Br. J. exp. Path. (1984) 65, 521–532

Generation of an interleukin-1-like lymphocyte-stimulating factor at inflammatory sites: correlation with the infiltration of polymorphonuclear leucocytes

Kumiko Goto, S. Nakamura, F. Goto and M. Yoshinaga
Department of Immunopathology, Kumamoto University Medical School, Kumamoto, Japan

Received for publication 29 November 1983

Summary. Using a mouse thymocyte co-stimulation assay, we demonstrated thymocyte-stimulating activity in murine peritoneal fluid obtained from the early stage (3 to 9 h) of casein-induced inflammation. This early inflammatory stage coincided with the time at which an influx of polymorphonuclear leucocytes (PMN) into the inflamed site was observed. Similar thymocyte-stimulating activity was produced *in vitro* by PMN purified from 4-h peritoneal exudate but not by purified PMN obtained at a later stage (24 h) of the inflammation. The inflammatory factor was interleukin (IL)-1-like; it was devoid of IL-2 activity when tested with IL-2-dependent cells. It could stimulate murine thymocytes to produce IL-2. On a Sephadex G-75 column, the factor was eluted between the molecular sizes of 10 000 and 30 000; its peak activity was at 21 000. The factor mainly consisted of two (pI 6.5 and pI 5.0) iso-electrophoretically different factors.

Keywords: inflammatory sites, lymphocyte-stimulating factor, polymorphonuclear leucocytes

The primary plaque-forming cell (PFC) response of mice to sheep red blood cells (SRBC) was strikingly enhanced when the antigen was introduced into the inflamed peritoneal cavity (Nakayama *et al.* 1982). This PFC enhancement was observed only when the antigen was introduced at the early, but not at the later, stage of inflammation. This observation suggests that early inflammatory polymorphonuclear leucocytes (PMN) may play an important role in this PFC enhancement, because it could be produced in normal mice by the adoptive transfer of the inflammatory PMN plus antigen.

During the course of our experiments, we detected lymphocyte-stimulating activity (LSA) in the early, but not the later, stage of inflammation. This led us to hypothesize that this lymphocyte-stimulating factor might be of biological importance in the enhancement of the immune response. There are two well-known immunologically non-specific factors that augment the lymphocyte proliferative response, *i.e.* lymphocyte-activating factor (LAF, or interleukin (IL)-1) (Gery *et al.* 1972; Economou & Shin 1978; Mizel 1979) and T cell growth factor (TCGF, or IL-2) (Morgan & Ruscett 1976; Gillis *et al.* 1978; Aarden *et al.* 1980). IL-1 is thought to be an inflammation-related hormone-like mediator, because it seems to induce acute-phase proteins (Sztein *et al.* 1981) and fever (Murphy *et al.* 1980). However, how the generation of lymphocyte-stimulating factor

Correspondence: M. Yoshinaga, Department of Immunopathology, Kumamoto University Medical School, 2-2-1 Honjo-machi, Kumamoto, 860, Japan.

relates to inflammation has not been studied in detail.

Therefore, we performed a detailed kinetic study of the generation of LSA in a casein-induced murine peritoneal inflammation. We examined the relationship between the appearance of this activity and leucocyte exudation into the peritoneal cavity and investigated the biological and biochemical nature of the LSA in the inflammatory site.

Materials and methods

Animals and induction of inflammation. C3H/HeN and C57BL/6N mice of both sexes, 7–12 weeks of age, were injected i.p. with 2 ml of 0.2% sodium caseinate in phosphate-buffered saline (PBS).

At appropriate intervals thereafter, peritoneal fluid was collected by washing the peritoneal cavity with 1 ml PBS. Small portions were removed for white cell count and smear and the rest of the exudate was centrifuged for 10 min at 1000 r/min. The final volume of the cell-free exudate was adjusted to 2 ml by adding PBS after 16 h dialysis against PBS. Then the cell-free peritoneal exudate fluid (PEF) was passed through a micropore filter (0.22 μm) and various assays were performed.

Thymocyte co-stimulation assay. A single-cell suspension of thymocytes (3×10^6/ml) from 7 to 12-week-old C3H mice was cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS; Flow Laboratories, Sydney, Australia), 2×10^{-5} M 2-mercaptoethanol (2-ME) and antibiotics, in the presence or absence of 1 μg/ml phytohaemagglutinin-p (PHA; Difco Laboratories, Detroit, Mich.). An appropriate amount of the inflammatory PEF was added to the culture and its effect on the DNA synthesis of the thymocytes was determined. DNA synthesis was assessed by the incorporation of ^{3}H-thymidine (^{3}H-TdR; 1 μCi/tube; 2 Ci/mmol) into the trichloroacetic acid (TCA)-insoluble fraction during the last 24 h of the 72-h culture period. The results were expressed as the mean ct/min of two or three experiments in triplicate cultures.

To calculate the biological units for the thymocyte co-stimulation activity, the enhanced ^{3}H-TdR uptake by the test sample was compared with that of a standard IL-1 sample from P388D$_1$ cells (Mizel 1980).

Standard IL-1 was produced according to a slight modification of the method of Mizel *et al.* (1978) from macrophage cell line P388D$_1$. The cells (2×10^6/ml) in RPMI 1640 medium containing 1% FCS, 2-ME and antibiotics were stimulated with 10 μg/ml endotoxic lipopolysaccharide, *Escherichia coli* 0111:B5 (Difco Laboratories), for 72 h. The culture supernatant was fractionated with ammonium sulphate. The precipitate produced between the 33% and 70% $(NH_4)_2SO_4$ saturation points was collected and eluted through a Sephadex G-75 column. The active fractions with a molecular weight between 12 500 and 24 000 were collected and concentrated on a YM-5 Diaflo membrane (Amicon Co., Lexington, Mass.).

One unit of thymocyte co-stimulation activity was determined as the amount necessary to produce a half-maximal DNA synthesis response by 3×10^6 thymocytes. The control maximal response was induced by stimulation with 1 μl PHA and 200 μl of 70 units/ml standard IL-1 preparation. This maximal response was usually 50 000 to 60 000 ct/min; it varied within 20% from experiment to experiment. When the activity in a sample was below 1 unit/400 μl test sample, the sample was concentrated by ultrafiltration on a YM-5 Diaflo membrane and tested again. Only in samples which showed no significant enhancement of the thymocyte ^{3}H-TdR uptake was a recording of 0 units made.

IL-2 assay. The inflammatory PEF and the active fraction from the PEF preparation were assessed for their ability to stimulate the uptake of ^{3}H-TdR in a cloned IL-2-dependent C3H/HeN anti-C57BL/6N cytotoxic mouse T cell line. The method used was essentially according to Gillis *et al.* (1978).

Gel filtration and iso-electrofocusing. The biological activity in the inflammatory PEF was assessed for its molecular size by gel filtration on a Sephadex G-75 column (2.5 × 40.5 cm, 200 ml). It was equilibrated with PBS and calibrated with the following markers; blue dextran, human serum albumin (HSA), ovalbumin (OVA), soy bean trypsin inhibitor (SBTI), cytochrome C(CC).

The biological activity in the active PEF was assessed for its iso-electric point. Inflammatory PEF (40 ml) was precipitated by 80% $(NH_4)_2SO_4$ from the supernatant fraction after 33% $(NH_4)_2SO_4$ precipitation and the resulting active fraction was dialysed against 1% glycine. This dialysed active fraction was incorporated into Ultrodex gel (LKB-2117) containing pH 3.5–10 carrier ampholite (LKB, Bromma, Sweden). Electrofocusing was carried out in an LKB Multiphor Apparatus (150-ml bed with a 23-cm length) at 8 W for 24 h. All the above procedures were according to the LKB manual. After electrofocusing, the bed was cut into 30 columns and each column was eluted with 3 ml PBS. Each 200 μl of the fraction was subjected to thymocyte co-stimulation assay after dialysis against PBS.

Separation of mouse PMN and stimulation in vitro. PMN were separated from 4-h or 24-h peritoneal exudate cells (PEC) by using discontinuous density gradients of colloidal silica (Percoll, Pharmacia, Uppsala, Sweden) according to Hanson *et al.* (1980). This method had been used for rabbit PMN, thus it required a slight modification. Five gradient layers were constructed with 30%, 50%, 60%, 65% and 70% Percoll in MEM medium and adjusted to pH 7.2 with 0.15 N NaOH and 10 mM Hepes buffer. Washed PECs (1×10^8) were layered onto the gradients in a 50-ml tube and centrifuged at 400 *g* for 60 min at 4°C. The cells in the most dense gradient layer were recovered, washed and assessed for their viability by trypan blue dye exclusion. Small samples were removed for cell count and smear. Purified PMN (1×10^6/ml) were cultured for 3 h in 6-cm Petri dishes containing 3 ml RPMI-1640 medium supplemented with 0·4% (w/v) HSA and 2×10^{-5} M 2-ME. The cells were stimulated with 1 mg/ml baked kaolin (Fisher Scientific Company, Fair Lawn, N.J.).

^{51}Cr-labelling of blood leucocytes and measurement of influx of labelled cells into the inflammatory site. This was essentially according to the method of Issekutz & Movat (1980). Leucocytes were harvested from blood and labelled with ^{51}Cr in a conventional way. The resulting ^{51}Cr-labelled leucocytes were calculated to contain 7 to 8×10^4 ct/min/10^6 cells.

The washed ^{51}Cr-leucocytes (2×10^6/100 μl; 14.2 to 16×10^4 ct/min) were i.v. injected at appropriate intervals after the induction of peritoneal inflammation with casein. One hour later, the mice were killed, their peritoneal cells were recovered and ^{51}Cr-labelled cells were counted using an LKB-gamma counter. The values for the ^{51}Cr-labelled cells in the peritoneal cavity were corrected by using the white blood cell counts which were simultaneously recorded during the course of inflammation.

Results

Generation of LSA during the course of murine peritoneal inflammation

The i.p. injection of sodium caseinate induced a mild acute inflammation. The inflammatory PEF was taken at intervals after casein injection and subjected to thymocyte co-stimulation assay.

Unstimulated mouse thymocytes (3×10^6) showed only low level of ^{3}H-TdR uptake (200–300 ct/min); a small increment was noted after stimulation with 1 μl PHA alone (2500–3000 ct/min). In the presence of 200 μl of the dialysed early PEF (within 9 h of casein injection) a marked enhancement of thymocyte DNA synthesis was observed. No such enhancement was induced by late PEF (20 to 96 h), irrespective of whether or not the PEF samples had been dialysed. The

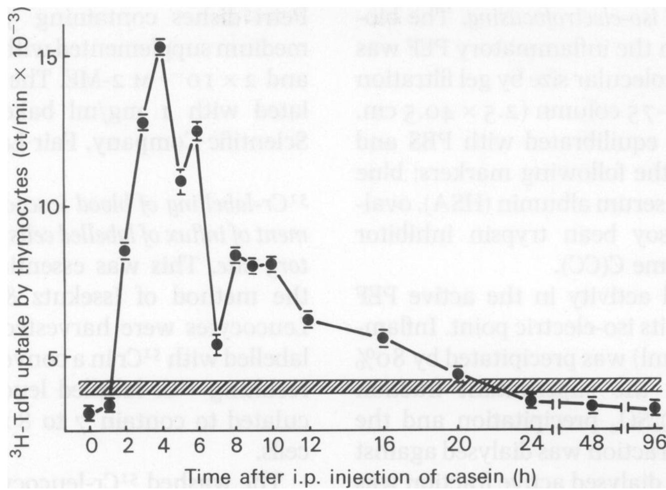

Fig. 1. Generation of thymocyte-stimulating activity during the course of peritoneal inflammation. Mice were injected i.p. with 2 ml of 0.2% casein. At the indicated times thereafter, they were killed and peritoneal exudate fluid (PEF) was removed. After dialysis against PBS, 200-μl samples were subjected to thymocyte co-stimulation assay. Control level of ^{3}H-TdR uptake by thymocytes, which were stimulated with PHA alone, is shown by the shaded zone. 0-h samples were obtained by washing the peritoneal cavity of untreated mice with 2 ml 0.2% sodium caseinate. Data are shown as the mean ^{3}H-TdR uptake ±SE ($n=6$).

strongest enhancement occurred in the presence of 4-h PEF. The addition of 24-h and 96-h PEF, slightly suppressed the DNA synthesis of PHA-stimulated thymocytes (Fig. 1).

As shown in Fig. 2, the DNA synthesis enhancement by 4-h PEF was dose-dependent; the 0·2% casein solution did not affect thymocyte DNA synthesis.

Kinetics of leucocyte accumulation and influx of leucocytes into the inflamed peritoneal cavity

At intervals after the i.p. injection of sodium caseinate, PECs were collected, leucocytes were counted and the leucocyte species were identified in Giemsa-stained smears.

As shown in Fig. 3, during the early stage of inflammation, most of the accumulated cells were PMN. In later stages the accumulation of lymphocytes and macrophages increased gradually. No red cells were detected during the 96-h observation period. In order to assess the influx of leucocytes into the inflamed peritoneal cavity, dx/dt values were calculated. The calculated dx/dt values suggest that the influx of PMNs into the peritoneal cavity occurred within 5 h of the induction of inflammation and that the influx of macrophages began at 7 h after injection and gradually increased until 96 h. The influx of lymphocytes was assumed to occur at a low but steady rate during the 96-h observation period.

Then, the influx of leucocytes was directly measured by using ^{51}Cr-labelling. Mixed blood leucocytes were labelled with ^{51}Cr without further cell purification because this required less cell manipulation *in vitro* as suggested by Issekutz & Movat (1980). We injected ^{51}Cr-labelled leucocytes i.v. at the indicated intervals after the i.p. injection of casein (Fig. 4). PEC, collected 1 h after ^{51}Cr-leucocyte injection, were assayed for radioactivity.

As shown in Fig. 4, the influx of ^{51}Cr-leucocytes peaked at 3–4 h after the induction of peritoneal inflammation. During the 8-h period in which a positive influx of ^{51}Cr-leucocytes was observed, most of the accumu-

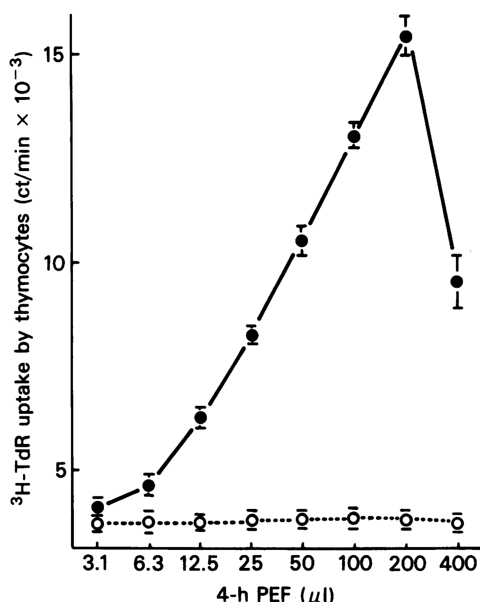

Fig. 2. Thymocyte co-stimulation activity in 4-h PEF. Two-fold dilutions of 4-h PEF (400 μl) were tested for thymocyte co-stimulation activity (●). In the controls, 0.2% sodium caseinate was doubly diluted and tested in the same manner (○). Data are expressed as the mean ^{3}H-TdR uptake ±SE in three experiments performed in triplicate (n=9).

lated cells at the inflamed site were PMN. The reason why the influx of ^{51}Cr-labelled leucocytes went undetected during the latter stage (16–96 h) of inflammation, may be ascribable to the rapid elimination of ^{51}Cr-labelled mononuclear cells from the circulation as stated by Issekutz & Movat (1980). These observations support the hypothesis that the influx of ^{51}Cr-leucocytes during the early stage of inflammation may roughly represent an influx of PMN.

The kinetics of the PMN influx into the inflamed peritoneal cavity coincided well with the profile obtained for the kinetics of the generation of thymocyte co-stimulation activity in the inflamed peritoneal cavity.

Production of thymocyte co-stimulation activity in vitro by 4-h and 24-h PEC

As thymocyte co-stimulation activity was detected only in early PEF, we compared the production of this activity *in vitro* by 4-h and 24-h PEC (cf. Fig. 1). PEC were collected from early and later lesions and washed three times with ice-cold HBSS. The 4-h PEC consisted of 86.1% PMN, 9.5% lymphocytes,

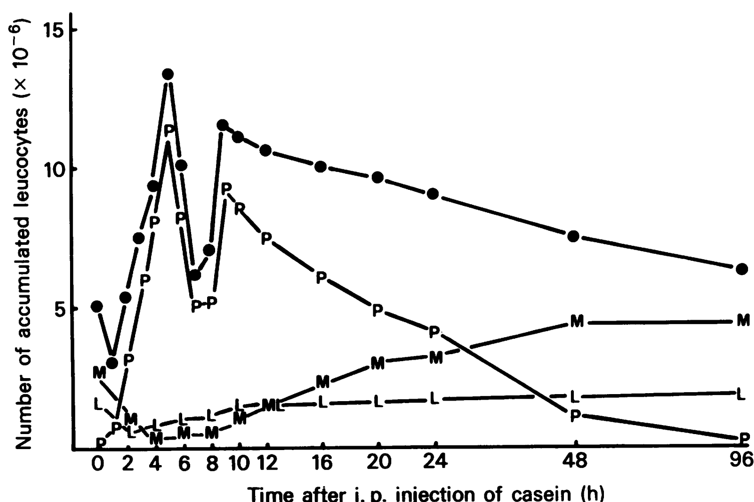

Fig. 3. Differential analysis of accumulated leucocytes in casein-induced peritoneal inflammation. Peritoneal inflammation was induced as in Fig. 1. The exuded cells were collected at the indicated intervals thereafter by washing the peritoneal cavity with 8 ml of ice cold HBSS and subjected to cell counting and differential analysis. During the entire post-injection time, no red cells were detected among the accumulated peritoneal cells. Solid circles represent the total of numbers exuded cells; M, macrophages; L, lymphocytes; P, PMN. All values represent the mean of three experiments.

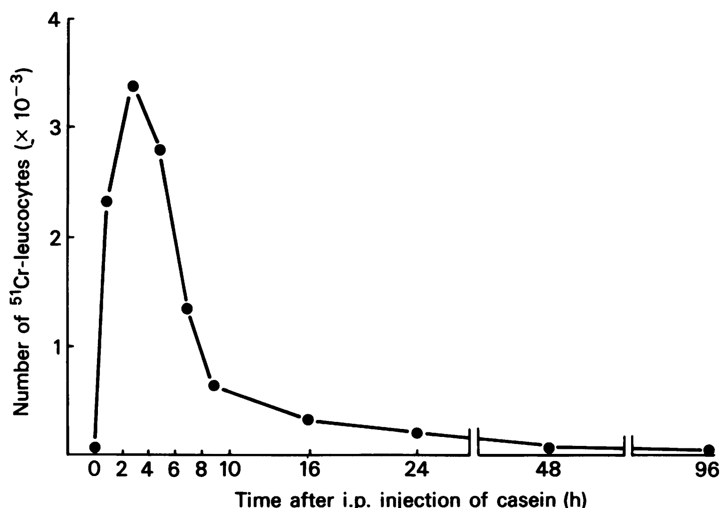

Fig. 4. Influx of ^{51}Cr-leucocytes into the casein-injected peritoneal cavity. Peritoneal inflammation was induced as in Fig. 1. At the indicated times, 2×10^6 ^{51}Cr-leucocytes were injected i.v. and 1 h later the radioactivity of washed peritoneal cells was determined. The number of ^{51}Cr-labelled cells was calculated on the basis of 0.08 ct/min/cell. Each point represents the mean cell number of two experiments.

3.7% macrophages, and 0.7% mast cells, the 24-h PEC of 45.4% PMN, 14.2% lymphocytes, 40.2% macrophages, and 0.2% mast cells. We tested several stimulants for the production of LSA by 4-h PEC, including kaolin, alum, polystyrene beads, formalinized sheep erythrocytes, opsonized sheep erythrocytes, muramyldipeptide and endotoxic lipopolysaccharide of *E. coli*. Of these kaolin was the strongest stimulant for the production of LSA and was therefore used as the stimulant in the subsequent experiments. PEC (1×10^6) were cultured with or without kaolin stimulation (1 mg/ml) and 3 h later, the culture supernatant was removed for thymocyte co-stimulation assay.

As shown in Table 1, 4-h PEC produced moderately high activity; 24-h PEC produced lower, but definitely positive thymocyte co-stimulation activity. The production of thymocyte co-stimulation activity by 4-h PEC

Table 1. Comparison of the production of thymocyte co-stimulation activity by 4-h and 24-h PEC

Cells*	Stimulation with kaolin	Thymocyte co-stimulation activity (units/3×10^6 cells)
4-h PEC	none	3.9
4-h PEC	1 mg/ml	15.6
24-h PEC	none	2.1
24-h PEC	1 mg/ml	<0.1

* 3×10^6 PEC, obtained 4 or 24 h after the i.p. injection of 2 ml of 0.2% casein, were cultured in 6-cm Petri dish containing 3 ml medium. The supernatant was harvested 3 h later and assayed for thymocyte co-stimulation activity.

Table 2. Elaboration of the thymocyte co-stimulation activity by purified PMN from 4-h and 24-h PEC

Exp.	Cells	Purity of PMN (%)	Kaolin stimulation	Thymocyte activity (units/3×10^6 cell)
1	4-h PMN	99	none	2.7
	4-h PMN	99	1 mg/ml	11.0
	24-h PMN	95	none	0
	24-h PMN	95	1 mg/ml	<0.1
2	4-h PMN	98	1 mg/ml	10.9
	24-h PMN	96	1 mg/ml	<0.1
3	4-h PMN	99	1 mg/ml	12.1
	24-h PMN	97	1 mg/ml	<0.1

PEC were obtained 4 or 24 h after the i.p. injection of 2 ml of 0.2% casein. PMN were isolated and these preparations (3×10^6) were cultured for 3 h. Supernatants were assayed for thymocyte co-stimulation activity.

was further enhanced by treatment with kaolin.

Next we separated PMN from 4-h PEC by discontinuous gradient centrifugation on Percoll. The resulting PMN showed 98 to 99% purity. The main contaminant in this preparation was lymphocytes; contamination by macrophages was less than 0.02%. In the control, PMN were separated from 24-h PEC. The purity of the PMN was 95 to 97%; they were contaminated with 3–5% lymphocytes, macrophage contamination was less than 0.04%. The isolated 4-h and 24-h PMN were cultured (1×10^6/ml) with or without kaolin stimulation (1 mg/ml). As described above, the supernatant was harvested and varying amounts were assayed for thymocyte co-stimulation activity.

As shown in Table 2, PMN purity differed somewhat from experiment to experiment. Culture supernatants from 4-h PMN showed strong thymocyte co-stimulation activity.

Concentration of the active factor in 4-h PEF by ammonium sulphate

In a preliminary experiment, we confirmed that the thymocyte co-stimulation activity appeared only in fractions 9–12 at void volume. Later fractions (Nos 14 to 25) were devoid of this activity when 4-h PEF was eluted directly through a 60-ml Sephadex G-25 column (data not shown). To concentrate the active factor in the 4-h PEF, 24 ml of 4-h PEF from 12 mice (total optical density at E_{280} was 64.5) were brought to 33% saturation with $(NH_4)_2SO_4$ and the resulting precipitate was collected. The supernatant was then brought to 80% $(NH_4)_2SO_4$ saturation and the resulting precipitate was again collected. The supernatant after 80% saturation was concentrated by pressure dialysis. These three fractions were desalted on a Sephadex G-25 column as described above. The resulting fractions (Nos 9–11) were pooled for the biological test. The protein content and the thymocyte co-stimulation activity in these fractions are presented in Table 3.

The biological activity was present in the precipitate fraction between the 33% and 80% $(NH_4)_2SO_4$ saturation points. The precipitate with 33% $(NH_4)_2SO_4$ was devoid of the biological activity and the supernatant fraction after 80% saturation contained only 0.2% of the activity. Therefore, we used the 33–80% ammonium sulphate fraction in the subsequent experiments. The yield of biological activity into the active fraction was 225% of the original 4-h PEF, suggesting the

Table 3. Ammonium sulphate fractionation of 4-h PEF

Sample	Total O.D. at E_{280}	Thymocyte co-stimulation activity (total units)
4-h PEF	64.5	48.0
Precipitate with 33% ammonium sulphate	0.5	0
Precipitate with 33%–80% ammonium sulphate	45.0	108.0
Supernatant after ammonium sulphate	5.7	0.2

PEF (24 ml) was obtained from 12 mice at 4 h after the i.p. injection of 2 ml of 0.2% casein and subjected to ammonium sulphate fractionation.

removal of an inhibitory activity from the original PEF.

Biological nature of the inflammatory factor

The active fraction used here contained 40 units/ml thymocyte co-stimulation activity after concentration by ultrafiltration on a YM-5 Diaflo membrane. The IL-2 content in this sample was tested by assessing the proliferative response of an IL-2 dependent cytotoxic T cell line. The inflammatory factor was devoid of IL-2 activity in 400 μl samples (data not shown). Next we examined the active fraction for its ability to induce IL-2 in C3H thymocytes. Con A stimulated (5 μg/ml) thymocytes (1.5×10^7) were cultured for up to 72 h in the presence or absence of 200 μl (8 units) of the inflammatory factor. In the absence of inflammatory factor, a

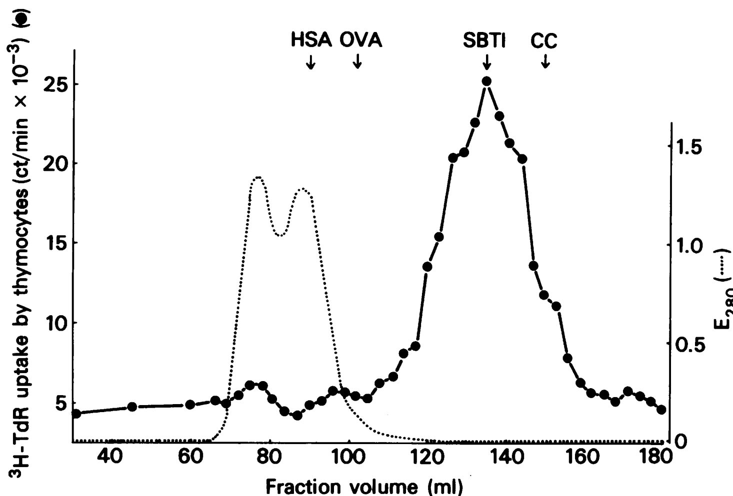

Fig. 5. Sephadex G-75 filtration of the inflammatory factor. Thymocyte co-stimulation activity in 200 μl of each fraction was expressed as the mean ^{3}H-TdR uptake by 3×10^6 thymocytes in the presence of 1 μl/ml PHA (triplicate cultures). Protein content of each fraction was monitored by absorbance at 280 nm (E_{280}). Kav values for marker molecules were as follows: 0.10 for HSA; 0.23 for OVA; 0.47 for SBTI and 0.62 for CC.

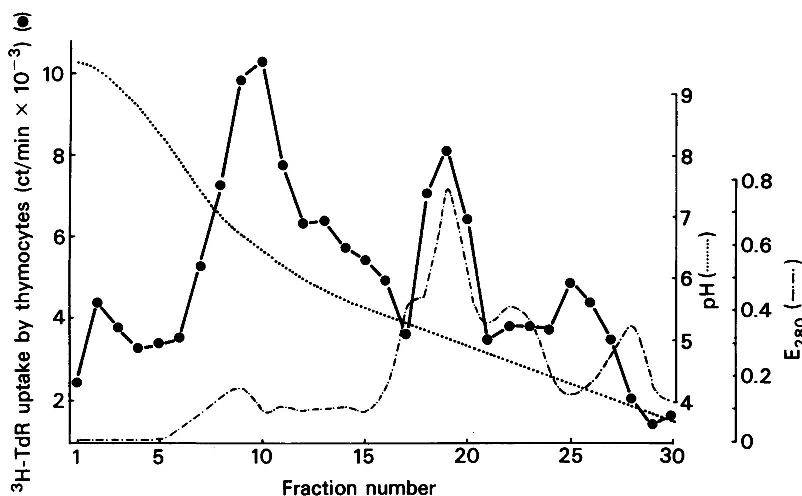

Fig. 6. Iso-electrofocusing of the inflammatory factor. Each fraction (200 μl) was tested for thymocyte co-stimulation activity expressed as the mean ^{3}H-TdR uptake by 3×10^6 thymocytes in triplicate cultures (●——●). Protein content of each fraction was monitored by absorbance at 280 nm (– · – · –); pH value of each fraction was presented (· · · · · · · ·).

weak IL-2 activity was observed in 18-h to 24-h thymocyte cultures. The IL-2 production at 18–24 h was greatly enhanced by the presence of the active fraction (data not shown).

Biochemical nature of the inflammatory factor

Four-hour PEF (20 ml) was precipitated with ammonium sulphate and the precipitate was admixed with 3 ml PBS. After brief dialysis against PBS, the precipitate fraction was eluted through a Sephadex G-75 column and 3-ml volumes of the effluent fractions were collected.

As shown in Fig. 5, the thymocyte co-stimulation activity showed a rather broad distribution profile, i.e. the activity appeared between the Kav value of 0.35 through 0.71. The molecular weight of the activity ranged between 10 000 and 30 000 daltons. This broad elution profile suggests that the active factor is heterogeneous.

The active ammonium sulphate fraction from 40 ml PEF was dialysed against 1% glycine and subjected to iso-electrofocusing at pH 3.5–10. Upon electrofocusing, the thymocyte co-stimulation activity separated mainly into two active fractions, one at a neutral pH range (peak at pH 6.5) and the other in a weakly acidic pH range (peak at pH 5.0) (Fig. 6).

Discussion

In the present report, we describe a thymocyte co-stimulation activity in casein-induced inflammatory peritoneal exudate of mice. This factor was functionally similar to IL-1. A concentrated active fraction from the 4-h inflammatory PEF revealed no IL-2 activity. In the presence of Con A stimulated thymocytes, the inflammatory factor induced the production of IL-2. This finding agrees with Smith *et al.* (1980) who showed that IL-1 acts as a mitogenic stimulus in lymphocytes by the induction of IL-2. The molecular size of the inflammatory factor ranged between 10 000 and 30 000 daltons suggesting it to be heterogeneous. Its molecular size is similar to that of murine macrophagic IL-1 (Gery & Handschumacher 1974; Economou & Shin 1978; Mizel 1979) and clearly different from that of murine IL-2 (Watson *et al.* 1979).

The IL-1 like activity in the inflammatory

peritoneal exudate was generated only during the early stage of inflammation. This coincided with the time during which the influx of PMN was observed. Therefore, we hypothesize that the PMN which have newly arrived at the inflammatory site may produce the active factor, while PMN which have been at the inflammatory site for some time may change their function and cease to produce the IL-1-like factor.

We demonstrated that purified PMN from the 4-h PEC could produce the IL-1-like activity *in vitro*. Therefore, we suggest that the IL-1-like activity in 4-h PEF and in culture supernatants of 4-h PEC is derived from PMN. However, the possibility remains that some of the IL-1-like activity may originate from macrophages for the 4-h PEC was contaminated with 3.7% macrophages. This number of macrophages, however, is incapable of producing the amount of IL-1-like activity we observed in the culture supernatant from 4-h PEC or in the 4-h PEF.

PEC obtained 24 h after casein injection produced less activity than 4-h PEC. Furthermore, purified PMN from 24-h PEC produced no IL-1-like activity, even after kaolin stimulation. This inability of the 24-h PMN to produce the IL-1-like activity coincides with the absence of the IL-1-like activity in 24-h PEF. At present we do not know why the IL-1-like activity was not detected in the 24-h PEF although 24-h PEC, especially macrophages, had the ability to produce the IL-1-like activity *in vitro*.

Our study showed that the function of PMN in the production of the IL-1-like activity differs according to the time lapsed following their influx into inflamed tissue. This observation is of interest in relation to findings by Hanson *et al.* (1980) who reported that purified rabbit PMN failed to produce endogeneous pyrogens (EP). There is persuasive evidence suggesting that IL-1 and EP are identical molecules (Rosenwasser *et al.* 1979; Murphy *et al.* 1980; Sztein *et al.* 1981). In their experiments, Hanson *et al.* (1980) used purified rabbit PMN from a 24-h inflammatory lesion. We suggest that the failure of purified rabbit PMN to produce EP may not be due to a species difference but rather to a difference in the timing of the PMN harvest after stimulation. It is possible that at later stages of inflammation, the PMN become exhausted or incapable of producing the factor through mechanism(s) as yet unknown. Alternatively the PMN active at the early stage of inflammation may be of a different subset from those in the later stage.

We previously reported the physicochemical properties of the IL-1-like factor in culture supernatant from murine 3-h PEC (Nakamura *et al.* 1982). The factor produced *in vitro* was composed of two iso-electrophoretically distinct, but gel-chromatographically similar factors. One is pI 9.8 with a molecular weight of 19 000 and the other is pI 5.4 with a molecular weight of 21 000. The active factor in the present 4-h PEF was mainly composed of two iso-electrophoretically different factors (pI 6.5 and 5.0). The reason for this difference in pI values is unknown at present. The pI value of the inflammatory factors may undergo a change due to the action of a hypothetical protease or a glycosidase at the inflammatory site.

Murine IL-1 from macrophages is microheterogeneous in both molecular size and pI value. The main components are pI 4·8 and pI 5·3 factors (Economou & Shin 1978; Mizel 1979) with molecular weights of 13 000 to 15 000. These pI values do not coincide with the pI value of the major inflammatory factor (pI 6.5).

The role of PMN in immune modulation has received little attention, although an interaction of PMN and lymphocytes may have important implications in the biological defence system. Several workers (Tchorzewski *et al.* 1973; Vischer *et al.* 1976; Yamasaki & Ziff 1977; Rodrick *et al.* 1982) have suggested that the participation of PMN in immune modulation and the generation of the IL-1-like activity in the early stage of inflammation may be related to immune enhancement during the inflammatory process. The direct and indirect splenic PFC responses were strikingly enhanced when an

antigen (SRBC) was injected at the early but not at a later, stage of inflammation. Inflammation-induced enhancement was reproducible by the adoptive transfer of early PEC, consisting mostly of PMN (Nakayama *et al.* 1982). Therefore, we propose that the IL-1-like factor produced in the early stage of inflammation may be of biological importance for immune enhancement *in vivo*. We reported previously on a IL-1-like factor present in synovial fluids of rheumatoid arthritis patients. That factor was found only during the active phase of the disease and correlated with the number of PMN in the RA synovial fluids, suggesting the IL-1-like factor to be of importance in that disease process also (Ise *et al.* 1982).

References

AARDEN L.A., BRUNNER T.K., CEROTTINI J.C. *et al.* (1980) Revised nomenclature for antigen–nonspecific T cell proliferation and helper factors. *Mol. Immun.* **17**, 641.

ECONOMOU J.S. & SHIN H.S. (1978) Lymphocyte-activating factor. I. Generation and physicochemical characterization. *J. Immun.* **121**, 1446.

GERY I., GERSHON R.K. & WAKSMAN B.H. (1972) Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. *J. exp. Med.* **136**, 128.

GERY I. & HANDSCHUMACHER R.E. (1974) Potantiation of the T-lymphocyte response to mitogens. III. Properties of the mediator(s) from adherent cells. *Cell. Immun.* **11**, 162.

GILLIS S., FERM M.M., OU W. & SMITH K.A. (1978) T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immun.* **120**, 2027.

HANSON D.F., MURPHY P.A. & WINDLE B.E. (1980) Failure of rabbit neutrophils to secrete endogenous pyrogen when stimulated with staphylococci. *J. exp. Med.* **151**, 1360.

ISE K., NAKAMURA S., OHKAWARA S. & YOSHINAGA M. (1982) DNA synthesis-potentiating activity on mouse thymocytes of synovial fluid of rheumatoid arthritis patients. *Acta. Path. Jap.* **32**, 491.

ISSEKUTZ A.C. & MOVAT H.Z. (1980) The *in vivo* quantitation and kinetics of rabbit neutrophil leukocyte accumulation in the skin in response to chemotactic agents and *Escherichia Coli. Lab. Invest.* **42**, 310.

MIZEL S.B., OPPENHEIM J.J. & ROSENSTREICH D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by a macrophage cell line, $P388D_1$. II. Biochemical characterization of LAF induced by activated T cells and LPS. *J. Immun.* **120**, 1504.

MIZEL S.B. (1979) Physicochemical characterization of lymphocyte-activating factor (LAF). *J. Immun.* **122**, 2167.

MIZEL S.B. (1980) Studies on the purification and structure-function relationships of murine lymphocyte activating factor (interleukin 1). *Mol. Immun.* **17**, 571.

MORGAN D.A. & RUSCETT F.W. (1976) Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science* **193**, 1007.

MURPHY P.A., SIMON P.L. & WILLOUGHBY W.F. (1980) Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte-activating factors made by rabbit alveolar macrophages. *J. Immun.* **124**, 2498.

NAKAMURA S., GOTO F., GOTO K. & YOSHINAGA M. (1982) Physicochemical characterization of a PMN-derived soluble factor that enhances lymphocyte DNA synthesis. *J. Immun.* **128**, 2614.

NAKAYAMA S., RODRIGUEZ-PINZON J., NAKAMURA S. & YOSHINAGA M. (1982) A possible role of PMN in a casein-induced enhancement of PFC response to sheep erythrocytes in mice. *Immunology* **45**, 669.

RODRICK M.L., LAMSTER I.B., SONIS S.T., PENDER S.G., KOLODKIN A.B., FITZGERALD J.D. & WILSON R.E. (1982) Effect of supernatants of polymorphonuclear neutrophils recruited by different inflammatory substances on mitogen responses of lymphocytes. *Inflammation* **6**, 1.

ROSENWASSER L.J., DINARELLO C.A. & ROSENTHAL A.S. (1979) Adherent cell function in murine T-lymphocyte antigen recognition. IV. Enhancement of murine T-cell antigen recognition by human leukocytic pyrogen. *J. exp. Med.* **150**, 709.

SMITH K.A., LACHMAN K.B., OPPENHEIM J.J. & FAVATA M.F. (1980) The functional relationship of the interleukins. *J. exp. Med.* **151**, 1551.

SZTEIN M.B., VOGEL S.M., SIPE J.D., MURPHY P.A., MIZEL S.B., OPPENHEIM J.J. & ROSENSTREICH D.L. (1981) The role of macrophages in the acute-phase response: SAA inducer is closely related to lymphocyte activating factor and endogenous pyrogen. *Cell. Immun.* **63**, 164.

TCHORZEWSKI H., SULOWSKA Z. & DENYS A. (1973) A new lymphocyte transforming factor derived from the lysosomes of polymorphonuclear leukocytes. *Experientia* **29**, 481.

VISCHER T.L., BRETZ U. & BAGGIOLINI M. (1976) *In*

vitro stimulation of lymphocytes by neutral proteinases from human polymorphonuclear leukocyte granules. *J. exp. Med.* **144**, 863.

WATSON J., GILLIS S., MARBROOK J., MOCHIZUKI D. & SMITH K. (1979) Biochemical and biological characterization of lymphocyte regulatory molecules. I. Purification of a class of murine lymphokines. *J. exp. Med.* **150**, 849.

YAMASAKI K. & ZITF M. (1977) Enhancement of *in vitro* immunoglobulin synthesis of human lymphocytes by lysosomal enzymes from polymorphonuclear leukocytes. *Clin. exp. Immun.* **27**, 254.