	0·02±0·01
	SCID	1·91±0·11	0·06±0·02	0·03±0·01
M. avium, day 30	CB-17	2·39±0·55	1·91±0·39	1·18±0·65
	SCID	0·49±0·08	0·04±0·00	0·25±0·06

Arithmetic mean ± 1 s.d. of the cell numbers of four mice.

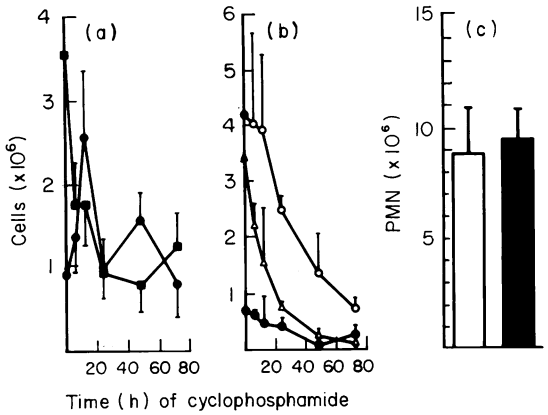

Fig. 4. Number of cells/ml of peripheral blood (a) and cells/peritoneal cavity (b, c) of C57BL/6 mice infected intraperitoneally for 14 days with 2×10^6 colony-forming units (CFU) of BCG. Cyclophosphamide (3 mg) was administered intravenously to infected animals at the indicated time intervals before peritoneal exudate harvest. (a) Number of mononuclear cells (squares) and neutrophils (circles) in the peripheral blood of infected animals; (b) number of macrophages (open circles), lymphocytes (open triangles), and neutrophils (closed circles) per peritoneal cavity of infected animals; (c) number of neutrophils per peritoneal cavity of mice infected for 14 days with BCG and treated for 8 h with 1 ml of 4% thioglycollate broth; one group of animals was given 3 mg of cyclophosphamide 48 h before being killed (■).

Cyclophosphamide and ionizing radiation reduce neutrophil accumulation

To evaluate the susceptibility of the peritoneal mechanisms responsible for the recruitment of neutrophils to anti-mitotic treatments, the effects of cyclophosphamide or X-ray irradiation were studied in animals infected intraperitoneally for 14 days with BCG and given the treatment up to 72 h before being killed. As shown by McGregor & Koster [8], cyclophosphamide is able to reduce the recruitment of neutrophils, through its effects on myelopoiesis, to peritoneal exudates induced by phlogistic agents after only 3 days of administration. Before that period of time, post-mitotic differentiation of neutrophil precursors in the bone marrow could deliver enough cells to enter acute inflammatory sites [8]. As shown in Fig. 4a, a single dose of cyclophosphamide was able to reduce the number of blood mononuclear cells significantly if given from 6 to 72 h before killing the animal. There was no reduction in the circulating numbers of neutrophils (Fig. 4a); at 12 h after drug administration the number of neutrophils was significantly higher than in untreated animals ($P < 0.05$). Treatment with cyclophosphamide induced a reduction in the numbers at day 14 of BCG infection of peritoneal macrophages and lymphocytes (significantly reduced at 24 h and after) and of neutrophils (significantly reduced at 48 and 72 h) (Fig. 4b). Figure 4c shows that BCG-infected animals given cyclophosphamide 48 h before cell harvest were able to recruit neutrophils to the same extent as infected but untreated controls after i.p. inoculation of 1 ml of thioglycollate broth. Furthermore, BCG-infected mice that had been irradiated with 750 cGy of X-rays showed a decrease in neutrophil number even if their bone marrow had been protected during or reconstituted after the irradiation (Table 2). In the two experiments, irradiated animals of both groups showed significantly reduced numbers of macrophages, lymphocytes and neutrophils.

Table 2. Number of macrophages, lymphocytes, and neutrophils ($\times 10^6$) in the peritoneal cavity of C57BL/6 mice infected for 14 days with 2×10^6 CFU of *M. bovis* BCG. One group of animals was irradiated by an X-ray source (7·5 Gy) 2 days before the study of the peritoneal exudate. Another group of animals was similarly irradiated but treated so as to maintain myelopoiesis (+BM) either by transfusing 50×10^6 bone marrow cells immediately after irradiation in experiment 1 or by protecting one leg during the irradiation procedure in experiment 2

	Macrophages	Lymphocytes	Neutrophils
Control			
Exp. 1	4·04±0·25	3·20±0·39	0·85±0·17
Exp. 2	3·92±0·36	4·21±0·99	0·94±0·21
7·5 Gy			
Exp. 1	1·67±0·38	0·22±0·07	0·38±0·22
Exp. 2	1·02±0·31	1·19±0·45	0·58±0·10
7·5 Gy+BM	1·46±0·29 Exp. 1	0·28±0·10 Exp. 1	0·49±0·10 Exp. 1
	1·45±0·28 Exp. 2	0·81±0·21 Exp. 2	0·25±0·08 Exp. 2

Anti-T cell treatments reduce neutrophil accumulation during BCG infection if given from the start of the infection

ATS was used to cause T cell depletion. We had shown in the previous experiment that the lysis or clearance of the neutrophil-recruiting cells 48 h before analysis of the peritoneal exudate led already to diminished neutrophil counts. In order to determine whether the T cells are involved in the direct recruitment of neutrophils and whether they are necessary to prime other cell types for such recruitment, BCG-infected mice were given ATS according to two protocols. In the first protocol, antibodies were given at the beginning of the infection, and in the second protocol the anti-T cell antibodies were administered 48 h prior to peritoneal exudate analysis. Mice inoculated with ATS from day 0 of infection showed a significant reduction in the numbers of peritoneal neutrophils compared with mice given NGS under a similar scheme (Table 3). In contrast, mice given ATS 48 h before being killed showed a lower reduction in neutrophil influx, compared with NGS-treated controls (Table 3). Similar results were obtained studying *M. avium* infection (Table 3). The extent of reduction in neutrophil influx was closely linked to the degree of reduction in peritoneal lymphoid cells achieved with the treatments. Although some of the results were not statistically significant, due to high variability of the results, the reductions in neutrophil accumulation were consistent in all experiments.

When ATS was given at different times of BCG infection, it could be observed that only ATS given at time 0 was sufficient to abrogate the neutrophilic response (Fig. 5a). Afterwards, ATS induced only a partial reduction in neutrophil recruitment (Fig. 5a). Again, the reduction in neutrophil numbers was closely linked to the degree of lymphocyte depletion obtained. To test whether these latter effects were not due to an inability of the serum to reach the peritoneal cavity, we tested the effect of i.p. administration of the serum during infection. Both treatments

Table 3. Number of neutrophils ($\times 10^6$) per peritoneal cavity of C57BL/6 mice infected intraperitoneally for 14 days with 10^6 CFU of *M. bovis* BCG or 2×10^8 CFU of *M. avium*. Animals received either anti-thymocyte serum (ATS) or goat non-immune serum (NGS) intravenously in the amounts of 0·3 ml (*), 0·1 ml (**), or 0·5 ml (***) per dose. Also shown are the numbers of lymphocytes in ATS-treated mice in percentage (%) of control, NGS-treated mice

Day of serum administration and infection	ATS	NGS	%
Days 0, 5 & 10			
BCG (*)	0·03±0·03†	0·45±0·31	12
BCG (**)	0·38±0·20	0·70±0·34	70
M. avium (*)	0·32±0·16†	1·66±0·31	47
Day 12			
BCG (*)	0·57±0·07	0·70±0·01	44
BCG (*)	0·50±0·09*	0·85±0·20	62
M. avium (***)	1·21±0·22	1·76±0·09	100

*$P = < 0{\cdot}01$.
†$P = < 0{\cdot}05$.

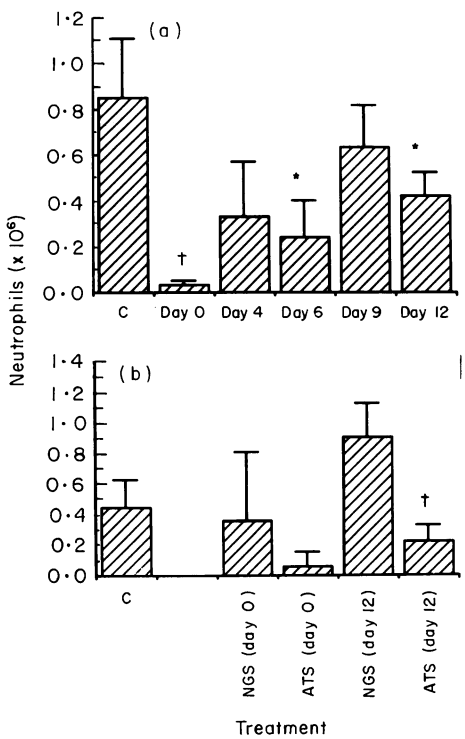

Fig. 5. Number of neutrophils per peritoneal cavity of C57BL/6 mice infected intraperitoneally for 14 days with 2×10^6 colony-forming units (CFU) of BCG. (a) Mice were treated intravenously with 0·3 ml of anti-thymocyte serum (ATS) at the times of infection shown and compared with untreated infected controls (C); the number of peritoneal lymphoid cells was reduced relative to the controls to 36% (day 0); 42% (day 4); 22% (day 6); 52% (day 9); and 31% (day 12) after administration of serum; (b) mice were treated intraperitoneally with ATS or normal goat serum (NGS) at either day 12 or at day 0 and compared with untreated infected controls (C); the numbers of peritoneal lymphoid cells were 42% of the control for the administration at 12 days and 150% for the administration at day 0. Statistical comparisons were made between ATS-treated *versus* untreated mice in (a) and between mice given ATS and mice given NGS in (b): *$P < 0{\cdot}05$; and †$P < 0{\cdot}01$.

led to a reduction in neutrophil influx, confirming the results obtained with the intravenously infused serum (Fig. 5b). However, the late administration of serum by i.p. (local) route was slightly more effective than by the i.v. (systemic) route. The lack of significance of the reduction observed with the early administration was associated with an increase in lymphocyte numbers relative to the controls.

DISCUSSION

Here we extended previous findings regarding the *M. avium*-induced peritoneal neutrophilia [3] by showing that another mycobacterium, *M. bovis* BCG, was also able to induce a persistent T cell-dependent accumulation of neutrophils in the peritoneal cavity of infected mice. We further documented the involvement of T cells in *M. avium*-induced neutrophilia by studying SCID mice. We showed that after the acute inflammatory response taking place during the first 24–48 h and characterized by an early recruitment of neutrophils peaking at 8 h post-inoculation, there was a second phase of neutrophil accumulation in the peritoneal cavity of mice infected with both mycobacteria which was dependent on the presence of T cells and enhanced by previous immunization of the challenged animals. This chronic neutrophilic response occurred when the bacteria were found inside macrophages in the peritoneal cavity. These facts suggest that neutrophils were recruited by immune rather than for non-specific mechanisms (e.g. complement activation).

We have already shown that immune T cells can attract neutrophils after stimulation with antigen [4–6]. Some of these cells may be proliferating, since they showed partial sensitivity to cyclophosphamide [5]. When the sensitivity of the cells responsible for the recruitment of neutrophils was studied when there was no depression in the circulating pool of neutrophils, we found that the treatment of BCG-infected mice with cyclophosphamide led to a reduction in the mononuclear cells (of the lymphoid and the mononuclear phagocyte lineages) in the peritoneal cavity, followed by a decrease in neutrophil influx. Whereas lymphocytes may be sensitive to anti-mitotic drugs when they are proliferating, macrophages are generally considered to be resting cells. We postulate that the killing of lymphocytes by the drug led to the stimulation of macrophage clearance from the peritoneum after phagocytosis of the dead cells. In any case, the decrease in both macrophage and lymphocyte number was associated with a decrease in neutrophil accumulation. This decrease in neutrophil numbers followed a delayed course compared with the one of the mononuclear cells suggesting a causal relationship between the two phenomena. We hypothesize that the elimination of the peritoneal mononuclear cells was linked to a decrease in the cells responsible for neutrophil recruitment.

We have shown here that the depletion of T cells during the entire course of the infection caused a complete abrogation in the chronic peritoneal neutrophilia induced by the mycobacterial infection. These results mimicked those obtained in the experiments using nude and SCID mice. However, the late administration of this anti-T cell treatment caused a smaller reduction in the neutrophilic response. In fact, when ATS was administered at different time intervals during infection, the most effective administration was the one taking place at time 0 of infection. From that time-point onwards, neutrophil counts

increased. These experiments suggest that T cells are essential for neutrophil recruitment. However, since the late depletion of T cells had a smaller effect on the recruitment of neutrophils, we conclude that the T cells are both directly involved in the recruitment of neutrophils and in the early priming of another cell type for the recruitment of neutrophils.

The treatment with cyclophosphamide differed in an important aspect from the treatment with ATS. Cyclophosphamide was able to promote a more pronounced depletion of neutrophils than ATS, when given 2 days before the analysis of the exudate. This could be explained by the observation that cyclophosphamide induced the disappearance of macrophages in addition to that of lymphocytes thus strengthening the notion that primed macrophages are also partly involved in the recruitment of neutrophils.

The role played by the neutrophils found during the chronic stages of mycobacterial infection is still not clear, since they are not engaged in phagocytosis. We have postulated that neutrophil molecules may enhance the anti-mycobacterial activity of macrophages after their uptake by these latter phagocytes [2]. We showed that neutrophil lysates could enhance *in vitro* the anti-mycobacterial activity of peritoneal macrophages infected with *M. microti* and *M. avium* [2]. Others have postulated different roles for neutrophils during infections by intracellular parasites, such as dissolution of listeria-infected hepatocytes [9] and phagocytosis of mycobacteria following their release from macrophages lysed by cytotoxic T lymphocytes [10].

Our results show that the chronic accumulation of neutrophils in the peritoneal cavity of mice infected with mycobacteria is dependent on T cells, strengthening the developing notion that T cells regulate neutrophil chemotaxis and function [11–17]. We postulate that T cells may directly attract neutrophils and that they also act by priming other cell types such as the macrophage to exhibit an enhanced ability to recruit neutrophils via the secretion of neutrophil chemotactic molecules.

ACKNOWLEDGMENTS

I am indebted to Dr M. T. Silva for helpful discussions, to J. Pedrosa for skillful technical assistance and to Dr F. Gonçalves for assisting in the flow cytometric analysis. This work was supported by grants from the I.N.I.C. and J.N.I.C.T. (Lisbon) and the Damien Foundation (Brussels).

REFERENCES

1 Edwards D, Kirkpatrick CH. The immunology of mycobacterial diseases. Am Rev Respir Dis 1986;**134**:1062–71.

2 Silva MT, Silva MNT, Appelberg R. Neutrophil-macrophage cooperation in the host defense against mycobacterial infections. Microbiol Pathog 1989; **6**:369–80.

3 Appelberg R, Silva MT. T-cell dependent chronic neutrophilia during mycobacterial infections. Clin Exp Immunol 1989; **78**:478–83.

4 Appelberg R, Pedrosa J, Silva MT. Host and bacterial factors control the *Mycobacterium avium*-induced chronic peritoneal granulocytosis in mice. Clin Exp Immunol 1991; **83**:231–6.

5 Appelberg R. $CD4^+$ T cells are required for antigen-specific recruitment of neutrophils by BCG-immune spleen cells. Immunology 1992; **75**:414–19.

6 Appelberg R. Mycobacterial infection primes T cells and macrophages for enhanced recruitment of neutrophils. J Leukocyte Biol 1992; **51**: in press.

7 Appelberg R, Sarmento AM. The role of macrophage activation and of *Bcg*-encoded macrophage function(s) in the control of *Mycobacterium avium* infection in mice. Clin Exp Immunol 1990; **80**:324–33.

8 McGregor DD, Koster FT. The mediator of cellular immunity. IV. Cooperation between lymphocytes and mononuclear phagocytes. Cell Immunol 1971; **2**:317–25.

9 Conlan JW, North RJ. Neutrophil-mediated dissolution of infected host cells as a defense strategy against a facultative intracellular bacterium. J Exp Med 1991; **174**:741–4.

10 Geertsma MF, Nibbering PH, Pos O, van Furth R. Interferon-γ-activated human granulocytes kill ingested *Mycobacterium fortuitum* more efficiently than normal granulocytes. Eur J Immunol 1990; **20**:869–73.

11 Campbel PA. The neutrophil, a professional killer of bacteria, may be controlled by T cells. Clin Exp Immunol 1990; **79**:141–3.

12 Steinbeck MJ, Roth JA. Neutrophil activation by recombinant cytokines. Rev Infect Dis 1989; **11**:549–68.

13 Czuprinsky CJ, Henson PM, Campbell PA. Enhanced accumulation of inflammatory neutrophils and macrophages mediated by transfer of T cells from mice immunized with *Listeria monocytogenes*. J Immunol 1985; **134**:3449–54.

14 Czuprinsky CJ, Brown JF. Dual regulation of anti-bacterial resistance and inflammatory neutrophil and macrophage accumulation by $L3T4^+$ and $Lyt2^+$ listeria-immune T cells. Immunology 1987; **60**:287–93.

15 Fong TAT, Mosmann TR. The role of IFNγ in delayed-type hypersensitivity mediated by Th1 clones. J Immunol 1989; **143**:2887–93.

16 Klasen IS, van der Kwast TH, Donselaar IG, *et al.* Flare-up of delayed-type hypersensitivity initially induced by murine cloned helper T cells. Cell Immunol 1987; **108**:235–41.

17 Ferrante A, Mocatta TJ. Human neutrophils require activation by mononuclear leucocyte conditioned medium to kill pathogenic free-living amoeba, *Naegleria fowleri*. Clin Exp Immunol 1984; **56**: 559–66.