Br. J. exp. Path. (1985) **66**, 257–270

Modifications of host defence mechanisms by an acute non-immunological inflammatory reaction

I. Florentin, J. Bird, Y. Le Garrec, V. Chung and J. P. Giroud

Institut de Cancérologie et d'Immunogénétique, Hopital Paul Brousse, Villejuif, Department de Pharmacologie, Pavillon Gustave Roussy, CHU Cochin-Port Royal, Paris and Laboratoire d'Immunothérapie Expérimentale, Institut Pasteur, Paris, France

Received for publication 29 May 1984

Summary. Mice developing an acute non-immunological inflammatory reaction were examined for modification of specific and non-specific defence mechanisms on the basis of previous observations that these animals displayed an increased resistance to bacterial and parasitic infections but an impaired resistance to neoplasia. Local acute inflammation was induced by injection into the pleural cavity of a non-antigenic, endotoxin-free irritant—calcium pyrophosphate microcrystals or low-molecular-weight dextran. Effector functions of macrophages at remote sites from the inflammatory focus were markedly stimulated. This was shown by: (a) an accelerated elimination of *Listeria monocytogenes* in the liver and spleen of mice with inflammation; (b) the acquisition of cytostatic activity for tumour cells by peritoneal macrophages; and (c) an enhancement of chemiluminescence emission and superoxide production in response to phagocytosis. Natural killer activity of spleen and peritoneal cells was stimulated in a biphasic manner. In contrast, cytolytic T cell differentiation upon *in vitro* immunization of spleen cells against allogeneic tumour cells was impaired. All these effects were observed very early (2 h) after the onset of inflammation and were still detectable at least 3 days after the inflammatory process had disappeared.

Keywords: host defence mechanisms, inflammation

In previous studies, we showed that mice developing an acute non-immunological inflammatory reaction induced by the injection of a non-antigenic irritant into the pleural cavity, have an increased resistance to infection. Protection against *Klebsiella pneumoniae* (Giroud *et al.* 1979) and *Trypanosoma cruzi* (Giroud *et al.* 1981*b*) and delayed mortality after acute *Candida albicans* infection (Drouhet *et al.* 1981) were observed when the inflammatory stimulus was applied before the inoculation of micro-organisms. In addition, these protective effects have been reproduced by injecting inflammatory serum into normal mice, 24 h before bacterial and parasitic challenge. On the other hand, the inflammatory process was able to influence primary tumour growth and metastatic development. Depending on the tumour model used and the time of application of the phlogistic stimulus with respect to tumour grafting, inhibition of facilitation of tumour progression was observed (Nolibe *et al.* 1980).

Correspondence: I. Florentin, Insitut de Cancérologie et d'Immunogénétique, Hopital Paul Brousse, 14 av. Paul Vaillant-Conturier, 94804 Villejuif Cedex, France.

The aim of the present work was to investigate whether this acute non-immunological inflammation interferes with the expression of specific and non-specific defence mechanisms in such a way that explains the modulation of host resistance. For this purpose, inflammation was induced in mice by an intrapleural injection of calcium pyrophosphate microcrystals or low-molecular-weight dextran. The inflammatory reaction, which is dominated by polymorphonuclear leucocytes (PMNs), reached its peak after 6 h and disappeared within 48 h (Willoughby *et al.* 1975).

Initially, we looked at modifications of macrophage effector functions at remote sites from the inflammatory focus. The evolution of bacterial growth in the spleen and liver of inflamed mice has been studied after *Listeria monocytogenes* infection since it can be considered as a good *in vivo* correlate of macrophage activation. Peritoneal macrophages were examined for their *in vitro* cytostatic activity for tumour cells which is also taken as a criteria of macrophage activation (North 1978). In addition, the chemiluminescence of peritoneal macrophages in response to opsonized zymosan particles was measured and we tried to correlate this activity with the release of superoxide anions as reactive oxygen metabolites are, at least in part, implicated in the bactericidal and tumoricidal activities of activated macrophages (Murray *et al.* 1979; Nathan *et al.* 1979).

Besides macrophages, natural killer (NK) cells are believed to play a role in non-specific defences against neoplasia and infections (Herberman 1982). Spleen and peritoneal cells from animals with inflammation were examined, therefore, for their NK activity against the highly sensitive YAC-1 lymphoma target cells.

In order to determine the effect of inflammation on specific immune responses, we looked at the differentiation of cytolytic T lymphocytes (CTL) during *in vitro* immunization of spleen cells from mice with inflammation against allogeneic tumour cells.

All the results obtained demonstrated that the most simple and short (48 h) inflammatory process, free of bacteria and endotoxin, was able to markedly affect host defence mechanisms against infection and neoplasia, long after the inflammatory process had disappeared.

Material and methods

Animals. Two-month-old pathogen-free DBA/2 and C57Bl/6 mice (Iffa Credo, l'Arbresle, France) were used.

Irritants. Calcium pyrophosphate (CaPP) microcrystals were kindly provided by J.L. Savio and R. Legros, Université Paul Sabatier, Toulouse.

Dextran, molecular weight 40 000, was obtained from Sigma Chemical Company, St Louis, USA.

Induction of the acute inflammatory reaction. Mice were injected intrapleurally, under aseptic conditions, with 0.7 ml of either a 1% suspension of calcium pyrophosphate microcrystals or 0.5 ml of a 12% solution of dextran, in pyrogen-free saline. The absence of pyrogens was verified by the Limulus assay (Industrie Biologique Française, Aubervilliers, France). Control animals were untreated since intrapleural injections of pyrogen-free balanced salt solution are known to be able to induce slight acute inflammatory reactions (Sultan *et al.* 1978).

Measurement of Listeria monocytogenes multiplication in vivo. Listeria monocytogenes, strain EGD, originally obtained from G.B. Mackaness, was stored frozen after a log-phase culture in trypticase Soy broth and diluted in 0.9% NaCl solution before inoculation.

Bacterial growth was monitored in the liver and spleen of four mice in each group at various times after bacterial challenge. The number of viable bacteria was determined by plating 10-fold dilutions of organ homogenates in saline on nutrient agar, and colony count was performed after 20 h incubation at 37°C.

A protection index (PI) was defined as the difference between the number (Log_{10}) of bacteria in inflamed and in control mice.

Measurement of chemiluminescence and superoxide production of peritoneal macrophages. Peritoneal cells were obtained from controls or mice with inflammation by washing the peritoneal cavity with 2 × 4 ml of cold phosphate-buffered saline (PBS). After washing twice, the cells were resuspended in phenol red-free Hanks balanced salt solution (HBSS) as described below.

Chemiluminescence was measured with a Packard Picolite luminometer using opsonized zymosan as the stimulus.

Opsonized zymosan was prepared by incubating 60 mg of zymosan (Sigma, USA) in 15 ml of 10% freshly prepared normal mouse serum diluted in PBS for 30 min at 37°C. After washing, the particles were resuspended in PBS and used at a concentration of 15 mg/ml.

Chemiluminescence was evaluated by introducing 150 μl of cell suspension (5×10^6 cells) into a 6 × 50 mm borosilicate tissue culture tube and placing it into the machine where it was maintained at 37°C in the dark. After equilibriation, 20 μl of luminol (Sigma, USA) solution was added to give a final concentration of 5×10^{-6} M. When background light emission became constant, 150 μl of the zymosan suspension was added and the photo-emission was recorded continuously for 20 min.

The production of superoxide by macrophages, when stimulated with opsonized zymosan, was assayed using a modification of the method described by Johnson *et al.* (1975).

Peritoneal cells (2.5×10^6 in 0.5 ml) were pre-incubated for 5 min at 37°C in HBSS with or without 200 μg of superoxide dismutase (SOD type I Sigma, USA) in a total volume of 1.15 ml. Aliquots (0.25 ml) of ferricytochrome C, 0.5 mM (type III, Sigma, USA) and 0·1 ml of opsonized zymosan (10 mg/ml), were added to start the reaction. After incubation for 10 min at 37°C, the reaction was stopped by immersing the tubes in an ice-bath. After centrifugation at 900 *g* for 5 min the absorbance of the supernatants at 550 nm was determined in a spectrophotometer. The results of quadruplicate tubes were averaged and converted to nanomoles of cytochrome C reduced, using the extinction coefficient:

$$E_{550\ nm} = 2.1 \times 10^4/M/cm.$$

Tubes containing SOD served as blanks. Results are expressed as the amount of superoxide released in response to zymosan minus the resting level of unstimulated cells.

Assay for macrophage cytostatic activity. Peritoneal cells were obtained by lavage of the peritoneal cavity with 4 ml HBSS. Washings from three mice in each group were pooled and centrifuged. Cells were resuspended in serum-free RPMI 1640 culture medium at a concentration of 2×10^6/ml and 200-μl aliquots were distributed in flat-bottomed microplates (No. 3040, Falcon Plastics, Oxnard, USA). After 2 h of incubation at 37°C in a 5% CO_2 in air atmosphere, the non-adherent cells were removed by three washes with culture medium and 2×10^4 L1210 leukaemic cells were added in 200 μl serum-free RPMI culture medium to the macrophage monolayers. Cultures were incubated for 24 h in the presence of 1 μCi [^{3}H]-methyl thymidine per well (^{3}H-TdR, specific activity 25 Ci/mmol, CEA, France) for the last 4 h of incubation. Tumour cells were collected on glass-fibre filters with a multiple automated sample harvester (MASH II, Microbiological Associates) and ^{3}H-TdR incorporation was measured in a liquid scintillation spectrometer (Rack Beta, LKB Instruments, Sweden).

Results were expressed as mean counts per minute (ct/min) ± standard error of the mean (SEM) from 12 replicate cultures.

Natural killer (NK) cytotoxicity assay. Spleen and peritoneal cells from five mice in each group were pooled and tested for their cytotoxic activity against YAC-1 tumour cell-

line. Varying numbers of effector cells were added to 1×10^4 ^{51}Cr-labelled YAC-1 tumour cells in the wells of round-bottomed microplates (Microtest plates, Nunclon, Denmark) in 200 µl of RPMI 1640 culture medium supplemented with 10% fetal calf serum (FCS, Gibco). The effector to target cell (E:T) ratios were 100:1, 50:1 and 25:1. After 4 h of incubation at 37°C, in a 5% CO_2 in air atmosphere, 100 µl of supernatant were collected in each well and counted in a gamma-counter (Rack Gamma, LKB Instruments).

Percentage cytotoxicity was determined by the formula:

$$\frac{E-S}{T-S} \times 100$$

where E is the ^{51}Cr released from the target cells in the presence of effector cells, S is the spontaneous ^{51}Cr release observed with unlabelled target cells in place of effector cells and T is the total amount of ^{51}Cr incorporated into the target cells. The mean percent cytotoxicity ±SEM was calculated from quadruplicate cultures.

In vitro generation of, and assay for cytotoxic T lymphocytes (CTL). Spleens from four mice in each group were pooled and prepared as single cell suspensions in RPMI 1640 culture medium supplemented with 10% FCS, 5×10^{-5} M 2-mercaptoethanol and with antibiotics. Spleen cells (5×10^6) were cultivated alone or with 6.25×10^4 mitomycin C-treated allogeneic P815 tumour cells in 1 ml of complete medium, into a 24-well microplate (No. 3037, Falcon Pastics). After 5 days of incubation at 37°C in a 5% CO_2 in air atmosphere, spleen cells were collected, washed once and their viability assessed by trypan blue exclusion.

CTL activity was measured by mixing varying numbers of viable cultivated spleen cells with 1×10^4 ^{51}Cr-labelled P815 or YAC-tumour cells as specific and non-specific target cells respectively, in 200 µl in wells of Microtest plates (Nunclon). The E:T ratios were 25:1, 12:1 and 6:1. After 4 h incubation at 37°C, the amount of ^{51}Cr released into 100 µl of supernatant in each well was determined in a gamma-counter. Percentage cytotoxicity was calculated from quadruplicate cultures as described above.

Statistical analysis. Results from treated and control groups were compared for statistical significance using Student's *t*-test.

Results

Multiplication of L. monocytogenes in mice developing an acute inflammation

A CaPP-pleurisy was induced in DBA/2 mice, which are highly susceptible to *Listeria* infection, 3 days before the bacterial challenge. This time interval was previously shown to be optimal for increasing mouse survival after lethal infection with *K. pneumoniae, T. Cruzi* and *L. monocytogenes* (Giroud *et al.* 1979, 1981*b*).

As shown in Fig. 1, the growth of the bacteria in the liver and spleen during the 2 days following the infection was attenuated in mice with inflammation. This was particularly evident 48 h after infection at which time a PI around 0.8 was observed in both organs.

In a further experiment, spleen and liver were removed 72 h after bacterial challenge, a time at which the specific immunity began to develop. As shown in Table 1, the number of bacteria was again significantly decreased in mice with inflammation as compared to control mice with a PI of 1.26 for the spleen and 1.13 for the liver.

Chemiluminescent (CL) response and superoxide release of peritoneal macrophages from mice developing an acute inflammation

From Fig. 2 it can be seen that peritoneal macrophages from mice developing a CaPP-induced pleurisy displayed an enhanced CL burst when exposed to zymosan which was particularly evident 24 h and 48 h after the onset of inflammation.

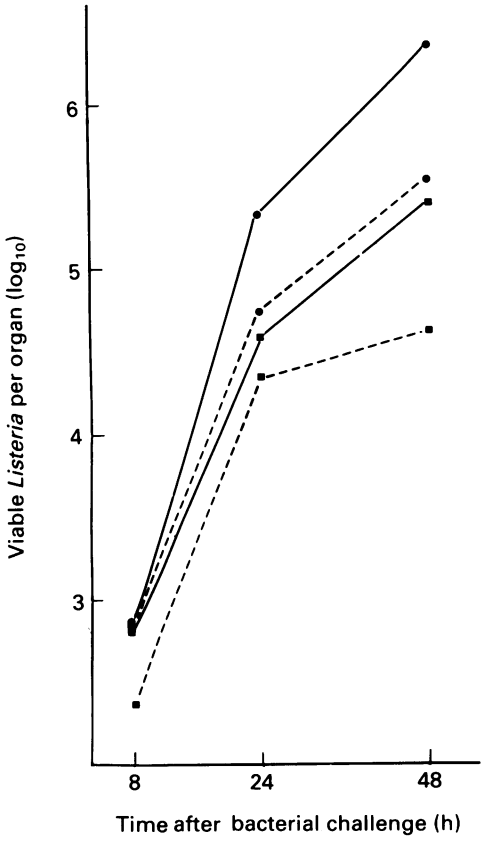

Fig. 1. Growth of *Listeria monocytogenes* in spleen (●) and liver (■) of normal (—) and inflamed (- - -) mice during the 48 h following bacterial challenge. Mice were inoculated i.v. with 3000 viable bacteria 3 days after induction of a CaPP-pleurisy. Mean from four mice in each group.

The measurement of superoxide release after *in vitro* exposure of macrophages to zymosan produced similar results (Fig. 3). Superoxide production was significantly enhanced in cells harvested 1, 2 and 3 days after application of the phlogistic stimulus. Inflammation did not modify the resting level of superoxide production in the absence of zymosan stimulus.

It must be emphasized that there was no change in the total cell number, general morphology or percentage of macrophages in the peritoneal cell populations from mice with inflammation.

Cytostatic activity of peritoneal macrophages in mice developing an acute inflammation or treated with inflammatory serum

As shown in Table 2, the uptake of ^{3}H-TdR by leukemic cells cultured on peritoneal macrophages, harvested as early as 2 h after the intrapleural injection of CaPP, was inhibited by 45% by comparison with the incorporation in the presence of normal macrophages. This cytostatic activity increased with time, reaching its maximal value (80% reduction of ^{3}H-TdR uptake by tumour cells) on day 3 after induction of the inflammatory reaction. It slightly decreased thereafter as, on day 5, a 59% inhibition of tumour cell proliferation was still observed.

Similar observations were made when

Table 1. Number of *Listeria monocytogenes* in spleen and liver of normal or inflamed mice, 3 days after bacterial challenge

Organ	Control mice* Mean number of bacteria ±SE (log_{10})	Inflamed mice *† Mean number of bacteria ±SE (log_{10})	PI
Spleen	5.78±0.73	4.52±0.17	1.26‡
Liver	4.85±0.70	3.72±0.23	1.13‡

* Normal and inflamed mice were inoculated i.v. with 2200 viable bacteria on day 0.

† CaPP-pleurisy was induced 3 days before bacterial challenge.

‡ PI, protection index, represents the difference between the number of bacteria in inflamed mice and in control mice.

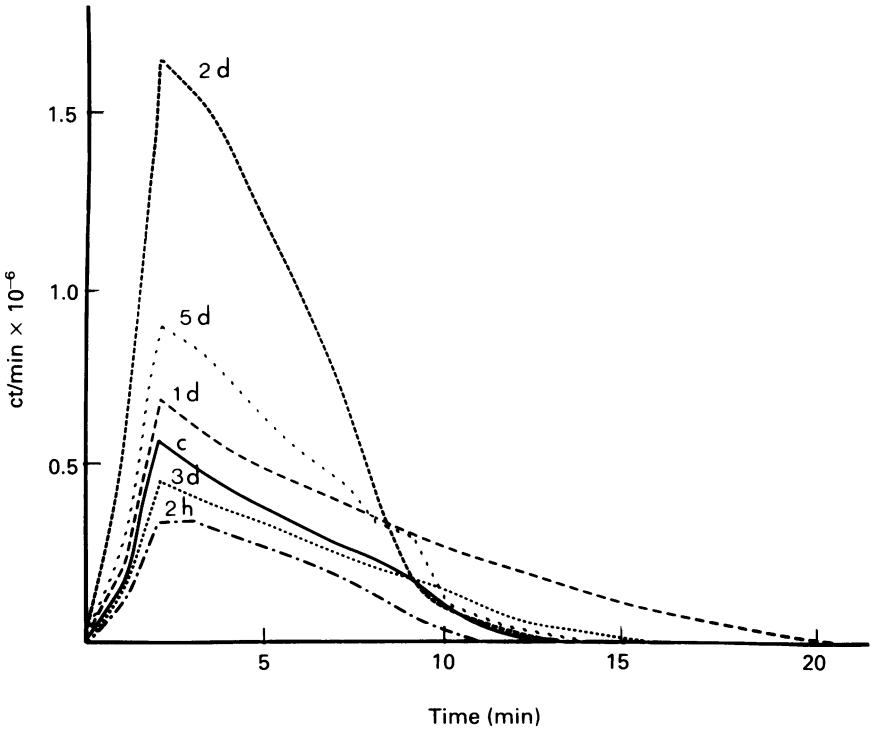

Fig. 2. Phagocytsis-induced chemiluminescence of peritoneal macrophages from normal (control, c) or inflamed mice. Chemiluminescence induced by opsonized zymosan particles in the presence of luminol was measured 2 h to 5 days after induction of a CaPP-pleurisy.

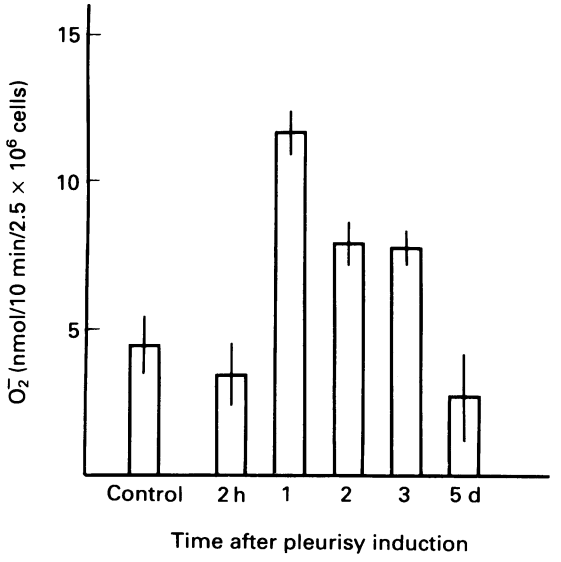

Fig. 3. Superoxide production by peritoneal macrophages from normal (control) or inflamed mice.

Table 2. Cytostatic activity of peritoneal macrophages from mice developing an acute inflammation

	Calcium pyrophosphate		Dextran	
Time after pleurisy	Mean ct/min ± SE*	% inhibition	Mean ct/min ± SE*	% inhibition†
Controls	14.941 ± 492	—	11.592 ± 521	—
2 h, 1 h	8.254 ± 247‡	45%	7.141 ± 701	38%
1 day	6.528 ± 227‡	56%	6.425 ± 361‡	45%
2 days	4·032 ± 231‡	73%	2.420 ± 245‡	80%
3 days	3.039 ± 181‡	80%	4.843 ± 398‡	58%
5 days	6.103 ± 210‡	59%	3.989 ± 306‡	66%

* ^{3}H-TdR incorporation by L1210 leukemic cells after 24 h exposure to macrophages from normal or inflamed mice. Mean from 12 replicate cultures.

† $100 - \left[\frac{\text{ct/min in cultures of inflamed macrophages}}{\text{ct/min in cultures of normal macrophages}} \times 100\right]$.

‡ Significantly different from control values.

dextran was used as the irritant (Table 2). Peritoneal macrophages displayed a significant cytostatic activity as early as 1 h after treatment. This activity was maximal on day 2 (80% inhibition of ^{3}H-TdR incorporation by tumour cells) and remained high on days 3 and 5.

To test the hypothesis that factors liberated into the circulation may be responsible for these effects at a distance from the inflammatory site, macrophage cytostatic activity was measured after intravenously injecting normal mice with serum collected 2 h after induction of a CaPP pleurisy. As

Table 3. Cytostatic activity of peritoneal macrophages after i.v. injection of inflammatory serum

	Treatment	Time after treatment	Mean ct/min ± SE†	% inhibition‡
Exp. 1	None	—	19.006 ± 816	—
	Normal serum	1 day	19.197 ± 718	—
	Inflammatory serum*	1 day	9.028 ± 520	53%
	Normal serum	2 days	14.728 ± 940	—
	Inflammatory serum*	2 days	7.914 ± 361	46%
Exp. 2	None	—	10.845 ± 724	—
	Normal serum	3 days	9.908 ± 842	—
	Inflammatory serum*	3 days	3.475 ± 277	65%

* Inflammatory serum was collected 2 h after CaPP-pleurisy induction.

† ^{3}H-TdR incorporation by L1210 leukaemic cells after 24 h exposure to macrophages from normal mice or mice injected with 0.5 ml normal or inflammatory serum. Mean from 12 replicate cultures.

‡ $100 - \left[\frac{\text{ct/min in cultures of macrophages from mice given inflammatory serum}}{\text{ct/min in cultures of macrophages from mice given normal serum}} \times 100\right]$.

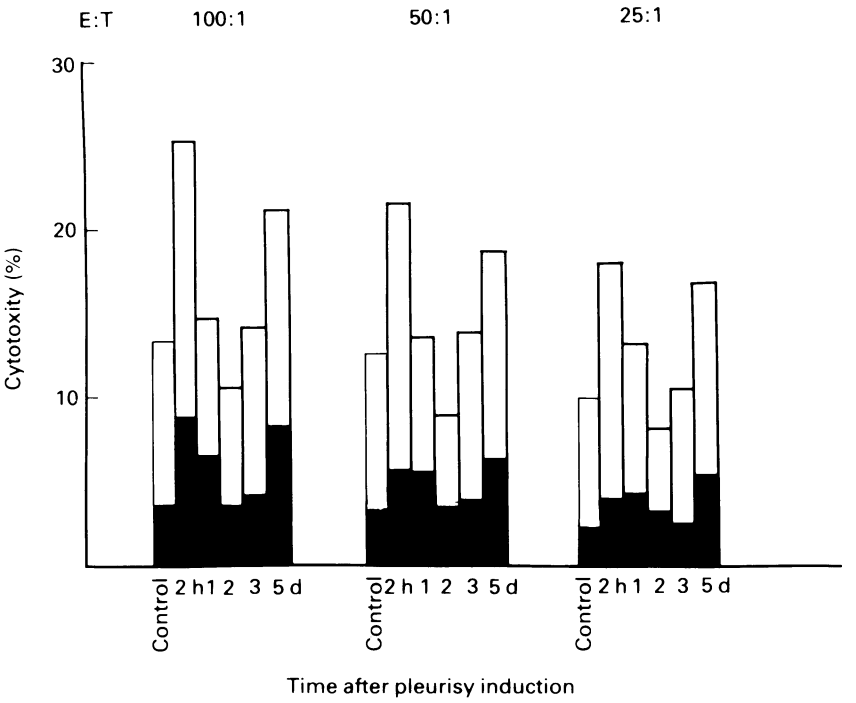

Fig. 4. Natural killer activity of spleen (□) and peritoneal (■) cells against YAC-1 tumour cells in normal (control) or inflamed mice. NK activity was measured 2 h to 5 days after induction of a CaPP-pleurisy using different effector to target cell (E:T) ratios.

shown in Table 3, peritoneal macrophages harvested 1, 2 and 3 days after injecting inflammatory serum, inhibited by about 50% tumour cell proliferation by comparison with macrophages from mice injected with normal serum.

NK activity of spleen and peritoneal cells in mice developing an acute inflammation

Spleen and peritoneal cells from mice with inflammation were examined for cytotoxic activity against YAC-1 tumour cells at different effector to target cell ratios. As shown in Fig. 3*a*, the NK activity of spleen cells was strongly enhanced (about 100%) 2 h after the induction of CaPP pleurisy. This activity returned to control values on day 1, and was slightly depressed (about 30%) on day 2. A second peak of enhanced cytotoxicity (60%) was observed on day 5 after induction of the acute inflammatory process.

Similar variations in the basically low NK activity of peritoneal cells were observed (Fig. 3*b*).

CTL differentiation in spleen cells from mice developing an acute inflammation

As shown in Fig. 4, after *in vitro* immunization against allogeneic P815 tumour cells, spleen cells from mice with inflammation were significantly less cytotoxic against the relevant target cells than similarly immunized normal spleen cells. This inhibitory effect on CTL differentiation was observable 2 h after injection of the irritant, was maximal at day 1 and still notable at day 5. This phenomenon was particularly evident when low effector to target cell ratios were used.

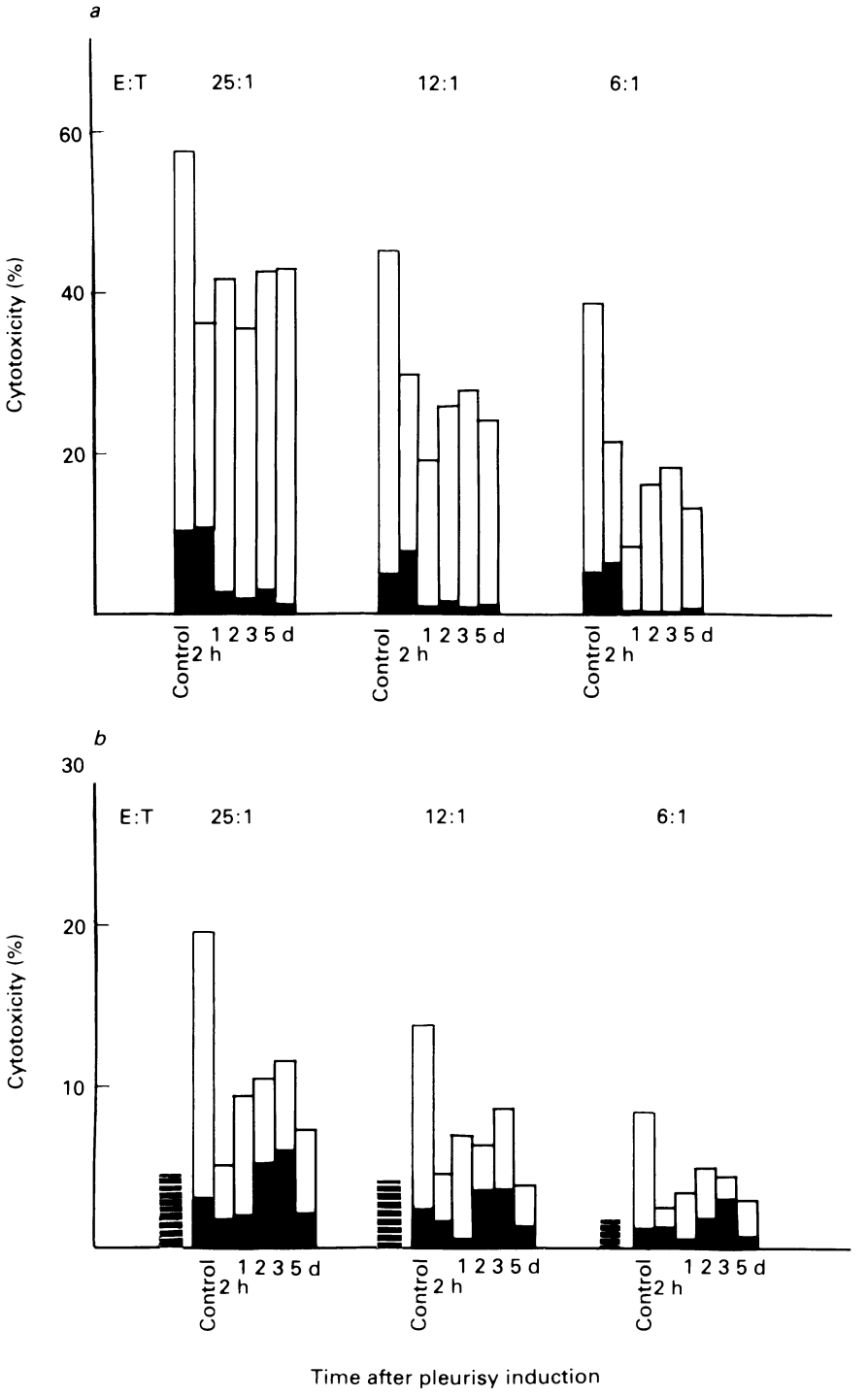

Fig. 5. Cytotoxicity of spleen cells from normal (control) or inflamed mice after *in vitro* immunization against allogeneic P815 tumour cells. (*a*) Cytotoxic activity of spleen cells against P815 target cells after 5 days of cultivation in the presence (□) or the absence (■) of P815 tumour cells. Different effector to target cell (E:T) ratios were used. (*b*) Cytotoxic activity of spleen cells against YAC-1 tumour cells after 5 days of cultivation in the presence (□) or the absence (■) of P815 tumour cells. The cytotoxic activity of non-cultivated (fresh) normal spleen cells (≣) was also measured. Different effector to target cell (E:T) ratios were used.

A significant cytotoxicity against P815 tumour cells also developed in the absence of spleen cell exposure to target cell antigens. These non-specific cytotoxic cells also were generated in cultures of spleen cells from 2-h inflamed animals but were nearly absent when spleen cells were derived from mice developing inflammation from 1 to 5 days (Fig. 4).

When YAC-1 lymphoma cells were used as target cells, marked cytotoxic activity was detected in cultures of P815-immunized normal spleen cells. This NK-like cytotoxicity was inhibited in cultures of spleen cells from mice with inflammation. In the absence of immunization with P815 tumor cells, cytotoxicity against YAC-1 lymphoma was low and was generally unaffected or even slightly increased when spleen cells came from mice with inflammation by comparison with controls (Fig. 5).

Discussion

In the present work, we demonstrated that a local acute non-immunological inflammation induced in mice by the injection of a non-antigenic, endotoxin-free irritant into the pleural cavity, is accompanied by marked modifications of specific and non-specific immune mechanisms which are known to play a role in defence against infections and neoplasia. These effects have been observed at remote sites from the inflammatory focus (spleen, peritoneum, liver) and lasted several days after the inflammatory process had subsided. These phenomena may be implicated in the increased resistance against bacterial, parasitic and fungal infections as well as in the modification of tumour development which have been previously described in animals with inflammation (Giroud *et al.* 1979, 1981*b*; Nolibe *et al.* 1980).

In this study, we showed that the multiplication of *Listeria monocytogenes* in the spleen and liver is decreased in mice developing inflammation from 3 days before the bacterial challenge. It is now well established that macrophages are implicated in the different phases of the resistance to *L. monocytogenes* (Fauve *et al.* 1966; Mitsuyama *et al.* 1978; Yoshikai *et al.* 1980). Acute inflammation may interfere first with the early step of this resistance which implies the proliferation of fixed macrophages (North 1969), as we have previously shown that a mitogenic factor for macrophages is liberated into the circulation very rapidly after the onset of the acute inflammatory reaction (Girre *et al.* 1981). Later, macrophages in animals with inflammation may undergo functional modifications which allow them to express increased bactericidal activity. Evidence consistent with this hypothesis was provided by subsequent experiments.

We observed that peritoneal macrophages from mice with inflammation emitted more chemiluminescence in response to opsonized zymosan particles than normal macrophages, from day 1 after the onset of inflammation. This enhancement of CL was usually concomitant with an increased production of superoxide anion, demonstrating stimulation of oxidative metabolism in the phagocytic cells. However, the slight discrepancy between the level of CL and superoxide release observed on day 3 after inflammation induction may be attributable to the fact that CL may involve contributions from other metabolic pathways such as arachidonic acid metabolism (Smith & Weideman 1980). Since superoxide anion and its related reactive oxygen intermediates are known to play an important role in the bactericidal activity of macrophages (Murray *et al.* 1979) these effects may account, at least in part, for the increased resistance to *Listeria monocytogenes* and other intracellular pathogens observed in animals with inflammation.

On the other hand, we also demonstrated that peritoneal macrophages became highly cytostatic for tumour cells very rapidly after initiation of the acute inflammatory process and remained in this state for several days after inflammation has disappeared. We were able to reproduce this phenomenon in normal mice by injecting serum from inflamed animals, collected 2 h after the onset

of inflammation. This strongly suggests that factors were released into the circulation very rapidly during the inflammatory process, which were implicated in the activation of peritoneal macrophages. It seems unlikely that interferon, which appears as a potent macrophage activating agent, could be responsible as its level was shown to be very low at the time (2 h) of serum collection (Chousterman *et al.* 1985).

It has been demonstrated that macrophages attracted locally by a sterile inflammation shared characteristics with immunologically activated macrophages, namely increased metabolic activity, digestive capacity and phagocytic activity (Karnovsky & Lazdins 1978). However, it is generally accepted that, contrary to activated macrophages, inflammatory macrophages do not exhibit cytostatic and tumoricidal nor bacteriostatic and bactericidal activities (North 1978). In our study, we demonstrate that both phagocytic and cytostatic activities were enhanced in macrophages collected at distance from the inflammatory site. That non-immunological inflammation might induce macrophage activation was already suggested by the observation of Poste (1979) which showed that cytotoxic macrophages could be recovered from coverslips implanted subcutaneously. Fauve *et al.* (1977) also reported that an extract from a granuloma, induced by a subcutaneous injection of talc particles, *in vitro* rendered normal macrophages cytostatic for tumour cells.

The suggestion that macrophages are functionally modified during the acute non-immunological process is reinforced by our previous observation that spleen cells from mice with inflammation exhibited a greatly enhanced antibody-dependent cellular cytotoxicity against antibody-coated chick erythrocytes; again, this effect was obtained in normal mice after injection of inflammatory serum (Kiger *et al.* 1979).

Besides macrophages, natural killer cells may destroy non-specifically tumour cells and cells infected with certain parasites. In the present work, we observed a marked increase of NK activity of spleen and peritoneal cells early (2 h) after the onset of inflammation. However, in contrast to the data seen with macrophages, this activity returned to normal baseline values and was even depressed from day 1 to 3, but was stimulated again on day 5. This implies that positive and negative regulatory influences of NK activity are successively predominant during the development of the inflammatory process. It was notable that NK activity was inversely correlated with interferon levels in the serum which are very low at 2 h, maximal at 48 h and vanish after 72 h (Chousterman *et al.* 1985). The depression of NK activity observed on day 2 coincided with maximal production of superoxide anion by macrophages; this active metabolite of oxygen inhibits NK cytoxicity (Seaman *et al.* 1982). Various mediators of inflammation can also stimulate or inhibit NK activity, viz, cyclic nucleotides (Katz *et al.* 1982), some metabolites of arachidonic acid (Brunda *et al.* 1980) and some acute-phase proteins (Hudig *et al.* 1981).

It is also possible that these factors, released into the serum during acute inflammation, which are low-molecular-weight peptides (phlogokines) capable of modifying various functions of macrophages, lymphocytes and polymorphonuclear cells (Giroud *et al.* 1981*a*), may also act on NK cells.

In contrast to its effect on non-specific anti-tumour effector mechanisms (macrophage cytostactic activity, NK cell activity), acute inflammation induced a marked depression of the specific T cell cytotoxicity against allogeneic tumour cells. This was observed up to 5 days after pleurisy induction. There was also a concomitant decrease of the non-specific cytotoxicity which develops during *in vitro* culture either in the presence or absence of the stimulating antigens, which can be linked to immunization against fetal serum constituents (Thorn 1980), to a polyclonal activation of CTL (Igarashi *et al.* 1977) or to the activation of cells distinct from CTL and NK cells (Seeley *et al.* 1979). Such an impairment of T cell

reactivity has already been observed in mice developing a CaPP-induced pleurisy. Spleen and lymph node cells show a decreased proliferative response to phytohaemagglutinin and concanavalin A at all time intervals after induction of the inflammatory process (Renoux *et al.* submitted for publication). This inhibition of both cytotoxic T cell differentiation and blastogenic response to mitogens can be related to a decrease in interleukin 2 production by T helper cells (Yao Jin Sheng *et al.* manuscript in preparation).

All these important alterations of the specific and non-specific defence mechanisms are probably involved in the modulation of the resistance of animals with inflammation to infections and neoplasia. The increase in phagocytic activity of macrophages associated with liberation of reactive species of oxygen may explain why mice with inflammation are protected against intracellular pathogens. Similar protection was observed in mice developing a sterile granuloma and this was also attributed to macrophage activation during the inflammatory process (Fauve *et al.* 1977). Stimulation of NK activity may also interfere with the resistance to parasites (Eugui & Allison 1980) which was markedly increased in mice with inflammation (Giroud *et al.* 1981*b*). The long-lasting inhibition of T cell cytotoxicity may explain why the resistance to various viral infections was not increased in mice developing a CaPP-induced granuloma (Zerial *et al.* 1980). As far as anti-tumour activity is concerned in mice, we have observed an enhancement of primary tumour growth and of lung metastatic development when inflammation was induced 3 days before inoculation of a few Lewis tumour cells (Nolibe *et al.* 1980). At that time, macrophage cytostatic activity was maximal and NK activity in the spleen was normal. Acute inflammation thus may impair the action of these effector cells either by preventing their migration to the tumour or by interfering with some non-immunological mechanisms involved in the metastatic process.

It is apparant that this inflammatory process, characterized by short duration and initiated by a non-antigenic, endotoxin-free and non-diffusable irritant, induced a cascade of events which, in turn, are able to modify at sites remote from the inflammatory focus, various functions of macrophages, lymphocytes, polymorphonuclear cells and their progenitors (Giroud *et al.* 1981*a*). Some of these activities have been related to low-molecular-weight serum factors which appear to be non-species specific. They are potentially of therapeutic value for they could be used to stimulate host-defence mechanisms against various bacterial and parasitic infections.

Acknowledgements

Mrs B. Chanaud, M. De Souza and S. Krzyzanowski are greatfully acknowledged for their expert technical assistance. This work was supported by a grant from the Association pour le Développement de la Recherche sur le Cancer (ADRC).

References

Brunda M.J., Herberman R.V. & Holden H.T. (1980) Inhibition of natural killer cell activity by prostaglandins. *J. Immun.* **124**, 2682–2687.

Chousterman S., Bird J., Yao J.S., Chelbi-Alix M., Giroud J.P. & Thang M.N. (1985) 2′5′-Oligoadenylate synthetase activity level, an interferon-induced enzyme, in macrophages derived from normal or inflamed mice. *Ann. Virol. (Inst. Pasteur)* in press.

Drouhet E., Dupont B., Pelletier M. & Giroud J.P. (1981) Acute nonspecific inflammatory reaction and protection against the *Candida albicans* experimental infections. *Agents Actions* **11**, 629–631.

Eugui E.M. & Allison A.C. (1980) Differences in susceptibility of various mouse strains to haemoprotozoan infections: possible correlation with natural killer activity. *Parasite Immun.* **2**, 277–281.

Fauve R.M., Douanchaud D. & Delaunay A. (1966) Cellular resistance to bacterial infection. IV—Active immunization and cellular resistance of macrophages from NCS mice to

infection with *Listeria monocytogenes, Corybacterium kutscheri* and *Brucella melitensis. Ann. Inst. Parsteur* **110**, (suppl. 3), 106-112.

FAUVE R.M., HEVIN B. & DAUGE M.C. (1977) Inflammation and activation of macrophages. In *Macrophage and Cancer.* Eds K. James, B. McBride & A. Stuart. Edinburgh: Econo Press. pp. 258–263.

GIRRE C., PELLETIER M. & GIROUD J.P. (1981) Mise en évidence au cours de réactions inflammatoires aigues d'un facteur sérique mitogène pour les macrophages. *J. Pharmacol. (Paris)* **12**, 147–159.

GIROUD J.P., BOREL J.P., CAM Y., CHEDID L., FLORENTIN I., JADIN J., NOLIBE D., PARANT M., PELLETIER M., ROCH-ARVEILLER M. & TISSOT M. (1981*a*) Acute inflammation and modulation of cells functions. Isolation and role of serum factors (phlogokines). In *Pharmacology of Inflammation and Allergy.* Eds F. Russo-Marie, B. B. Vargaftig & B. Benveniste. Paris: Editions INSERM. pp. 223–250.

GIROUD J.P., FLORENTIN I., PELLETIER M. & NOLIBE D. (1981*b*) Influence of an acute non-immunological inflammation on resistance to infection and neoplasia. In *Advances in Immunopharmacology.* Eds J. Hadden, L. Chedid, P. Mullen & F. Spreafico. Oxford: Pergamon Press. pp. 249–257.

GIROUD J.P., PARANT M., PARANT F., PELLETIER M. & CHEDID L. (1979) Effet d'un processus inflammatoire aigu sur la résistance anti-infectieuse des souris. *C.R. Acad. Sci. Paris Série D* **289**, 1945–1948.

HERBERMAN R.B. (ed.) (1982) *NK Cells and Other Natural Effector Cells.* New York: Academic Press.

HUDIG D. HAVERTY T., FULCHER C., REDELMAN D. & MENDELSOHN J. (1981) Inhibition of human natural cytotoxicity by macromolecular proteases *J. Immun.* **126**, 1569–1574

IGARASHI T., OKADA M., KISHIMOTO T. & YAMAMURA Y. (1977) In vitro induction of polyclonal killer T cells with 2-mercaptoethanol and the essential role of macrophages in this process. *J. Immun.* **118**, 1697–1703.

JOHNSTON R.B., KEELE B.B., MISRA H.P., LEHMEYER J.E., WEBB L.S., BAEHNER R.L. & RAJAGOPALAN K.V. (1975) The role of superoxide anion generation in phagocytic bactericidal activity. Studies with normal and chronic granulomatous disease leukocytes. *J. clin. Invest.* **55**, 1357–1372.

KARNOVSKY M. & LAZDINS J.K. (1978) Biochemical criteria for activated macrophages. *J. Immun.* **121**, 809–813.

KATZ P., ZAYTOUN A.M. & FAUCI A.S. (1982) Mechanisms of human cell-mediated cytotoxicity. I—Modulation of natural killer cell activity by cyclic nucleotides. *J. Immun.* **129**, 287–296.

KIGER N., PELLETIER M. & GIROUD J.P. (1979) Effect of a non-specific acute inflammatory stimulus on antibody-dependent cellular cytoxicity: role of a serum factor. *Int. J. Immunopharmac.* **1**, 127–132.

MITSUYAMA M., TAKEYA K., NOMOTO K. & SHIMOTORI S. (1978) Three phases of phagocyte contribution to resistance against *Listeria monocytogenes. J. gen. Microbiol.* **106**, 165–171.

MURRAY H.W., JUANGBHANICH B.W., NATHAN C.F. & COHN Z.A. (1979) Macrophage oxygen-dependent: antimicrobial activity I. The role of oxygen intermediates. *J. exp. Med.* **150**, 950–964.

NATHAN C.F., SILVERSTEIN S.C., BRUKNER L.H. & COHN Z.A. (1979) Extracellular cytolysis by activated macrophages and granulocytes. II—Hydrogen peroxide as a mediator of cytotoxicity. *J. exp. Med.* **149**, 100–113.

NOLIBE D., FLORENTIN I., PELLETIER M., MASSE R. & GIROUD J.P. (1980) Effect of an acute inflammatory process on tumor development. In *Inflammation-Mechanisms and Treatment.* Eds D. A. Willoughby & J. P. Giroud. Lancaster: MTP Press. pp. 663–690.

NORTH R.J. (1969) Cellular kinetics associated with the development of acquired cellular resistance. *J. exp. Med.* **130**, 299–314.

NORTH R.J. (1978) The concept of the activated macrophage. *J. Immun.* **126**, 806–809.

POSTE G. (1979) The tumoricidal properties of inflammatory tissue macrophage and multinucleate giant cells. *Am. J. Path.* **96**, 595–611.

SEAMAN W.E., GINHART T.D., BLACKMAN M.A., DAZAZ B., TALAL N. & WERG Z. (1982) Suppression of natural killing in vitro by monocytes and polymorphonuclear leukocytes. Requirement for reactive metabolites of oxygen. *J. clin. Invest.* **69**, 876–888.

SEELEY J.K., MASUCCI G., POROS A., KLEIN E. & GOLUB S.H. (1979) Studies on cytotoxicity generated in human mixed lymphocytic cultures. II. Anti-K562 effectors are distinct from allospecific CTL and can be generated from NK-depleted T cells. *J. Immun.* **123**, 1303–1311

SMITH R. L. & WEIDEMANN M.J. (1980) Reactive oxygen production associated with arachidonic acid metabolism by peritoneal macrophages. *Biochem. Biophys. Res. Commun.* **97**, 973–984.

SULTAN A.M., DUNN C.J., MIMMS P.C., GIROUD J.P.

& WILLOUGHBY D.A. (1978) The leucocyte disappearance reaction in non immune acute inflammation. *J. Path.* **126**, 221–229.

THORN R.M. (1980) Murine T-cell mediated cytotoxicity against syngeneic and allogeneic cell lines induced by fetal calf serum. *Cell Immun.* **54**, 203–214.

WILLOUGHBY D.A., DUNN C.J., YAMAMOTO S., CAPASSO F., DEPORTER D.A. & GIROUD J.P. (1975) Calcium pyrophosphate induced pleurisy in rats: a new model of acute inflammation. *Agents Actions* **5**, 35–38.

YOSHIKAI Y., MIAKE S., MATSUMOTO T., NOMOTO K. & TAKEYA K. (1980) Relationship between non-specific activity of macrophages and immune response to *Listeria monocytogenes*. *Immunology* **40**, 295–302.

ZERIAL A., FLOC'H F. & WERNER G.H. (1980) Comparative effects of an inflammatory reaction on the resistance of mice to bacterial and viral infections. *Ann. Immun. (Institut Pasteur)* **131** C, 177–184.