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Ciprofloxacin enhances T cell function by modulating interleukin activities

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SUMMARY

Ciprofloxacin (CIP) is a quinolone carboxylic acid derivative with a broad spectrum of antibacterial activity. CIP (0·1–30 μg/ml) enhanced DNA synthesis of mouse spleen cells and human peripheral blood lymphocytes (PBL) that had been activated with T cell mitogens or with alloantigens. In addition, CIP increased the amount of IL-2 found in the supernatants of phytohaemagglutinin (PHA)-stimulated human PBL. The presence of CIP in the medium (0·3–10 μg/ml) increased the levels of IL-1 found in the culture supernatants of adherence-enriched mouse macrophages, human monocyte/macrophages and a human monocytic cell line stimulated with lipopolysaccharide. In contrast there was no effect of CIP on the release of IL-1 by freshly isolated human monocytes or by cells of the keratinocyte line, A431. CIP alone had no influence on the basal release of IL-2 by NOB-1 cells, a T cell line that responds to IL-1 with an increase in IL-2 synthesis, but, in combination with recombinant IL-1, CIP significantly enhanced the release of IL-2 by these cells. The results of this study suggest that CIP modulates the immune response at two levels—the production of IL-2 by activated T cells and the production of IL-1 by activated monocyte/macrophages. However, CIP did not affect the primary antibody response *in vitro* or *in vivo* against sheep erythrocytes and ovalbumin respectively. Thus the enhancing action of ciprofloxacin on the immune system appears to be restricted to T cell function and macrophage/T cell interactions.

Keywords ciprofloxacin immune response lymphocyte monocyte macrophage interleukin

INTRODUCTION

Severe bacterial and fungal infections are often accompanied by impaired immune function. Clinical experience in patients with a primary or secondary immunodeficiency syndrome has clearly demonstrated that a successful antibiotic treatment of patients with bacterial infections depends on an effective host defence system based not only on non-specific cellular events like phagocytosis but also on specific humoral and cellular defence mechanisms of lymphocytes. However, a number of antibiotics have been shown to interfere detrimentally with the immune system (reviewed by Hauser & Remington, 1982; Potts *et al.*, 1983; Roszkowski *et al.*, 1985).

No such inhibitory effects have been seen with clinically relevant doses of ciprofloxacin (CIP) which is a new quinolone carboxylic acid derivative with a broad spectrum of antibacterial activity. These data were obtained in studies on *in vitro* phagocytic cell functions such as chemotaxis, phagocytosis and bactericidal activity (Wise, Andrews & Edwards, 1983; Delfino *et al.*, 1985). Indeed, a more recent study of the effects of fluorinated 4-quinolones on lymphocyte function showed that clinically achievable concentrations induce a pronounced hyperproduction of IL-2 by phytohaemagglutinin (PHA)-stimulated human lymphocytes (Riesbeck *et al.*, 1989). In this study, ciprofloxacin was tested for its effects on humoral and cellular immune responses and the production of cytokines known to be essential for B and T cell activation and proliferation. The results show that the cellular immune response of murine and human lymphocytes was significantly elevated by CIP in *in vitro* and *ex vivo* lymphocyte transformation experiments. We further show that this effect was paralleled by an increased synthesis of both IL-1 and IL-2. In contrast, the humoral arm of the immune response, evaluated in different test systems, remained unaffected.

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MATERIALS AND METHODS

Reagents

Ciprofloxacin (Lot No. 907 337) was provided by Dr H. Grohe, Bayer Leverkusen and contained neither stabilizer nor preservative. For *in vivo* experiments CIP was injected s.c. at daily intervals over a period of 7 or 9 days depending on the test model (see below). Human recombinant IL-2 (rhIL-2) was obtained from Boehringer-Mannheim and used as an internal standard and for maintenance of the IL-2 dependent T cell line. Recombinant human IL-1α was obtained from Genzyme (IC Chemikalien GmbH, Munich, Germany). Lipopolysaccharide (LPS) from *Salmonella typhimurium* was obtained from Difco (Biotest AG, Dreieich).

Cell lines

The mouse thymocyte NOB-1 line used in these studies was originally described by Gearing & Bird (1987) and obtained from the European Collection of Animal Cell Cultures (ECACC; Porton Down, UK). The IL-2 responsive mouse CTLL line and the human epidermal cell line A431 were also obtained from the ECACC. The human monocytic cell line MonoMac-6 was kindly provided by Dr Ziegler-Heitbrock and cultured according to the published method (Ziegler-Heitbrock *et al.*, 1988). All cell lines were maintained in RPMI 1640 (Flow GmbH, Meckenheim) supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine. In the case of CTLL this medium was also supplemented with 20 U/ml rhIL-2.

Animals

In-bred female BALB/c, DBA, C57Bl/6 and outbred NMRI mice 8–12 weeks of age were purchased from Bomholtgaard (Rye, Denmark). The animals were housed under standard conditions on a standard diet and water *ad libitum*.

In vivo *treatment by anti-Thy 1.2 MoAb*

The monoclonal anti-Thy 1.2 antibody (clone C) was injected at a dose of 0·3 ml i.p. 3 days before mice were killed and the spleens removed. *In vivo* treatment with anti-Thy 1.2 causes almost complete inhibition of the T lymphocyte stimulatory capacity in a mitogen stimulation assay with concanavalin A (Con A) and PHA (Opitz *et al.*, 1982; Oschilewski *et al.*, 1986).

Preparation of mouse spleen cells

Spleens were removed under sterile conditions and teased out with forceps into ice-cold balanced salt solution (BSS). After brief sedimentation to remove coarse particles, the cell suspension was washed twice in 20 ml of cold BSS and subsequently resuspended in culture medium (RPMI 1640 (Flow) supplemented with 2 mM L-glutamine and 5% FCS). Cell counts and viability testing by the dye exclusion method (0·5% trypan blue) were performed in a Neubauer haemocytometer.

Preparation of mouse peritoneal macrophages

Mice were killed and injected i.p. with 4 ml of ice-cold BSS supplemented with 10% FCS. After repeated massaging the abdominal cavity was opened aseptically and the lavage fluid of the peritoneum was aspirated with a syringe. Cells were pelleted by centrifugation, washed twice and the viable cell count was estimated. Cells were diluted to $1{\cdot}2 \times 10^6$/ml with RPMI 1640 containing 10% FCS and distributed in Costar 24-well cluster plates (1 ml per well). Plates were incubated overnight at 37°C. Non-adherent cells were carefully removed by repeated washes using warmed culture medium. The actual cell count of adherent cells was estimated by re-collecting and recounting the removed cells. The average cell count per well was approximately 4×10^5.

Preparation of human lymphocytes

Fresh peripheral blood lymphocytes (PBL) from healthy volunteers were isolated by Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) centrifugation (1·077 g/cm^3; 400 *g*, 40 min, 20°C). PBL were also stored in liquid nitrogen and recovered by rapid thawing at 40°C. They were then washed twice in 20 ml BSS containing human serum albumin (HSA) and resuspended in RPMI 1640 medium at $6{\cdot}6 \times 10^6$ or 5×10^6 cells/ml.

In addition, human peripheral blood monocytes were provided by Cell Diagnostica (Münster, Germany) after preparation by elutriation. The cells were cultured at 37°C in Teflon-coated bags in RPMI 1640 supplemented with 5% human serum.

Culture conditions for mitogenic activation

MSC (5×10^5) were cultured in 96-well cluster plates (Costar, Cambridge, MA) in 0·2 ml RPMI 1640 supplemented with 5% fetal calf serum and either 5 μg/ml Con A (Pharmacia), 10 μg/ml PHA (Wellcome GmbH, Burgdorf, Germany) or 20 μg/ml LPS.

PBL (1×10^5) were cultured under the same conditions in RPMI 1640 but supplemented with 10% human AB serum as previously described (Munker *et al.*, 1983). Cowan I strain of *Staphylococcus aureus* (SAC; Calbiochem GmbH, Frankfurt, Germany) was used as B-lymphocyte mitogen (Forsgren, Svedjelund & Wigzell, 1976).

^{3}H-thymidine (1 μCi, Amersham-Buchler, Braunschweig, Germany; specif. activity 20 Ci/mmol) was added to cells during the last 16 h of the culture period (72 h for MSC; 96 h for PBL). Cells were harvested using a Skatron cell harvester and the thymidine uptake determined by liquid scintillation spectrometry.

Culture conditions for allogeneic activation

Allogeneic mixed lymphocyte reactions (MLC) with mouse spleen cells were carried out after T lymphocyte enrichment using nylon wool columns. The method of pretreatment of the nylon wool (LP-1 Leuco pak Leukocyte Filter, Fenwal, USA), setting up of the column and the procedure of T cell purification was adapted from the method of Eisen, Wedner & Parker (1972).

Standard one-way MLC were assayed in 96-well cluster plates by mixing (1:1) nylon wool enriched MSC ($2{\cdot}5 \times 10^5$) of C57Bl/6 mice (=responder) with unseparated mitomycin-C-treated spleen cells of DBA (=stimulator). Syngeneic co-cultures served as internal controls.

During the last 16 h of the 96 h culture period, ^{3}H-thymidine (see above) was added, and thymidine uptake determined by liquid scintillation spectrometry.

Human MLCs were performed directly with Ficoll–Hypaque isolated PBL. Pretreated donor combinations (1×10^5 per donor) were used according to the procedure described previously (Munker *et al.*, 1983). During the last 16 h of the 6 day culture period ^{3}H-thymidine was added to monitor DNA synthesis.

Primary immune response in vitro *and measurement of antibody synthesis*

The primary immunization of cultured spleen cells with sheep erythrocytes was performed according to the method originally described by Mishell & Dutton (1967) and modified as previously described (Opitz *et al.*, 1982). When spleen cells of anti-Thy 1.2 pretreated mice were used for primary immunization in a T-cell-depleted Mishell-Dutton system, T-cell-replacing factor (TRF) was added 2 days after starting the culture (Schimpl & Wecker, 1972). A haemolytic plaque assay, originally described by Jerne & Nordin (1963), was used to quantify the antibody production on day 5 of culture.

Table 1. Effect of CIP on the stimulation of mouse splenocytes with PHA (mean values of triplicate samples ± s.d.)

Ciprofloxacin (μg/ml)	^{3}H-thymidine incorporation (ct/min × 10^3)	
	PHA	Control
0	76 ± 1.3	4 ± 0·2
1	81 ± 6·2	4 ± 0·7
3	79 ± 0·9	3 ± 1·3
10	109 ± 9·7*	6 ± 1·0
30	155 ± 2·5*	4 ± 0·8

* Statistically significant, $P \leqslant 0{\cdot}05$.

Table 2. Effect of CIP on the stimulation of human PBL with PHA (mean values of triplicate samples ± s.d.)

Ciprofloxacin (μg/ml)	^{3}H-thymidine incorporation (ct/min × 10^3)	
	PHA	Control
0	102 ± 7·0	0·3
1	110 ± 2·3	0·3
3	106 ± 0·4	0·4
10	145 ± 9·5*	0·3
30	171 ± 1·2*	0·4

* Statistically significant, $P \leqslant 0{\cdot}05$.

Immunization in vivo *and determination of specific antibody titres*
Mice were immunized with a suboptimal concentration of the soluble antigen ovalbumin (3 μg/mouse; s.c.). Ten days later, animals were bled. The titre of specific antibodies was determined in an indirect haemagglutination test, originally described by Onkelinx *et al.* (1969).

Bioassay for IL-1 and IL-2
A two-stage interleukin assay was used with NOB-1 as an IL-1 sensitive producer of IL-2 and CTLL as the responder cell line for IL-2 (Gearing & Bird, 1987). The amounts of IL-2 present in the test supernatants were calculated according to the area-under-the-curve (AUC) method described by Hewlett, Stünkel & Schlumberger (1989). IL-1 activity was estimated on the basis of IL-2 production by the NOB-1 cells. Before bioassay was carried out, all test supernatants were dialysed at +4°C against 100-fold volumes of culture medium (two changes over 2 days) in order to reduce levels of ciprofloxacin which might otherwise inhibit the assay system. Microbiological assays were carried out by Dr R. Endermann as detailed below and showed that such dialysed supernatants contained less than 0·01 μg/ml ciprofloxacin (data not shown).

Determination of serum levels of CIP
Measurement of drug levels in serum of mice (NMRI) was performed by an agar diffusion technique (Well method, Iso-Sensitest agar, *E. coli* ICB 4004 as test organism, Nunc-bioassay dishes). Each serum dilution, 0·1 ml, in phosphate-buffered saline was pipetted into the wells of plates inoculated with the test strain. After incubation for 18–24 h at 37°C the inhibition zones were measured and the amount of drug concentration calculated using a calibration standard on the same plate as reference.

Statistical analysis
Statistical analysis of differences was performed by Student's *t*-test (one-tailed) for independent samples.

RESULTS

Effect of CIP on T cell responses
The stimulation of murine spleen cells with the T cell mitogen PHA in the presence of increasing amounts of CIP (1–30 μg/ml) led to enhanced cell proliferation indicated by ^{3}H-thymidine

Table 3. Effect of CIP on the proliferation of human PBL in the mixed lymphocyte cultures (^{3}H-thymidine incorporation (ct/min × 10^3); mean values of triplicate samples ± s.d.)

Stimulator cells	Ciprofloxacin (μg/ml)				
	0	0·1	1	3	10
Donor 1					
Autologous	5 ± 3·1				
Allogeneic	17 ± 0·7	21 ± 1·7	29 ± 3·9	28 ± 1·2	32 ± 5·2
Donor 2					
Autologous	1 ± 0·5				
Allogeneic	42 ± 7·9	65 ± 8·9	89 ± 8·9	83 ± 1·2	72 ± 7·7

All values differ significantly from their respective controls.

Table 4. Effect of repeated subcutaneous treatment of mice with CIP on proliferation of splenocytes in response to mitogenic (PHA) and allogeneic stimulation *ex vivo* (^{3}H-thymidine incorporation (ct/min × 10^3); mean of triplicate samples ± s.d.)

Ciprofloxacin dose (s.c.) (mg/kg)	Stimulus			
	PHA	None	alloantigen	Syngeneic control
0	65 ± 2·9	0·5 ± 0·1	14 ± 0·1	2 ± 0·3
0·1	85 ± 7·6*	0·5 ± 0·1	15 ± 2·3	5 ± 0·9
1·0	109 ± 4·3*	0·9 ± 0·1	15 ± 1·5	4 ± 0·5
20·0	142 ± 2·9*	0·4 ± 0·1	25 ± 1·9*	3 ± 0·7
80·0	90 ± 8·1*	0·7 ± 0·1	23 ± 2·0*	3 ± 0·2

Cultures without mitogen or cocultures with syngeneic cells served as controls.
* Statistically significant, $P \leqslant 0{\cdot}05$.

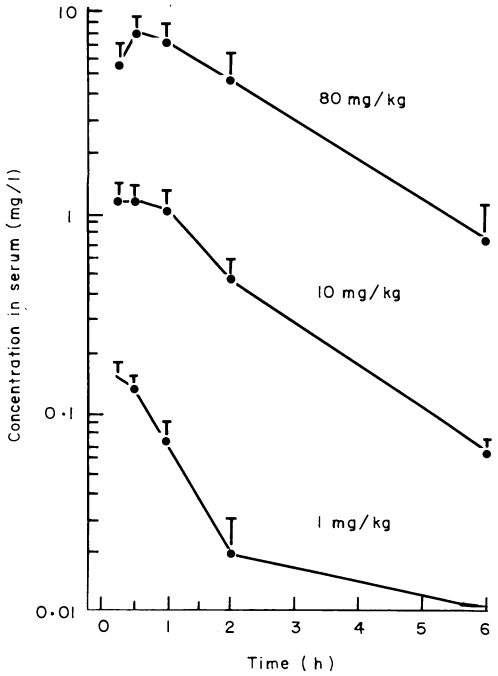

Fig. 1. Serum pharmacokinetics of ciprofloxacin in mice; NMRI mice were treated with a single subcutaneous administration of CIP at the indicated doses at time zero. Serum samples were obtained at the times shown and analysed for CIP activity in an agar diffusion test.

Table 5. Effect of CIP on the primary humoral immune response of mouse spleen cells to sheep erythrocytes in culture (plaque forming cells/10^7; mean of triplicate samples)

	Ciprofloxacin (μg/ml)					
Type of experiment	0	0·1	0·3	1	3	10
Spleen untreated	1990	2360	ND	2180	ND	2960
Spleen, T-depleted − TRF	115	220	155	190	110	180
Spleen, T-depleted + TRF	43200	39300	40680	36360	38380	36200

No values differ significantly from their respective controls.

uptake (Table 1). A less pronounced effect was seen when using the T cell mitogen Con A. Unstimulated cultures were not affected in the presence of CIP, i.e. CIP alone had no mitogenic potential. Enhanced T cell proliferation was also observed for human peripheral blood lymphocytes in the presence of PHA (Table 2). Again there was no CIP-dependent influence on unstimulated lymphocytes. The alloactivated one-way mixed lymphocyte culture (MLC) was chosen as a suitable test model for antigen-specific T cell proliferation. The results in Table 3 demonstrate an enhanced proliferative response of the activated human T cells at doses of 0·1–10 μg CIP/ml.

To evaluate the effect of chronic *in vivo* administration of CIP on lymphocyte functions, mice were treated daily with CIP at doses of 0·1–80 mg/kg subcutaneously for 7 days. On day 8 the mice were killed and cultures of their spleen cells were

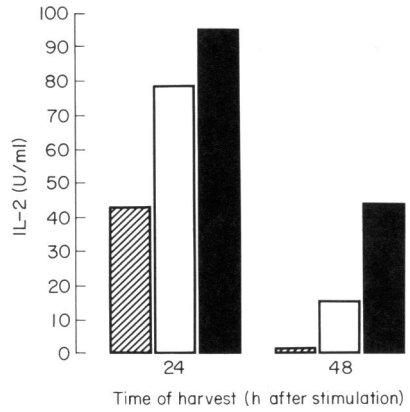

Fig. 2. Effect of CIP on levels of IL-2 in supernatants of PHA-stimulated human PBL; supernatants were harvested at the times shown, dialysed and analysed for IL-2 with the CTLL bioassay. ▨, Control; □, CIP 3 μg/ml; ■, CIP 30 μg/ml.

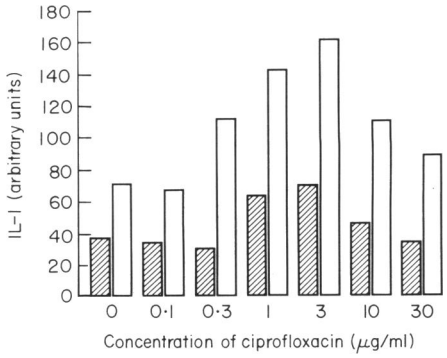

Fig. 3. Effect of CIP on levels of IL-1 in supernatants of mouse peritoneal macrophage cultures; supernatants of LPS-stimulated (□) and control (▨) macrophages were harvested, dialysed and assayed as described.

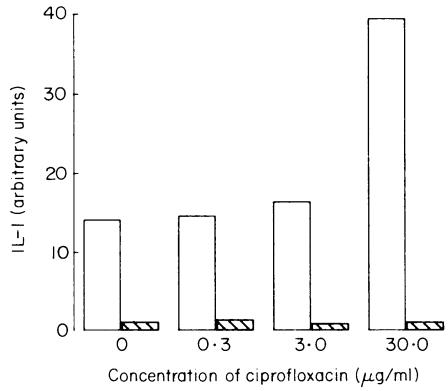

Fig. 4. Effect of CIP on levels of IL-1 in supernatants of the human MonoMac-6 cell line in the presence (□) and absence (▧) of 30 ng/ml lipopolysaccharide. Supernatants were assayed as described.

Table 6. Effect of CIP on the LPS-induced release of IL-1 (expressed in arbitrary units) by human PBL-monocyte cultures of different ages

Ciprofloxacin (μg/ml)	Age of monocyte cultures	
	0–3 days culture	28 days culture
10	25	36
3	42	92
1	44	70
0·1	48	107
Control	57	54

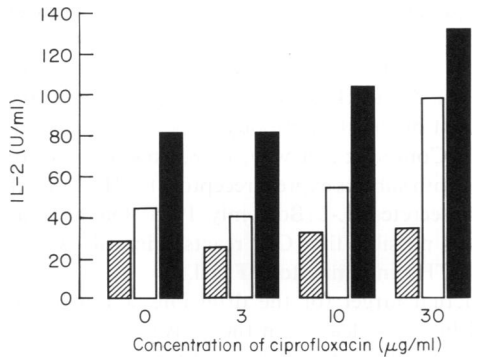

Fig. 5. Effect of CIP on the production of IL-2 by NOB-1 cells responding to defined concentrations of recombinant human IL-1; culture supernatants were harvested after 24 h of treatment, dialysed and analysed for IL-2. ■, without IL-1; □, 50 mU/ml IL-1; ■, 200 mU/ml IL-1.

stimulated non-specifically with the mitogen PHA. The results revealed that treatment of mice with 1–80 mg of CIP/kg body weight led to an enhanced T cell response (Table 4). Evaluation of serum levels in mice after subcutaneous administration of 1, 10 and 80 mg CIP/kg revealed that, at the highest dose, mean peak concentrations of 8 mg/*l* were obtained 30 min after injection of the drug. With the 10 or 1 mg/kg dose, concentrations of approximately 1 and 0·15 mg/*l*, respectively, were measured 30 min after administration of ciprofloxacin (Fig. 1). These concentrations are similar to those measured after oral administration of a 500 or 750 mg dose to humans (Gonzales *et al.*, 1984; Wise *et al.*, 1984).

Ex vivo alloantigen-stimulated T cells from these pretreated mice also showed a significant increase in ^{3}H-thymidine incorporation (Table 4).

Effect of CIP on the primary humoral immune response

Polyclonal stimulation of splenocytes or human PBL with LPS or SAC showed that B cell proliferation was not affected by CIP at concentrations between 0·1 μg/ml and 30 μg/ml (data not shown). In order to investigate further the interaction of CIP with B cells, the influence of CIP on the specific humoral immune response was tested in the Mishell–Dutton system using sheep erythrocytes as a T-cell-dependent, particulate antigen. CIP did not modulate the development of antibody-producing cells in this *in vitro* system. Similar observations were made in the T cell-depleted Mishell–Dutton system, with or without the addition of T cell replacing factor (Table 5). Even a 9 days treatment of mice with CIP at dosage of 0·1–80 mg CIP/kg injected s.c., as described above, did not influence the *ex vivo* antibody production in the Mishell–Dutton culture of unprimed splenocytes (data not shown).

Additional studies were carried out *in vivo* using ovalbumin as soluble antigen. Immunized mice were treated with CIP daily for a perioid of 9 days (dose range 0·1–80 mg/kg body weight) immediately following immunization but there was no effect of treatment on the agglutinating antibody titre (data not shown).

Effect of CIP on lymphokine (IL-1, IL-2) production

The above results demonstrate that CIP influences T-cell-related functions more than those of B cells. Since it is known that T cells produce and respond to a variety of cytokines we tested the effect of CIP on the synthesis of two such factors, IL-1 and IL-2.

Figure 2 shows that the presence of CIP in cultures of PHA-stimulated human PBL led to a dose-dependent increase in the levels of IL-2 present in the respective supernatants as assayed in the CTLL bioassay. Even after 48 h of stimulation with PHA, when the control cultures showed low levels of IL-2 (presumably due to consumption of IL-2 by the dividing T cells), the CIP-treated cultures still exhibited significant levels of IL-2 activity. Thus the increased mitogenic response of such lymphocytes (see Table 2) is paralleled by a hyperproduction of IL-2.

Macrophages release IL-1 after stimulation with bacterial lipopolysaccharide, The presence of CIP (0·3–30 μg/ml) increased the levels of IL-1 found in the culture supernatants of adherence-enriched murine peritoneal macrophages stimulated with LPS at a dose range of 0·3–3 μg CIP/ml (Fig. 3). The most effective concentration was 3 μg CIP/ml and a fall in IL-1 levels was seen at CIP concentrations of 10 μg/ml and above. There was also a slightly enhanced release of IL-1 in the absence of LPS stimulation at concentrations of 1–10 μg CIP/ml.

When we examined primary cultures of human peripheral blood monocytes we did not observe any stimulatory effect of CIP until the cells had first been in continuous culture for a period of 4 weeks before treatment with LPS and CIP (Table 6). There is a remarkable similarity between the responses of the mouse macrophages to CIP (Fig. 3) and those of the long term cultures of human peripheral blood monocytes (Table 6) in that the IL-1 production of both populations was stimulated by concentrations of CIP up to 3 μg/ml and that there was a reduction in levels of secreted IL-1 at 10 μg/ml CIP.

In order to confirm the effect of CIP on the macrophage/monocyte cell and to exclude the possibility that the observed effects were due to contaminating lymphocytes in the various macrophage/monocyte preparations, we examined the response of the human monocytic cell line, MonoMac-6, to CIP. Figure 4 shows that the LPS-induced release of IL-1 by MonoMac-6 is greatly affected by CIP.

Since CIP has such a significant effect on IL-1 production it was of interest to examine whether the antibiotic would also modulate IL-1 production by a non-haematopoietic cell. When A431 keratinocytes were incubated with CIP for 24 or 72 h and the supernatants examined for IL-1 activity, there was no obvious effect of the antibiotic on the amount of IL-1 released into the medium (data not shown).

In titrations with NOB-1 cells, a T cell line which shows an increased secretion of IL-2 in response to IL-1, it was found that levels of released IL-2 were significantly increased in cultures containing 10 or 30 μg CIP/ml (Fig. 5). In the absence of IL-1, CIP had only a marginal effect.

DISCUSSION

The present report demonstrates that the quinolone derivative, ciprofloxacin, augments ^{3}H-thymidine incorporation by mouse splenocytes and human PBL stimulated with T cell mitogens or alloantigens. This effect was even more pronounced after administration of the compound to mice for a period of 7 days before testing *in vitro*. These findings are not in accordance with the results of Gollapudi, Prabhala & Thadepalli (1986) who did not see any effect of the compound on non-specifically stimulated mouse spleen T cells and human peripheral blood T cells. Our findings do, however, confirm the results of Forsgren and collaborators (1986) who were also interested in investigating the influences of CIP on human lymphocytes stimulated with the T cell mitogen PHA. They observed an increase in ^{3}H-thymidine incorporation at concentrations of CIP in the range of 0·8 to 50 μg/ml. In contrast to our findings, they found an enhancing effect of CIP on polyclonally proliferating B cells, too. We therefore investigated specifically induced primary immune responses against sheep erythrocytes *in vitro* and against the soluble antigen ovalbumin *in vivo*. None of these tests revealed influences of CIP on primary humoral immune responses.

These observations led to a more detailed investigation of the effects of CIP on T cell activation and proliferation. It is well documented that IL-1 and IL-2 are essential cytokines for T cell activation and proliferation, so the influence of CIP on the release of both cytokines was studied *in vitro*. The results revealed that the production of IL-2 by stimulated human PBL is significantly increased in the presence of CIP, even at a dose of 3 μg/ml. This increase in IL-2 production appears to be the result of a two-step process. The dissection of the activity of CIP showed that the first event occurs at the macrophage/monocyte level. Activated mouse peritoneal macrophages, 4-week-old human monocyte cultures and the human MonoMac-6 cell line released significantly more IL-1 into the culture supernatant in the presence of CIP than in its absence. In contrast, CIP does not appear to influence IL-1 production of freshly isolated human peripheral blood monocytes or of cells like A431 keratinocytes which have also been shown to produce IL-1 activity (Luger, Köck & Danner, 1985). This suggests that the monocyte/macrophage has to attain a certain level of differentiation before it can respond to CIP. Our data also suggest that, on the basis of IL-1 production, the MonoMac-6 cells behave more like macrophages than monocytes.

The second interaction of CIP is at the T cell level itself. This was demonstrated with NOB-1 cells in the presence of IL-1 although in this case significant effects were first seen at concentrations of CIP above 3 μg/ml. In contrast to our results, Roche *et al.* (1987a, b) did not find any effects of therapeutically relevant concentrations (<25 μg/ml; Bergan, Dalhoff & Rohwedder, 1988) of CIP on lymphocyte proliferation. However, they obtained similar results to ours when stimulated T cells were investigated for their release of IL-2 in the presence of the antibiotic (Roche, Fay & Gougerot-Pocidalo, 1988). In addition, their data supports our results with freshly cultured human blood monocytes in that they did not find any influence of the quinolone on the production of IL-1 by human monocytes at a concentration of 10 μg/ml. More recently, Riesbeck *et al.* (1989) demonstrated that fluorinated 4-quinolones not only resulted in a hyperproduction of IL-2, there was also a significant elevation in the level of specific human IL-2 mRNA in treated cells.

The results obtained in the mouse system indicate that CIP influences T cells but not B lymphocytes. This means that the increased T cell proliferation indicated by the enhanced ^{3}H-thymidine uptake leads to an expanded T cell subpopulation which does not have any modulating effect on B cell differentiation and function during the primary humoral immune response. Functional and phenotypical dissection of CD4$^+$ T cells has revealed that in fact at least two CD4$^+$ T cell subpopulations exist. One of these released IL-2, IFN-γ and TNF-β but no IL-4 (defined as CD4$^+$ TH1/CTL in the murine system which might have its equivalent in the CD4$^+$2H4$^+$ in the human system), the other subset (CD4$^+$TH2 which may have its equivalent in CD4$^+$2H4$^-$ or CD4$^+$UCHL1$^+$ cells, respectively) releases IL-4 as well as IL-5 and IL-6 which are cytokines essential for the development of specific antibody responses (Cherwinski *et al.*, 1987; Poo, Conrad & Janeway, 1988; Powrie & Mason, 1988). However, both subsets express receptors for IL-2 and, therefore, respond to secreted IL-2 (Bottomly, 1988). On the basis of these reports it is possible that CIP reacts primarily with the IL-2-producing TH1/inflammatory T cell.

The actual target for the drug interaction has yet to be identified but it is clear from the above results that CIP only affected the function of cells that had previously been activated.

Most antimicrobial agents do not affect T or B lymphocytes but some inhibit mitogenic, cellular and humoral immune responses (Forsgren & Banck, 1978; Roszkowski *et al.*, 1985). The observation that activated mouse and human T lymphocytes show increased proliferation, irrespective of the mode of their activation, in the presence of ciprofloxacin emphasizes the potential importance of this new quinolone for the treatment of immune-compromised patients. This conclusion is supported by pharmacokinetic data obtained from healthy volunteers and patients which show that administration of therapeutic doses of ciprofloxacin leads to concentrations of substance in serum and tissues which, in our hands, affected specific T cell functions (Bergan *et al.*, 1988; Gonzales *et al.*, 1984; Wise *et al.*, 1984).

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