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Aryl-Fluoroquinolone Derivatives A-56619 (Difloxacin) and A-56620 Inhibit Mitogen-Induced Human Mononuclear Cell Proliferation

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Aryl-fluoroquinolone derivatives A-56619 (difloxacin) and A-56620 were found to inhibit human peripheral blood mononuclear cell (MNC) proliferation (measured by [^{3}H]thymidine uptake) that was induced by concanavalin A or monoclonal antibody OKT3. These antimicrobial agents exert their maximum suppressive effect when added within the first 24 h after the onset of culture with concanavalin A. No increase in the concentration of mitogen or the duration of incubation of MNC cultures reversed this inhibitory effect, but the removal of the drug from cultures reversed the suppression of DNA synthesis. A-56619 appeared not to interfere with the triggering of MNC activation by mitogen because it did not inhibit mitogen-induced increase in protein synthesis (measured by [^{3}H]leucine incorporation), interleukin-2 receptor expression (measured by the binding of fluorescein-conjugated monoclonal antibody against interleukin-2 receptor), and cell volume. These findings are considered in terms of possible interference of aryl-fluoroquinolones with mammalian topoisomerase and DNA polymerases.

Certain cephalosporins, such as cephalothin, cephalexin, cephradine, cefamandole, cephapirin, cefoxitin, moxalactam, and cefuroxime, and tetracyclines, such as doxycycline and minocycline, have been shown to produce a dose-dependent suppression of human peripheral blood mononuclear cell (MNC) proliferation induced by polyclonal mitogens concanavalin A (ConA) and phytohemagglutinin (1, 6, 12, 15, 16, 18). However, penicillins, including carbenicillin and benzylpenicillin, do not inhibit the proliferative responses of MNCs (1). These reports indicate that some antibiotics adversely affect the metabolic processes of MNCs and consequently may interfere with their function.

Quinolones represent a new class of antimicrobial agents derived from nalidixic acid, with broad-spectrum antimicrobial activity (20). Relatively little is known about the effects of quinolones on the proliferative functions of MNCs. We previously reported that ciprofloxacin, a carboxy quinolone derivative, had no adverse effects on mitogen-induced MNC proliferation (9). In this study, we examined the influence of two new aryl-fluoroquinolone derivatives, A-56619 (difloxacin) and A-56620, on MNC activation and proliferation induced by ConA and monoclonal antibody OKT3 (OKT3 MAb).

Triggering of MNCs by mitogens can be operationally divided into the following stages: (i) activation or exit from G_0 and (ii) commitment to DNA synthesis with different signal requirements (2, 5, 14). The results reported in this communication show that both A-56619 and A-56620 block the entry of activated cells into S phase (DNA synthesis).

MATERIALS AND METHODS

Antimicrobial agents. A-56619 and A-56620 were supplied by Abbott Laboratories, North Chicago, Ill. Prior to use, the drugs were dissolved in culture medium, and the pH of the medium was adjusted to 7.4.

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Mitogens. Concanavalin was purchased from Sigma Chemical Co., St. Louis, Mo., and monoclonal pan T-cell antibody OKT3 was purchased from Ortho Diagnostics, Inc., Raritan, N.J.

Cell culture medium. Medium 199 (Irvine Scientific, Irvine, Calif.) containing 10% heat-inactivated fetal calf serum (Irvine Scientific), 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) at pH 7.4, and 2 mM glutamine, hereafter termed culture medium, was used in all experiments.

Preparation of MNCs. Heparinized (20 U/ml) peripheral blood was obtained from healthy volunteers, and MNCs were isolated by Ficoll-Hypaque density gradient centrifugation (4). The cells were washed three times with Hanks balanced salt solution (Irvine Scientific) and suspended in culture medium.

Cell proliferation assay. Proliferation was assessed by measuring [^{3}H]thymidine uptake in mitogen-stimulated lymphocytes as previously described (10). Cells were cultured in triplicate in 96-well round-bottom tissue culture plates (Linbro Plastics, Flow Laboratories, McLean, Va.) at 37°C in humidified 5% CO_2 in the presence or absence of ConA (5 μg/ml) or OKT3 MAb (25 ng/ml) with or without A-56619 or A-56620. The concentrations of A-56619 and A-56620 ranged between 1 and 25 μg/ml, and the drugs were added either at the beginning of the culture or at various times during the culture period. At 24 h prior to termination of culture, 1 μCi of [^{3}H]thymidine (specific activity, 6.9 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added to each well. The cultures were harvested onto a glass fiber filter by using an automated cell harvester (Skatron, McLean, Va.), and [^{3}H]thymidine incorporation was assessed by a liquid scintillation counter. Variation among triplicates did not exceed ±15%.

Protein synthesis. MNCs (10^6/ml) were cultured in tissue culture tubes (12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.) with or without ConA (5 μg/ml) in the presence or absence of A-56619 (25 μg/ml) for 42 h. The cells

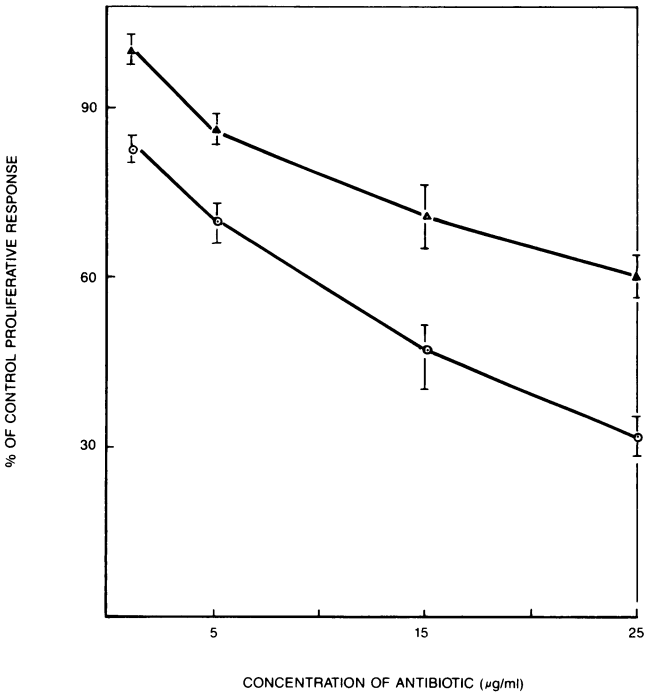

FIG. 1. Effects of A-56619 and A-56620 on ConA-induced proliferation. Cells were cultured in the presence or absence of indicated concentrations of A-56619 (⊙) or A-56620 (△) and were stimulated with ConA (5 μg/ml). Each point shows the mean ± the standard error of the mean of six experiments, with each experiment done in triplicate on MNCs from a different donor. The data are normalized to the [^{3}H]thymidine incorporation in MNCs cultured with ConA alone and are expressed as % of control = (cpm of MNC + ConA + drug/cpm of MNC + ConA) × 100.

were then washed three times with Hanks balanced salt solution, suspended in leucine-deficient medium, and incubated for 1 h at 37°C. [^{3}H]leucine (1.5 μCi; specific activity, 50 μCi/mmol; ICN Pharmaceuticals, Inc., Costa Mesa, Calif.) was added, and the cells were incubated for 4 h more. At the end of the incubation, cells were centrifuged, and the supernatant containing radioactivity was discarded. Cells were washed twice with phosphate-buffered saline, and the cell pellet was suspended in 180 μl of phosphate-buffered saline and 20 μl of carrier protein (bovine serum albumin, 1 μg/ml). Trichloroacetic acid (200 μl) was then added, and labeled proteins were precipitated in the cold for 1 h. The resultant precipitate was washed twice with 5% ice-cold trichloroacetic acid, incubated with 5% trichloroacetic acid at 90°C for 25 min, boiled in 2% sodium dodecyl sulfate for 1 min, and counted in Aquasol scintillation fluid, and the incorporation of [^{3}H]leucine was measured in a liquid scintillation counter. All experiments were performed in triplicate.

IL-2 receptor expression. Fluorescein-conjugated monoclonal antibody against IL-2 receptor, Tac, was purchased from Becton Dickinson and Co., Sunnyvale, Calif. The binding of anti-Tac to activated lymphocytes was analyzed by direct immunofluorescence with a fluorescence-activated cell sorter (FACS) analyzer (Becton Dickinson). MNCs (10^6) were cultured in the presence or absence of 25 μg of A-56619 per ml and were stimulated with MAb. At 24 h after incubation, the cells were washed three times with Hanks balanced salt solution containing sodium azide (0.1%), suspended in 100 μl of the appropriate dilution of fluorescein-conjugated anti-Tac, and incubated for 30 min at 4°C. The cells were then washed, suspended in phosphate-buffered saline containing 0.1% azide, and evaluated with a FACS analyzer. Fluorescence data were collected for 10^4 cells.

Cell volume determination. Cell volume analysis was performed with a FACS analyzer by the technique described by Herzenberg and Herzenberg (13). MNCs (10^6/ml) were cultured with or without OKT3 MAb (25 ng/ml) in the presence or absence of A-56619 (25 μg/ml) for 18 h. The cells were then washed two times and suspended in culture medium, and cell volume was determined with a FACS analyzer.

RESULTS

The effects of A-56619 and A-56620 on ConA- and OKT3 MAb-induced MNC proliferation as assessed by [^{3}H]thymidine incorporation are shown in Fig. 1 and Fig. 2. In these experiments, MNCs were incubated with 1 to 25 μg of A-56619 or A-56620 per ml with optimal concentrations of ConA or OKT3 MAb. Each drug was tested on cells from six normal subjects. Both A-56619 and A-56620 were inhibitory at and above 5 μg/ml to cells of all subjects.

To exclude the possibility that the suppression was due to toxicity of drugs to MNCs, viability was assessed in controls containing drugs at the highest concentrations. At the end of a 3-day culture period, 91 to 92% of the cells cultured with 25 μg of A-56619 or A-56620 per ml were found to be viable as judged by the trypan blue exclusion method. This concentration of A-56619 and A-56620 caused substantial inhibition of [^{3}H]thymidine incorporation (Table 1).

To assess the reversibility of antibiotic-induced suppression, MNCs were incubated for 3 h in the presence of A-56619 or A-56620, washed to remove these drugs, suspended in fresh culture medium, and cultured with ConA. Such treatment had no inhibitory effect on the subsequent proliferative response of the cells (Table 2), suggesting that

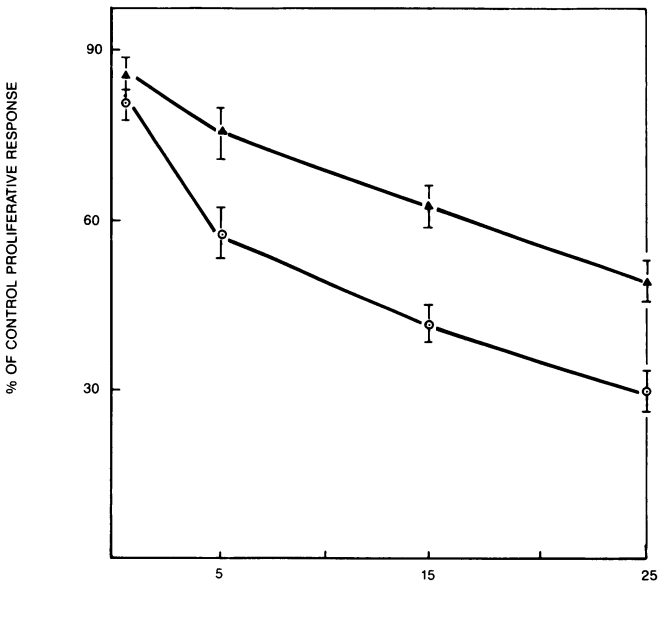

FIG. 2. Effects of A-56619 and A-56620 on OKT3-induced proliferation. A-56619 (⊙) and A-56620 (△) were added at culture initiation, and [^{3}H]thymidine incorporation was measured at 72 h. Results are shown for the mean of six experiments ± the standard error of the mean.

TABLE 1. Viability of lymphocytes cultured in the presence of A-56619 and A-56620

Cells cultured[a] with:	% Viability	[^{3}H]thymidine incorporation (cpm)
Control	88	1,906
ConA	91	33,968
ConA + A-56619	92	12,251
ConA + A-56620	91.5	18,339

[a] MNC were incubated at 37°C for 72 h in the presence of ConA (5 μg/ml) and 25 μg of A-56619 or A-56620 per ml. Control MNCs were cultured in medium alone for the same length of time. Viability was assessed by the trypan blue dye exclusion method. The results represent the mean value of cells cultured in triplicate in one representative experiment.

inhibitory effects were reversible and required the continuous physical presence of quinolone derivatives.

To determine whether the decrease responses of MNCs in the presence of quinolone derivatives reflected a dose or a kinetics phenomenon, various mitogen concentrations and incubation periods were examined. The data in Table 3 show that the inhibitory effects of A-56619 and A-56620 were not dependent on the concentration of mitogen. Figure 3 shows data from representative experiments on the effect of duration of culture on quinolone derivative-induced inhibition of DNA synthesis. Clearly, suppression was consistent through 5 days of the culture period, indicating that the inhibition exerted by A-56619 and A-56620 was not a result of an alteration of the time course of proliferative response.

Mitogens must remain in contact with the lymphocyte membrane for at least 10 to 20 h to irreversibly commit the cells for proliferation (3, 11). To exclude the possibility that A-56619 and A-56620 interfered with the binding of mitogen to cell surface receptors, the effect of delayed addition of these drugs to MNC cultures was studied. A-56619 and A-56620 were added to separate cultures at various times after ConA addition and were left in culture until [^{3}H]thymidine incorporation was measured. A significant inhibition was observed when the drugs were added during the first 24 h of culture (Fig. 4). No significant inhibition was observed when these agents were added at 48 or 72 h after the addition of ConA. These results indicate that A-56619 and A-56620 do not block mitogen interaction with cell surface receptors, but interfere with the generation of subsequent events involved in cell activation and proliferation.

To evaluate whether or not quinolone derivatives interfered with early events in cell activation, the effect of A-56619 on mitogen-stimulated protein synthesis, expression of IL-2 receptors, and increase in cell volume was examined. The data in Table 4 show that A-56619 did not inhibit protein synthesis as measured by [^{3}H]leucine incorporation into acid-insoluble protein at a concentration that markedly inhibited DNA synthesis. The results in Table 5 show that OKT3-induced IL-2 receptor expression, as identified by anti-Tac binding, was not inhibited by an addition of 25 μg of A-56619 per ml. This dose of drug caused inhibition of [^{3}H]thymidine incorporation in the samples of the same culture used for fluorescence Tac analysis. Parallel cultures were also analyzed with the FACS analyzer for increase in cell volume. The data in Fig. 5 show that A-56619 had no effect on OKT3-induced increase in cell volume.

TABLE 2. Reversibility of A-56619- and A-56620-induced inhibition of MNC proliferation[a]

Cells preincubated with:	Washed cells cultured with:	[^{3}H]thymidine incorporation (cpm ± SD)
Nothing	Nothing	1,684 ± 240
ConA	ConA	23,401 ± 1,357
ConA + A-56619	ConA + A-56619	6,563 ± 351
ConA + A-56619	ConA	21,645 ± 1,741
ConA + A-56620	ConA + A-56620	13,098 ± 560
ConA + A-56620	ConA	23,493 ± 2,755

[a] MNC were preincubated for 3 h in the presence or absence of A-56619 (25 μg/ml) or A-56620 (25 μg/ml) with or without ConA (5 μg/ml), washed, and recultured as indicated. Results represent mean [^{3}H]thymidine incorporation in cells cultured in triplicate.

TABLE 3. Effect of concentration of mitogen on drug-induced inhibition of proliferation of MNCs[a]

Concn of mitogen (μg/ml)	% ConA response with:	
	A-56619	A-56620
1	24.5	32
5	26.5	30
10	21.7	30.6

[a] MNCs were cultured in the presence or absence of A-56619 (25 μg/ml) or A-56620 (25 μg/ml) and were stimulated with the indicated concentrations of ConA. Thymidine incorporation was measured at 72 h, and the results, expressed as a percentage of the control response, were determined by the formula (cpm in cultures containing experimental antibiotic/cpm in cultures without antibiotic) × 100. The incorporation of [^{3}H]thymidine in MNCs stimulated with 1, 5, or 10 μg of ConA per ml in the absence of quinolone derivatives was 17,000, 37,599, and 35,825 cpm, respectively. Results represent the mean of two experiments.

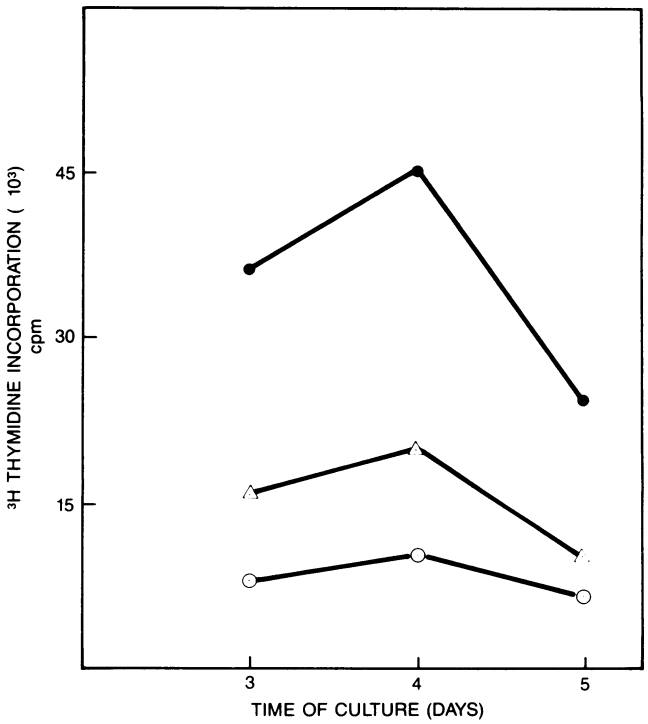

FIG. 3. Effects of quinolone derivatives on the proliferative response on different days of cultures. MNCs were stimulated with 5 μg of ConA per ml in the absence (●) or presence of 25 μg of A-56619 (⊙) or A-56620 (△) per ml for the indicated times. Proliferation was assessed by measuring [^{3}H]thymidine uptake, and results represent the mean of triplicate cultures.

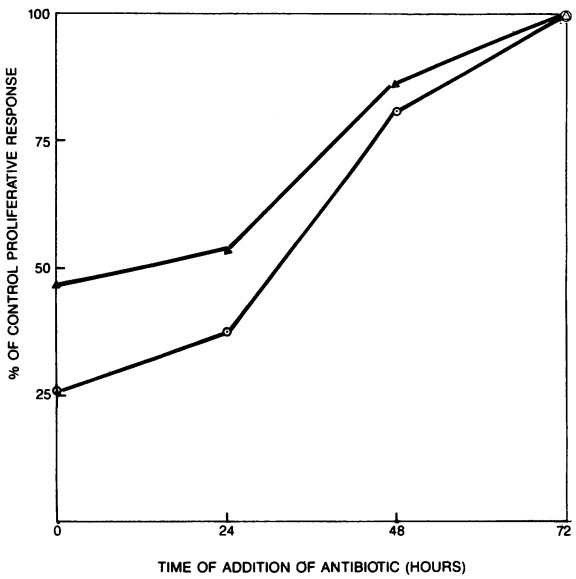

FIG. 4. Temporal sensitivity to drug inhibition of proliferation. ConA was added at time zero, and 25 μg of A-56619 (○) or A-56620 (△) per ml was added at the times shown on the abscissa. Each point is the mean of two experiments, with each experiment done in triplicate on MNCs from different donors. The data are normalized to the [^{3}H]thymidine incorporation in ConA-activated MNCs cultured in the absence of drugs.

DISCUSSION

The results presented herein demonstrate that arylfluoroquinolone derivatives A-56619 and A-56620 inhibit proliferation (DNA synthesis) of MNCs stimulated by ConA and OKT3 MAb. Rosoxacin, (J. P. Manzella and J. Clark, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 485, 1985) and ofloxacin (C. DeSimone, A. Cilli, A. Zanzoglu, L. Lucci, S. Delia, and F. Solice, 14th Int. Congr. Chemother., S-17-8, 1985) have been reported to cause significant depression of mitogenic responses of lymphocytes. However, ciprofloxacin, amifloxacin, norfloxacin (Manzella and Clark, 25th ICAAC), and nalidixic acid (1) appear to have no inhibitory effects on MNC proliferation. The reason for these differences is not clear. They may be due to a failure of certain quinolone derivatives to penetrate into or accumulate in cells responding to T-cell activators. Two other possibilities are that ciprofloxacin, amifloxacin, norfloxacin, and nalidixic acid either do not interact with MNCs or are inactivated by MNCs.

Several lines of evidence presented in this study indicate that suppression of DNA synthesis by A-56619 and A-56620 is unlikely to be a result of nonspecific cytotoxicity because (i) the cell viability, as judged by trypan blue exclusion, was unaffected at concentrations that inhibited DNA synthesis (Table 1); (ii) the inhibition due to A-56619 and A-56620 was reversible on their removal from cell culture (Table 2); (iii) cells cultured in the presence of A-56619 and stimulated with mitogen continued to synthesize proteins to the same extent as cells cultured in the absence of A-56619 (Table 4); and (iv) the inhibitory effects of A-56619 and A-56620, which were substantial when either drug was added at time zero or 24 h after culture, decreased with delayed addition (Fig. 4). Taken together, the above data suggest that the suppression of DNA synthesis is likely due to interference of A-56619 and A-56620 with cellular events leading to cell proliferation.

Our findings that the observed suppression could not be circumvented by increasing mitogen concentration (Table 3) and that these drugs significantly inhibited proliferation even when added 24 h after the addition of mitogen supports the theory that A-56619 and A-56620 do not interfere with ligand-receptor interaction but, rather, block subsequent triggering and entry of cells into the cell cycle. The facts that A-56619 did not inhibit mitogen-induced protein synthesis (Table 4), expression of receptors for cell growth factor (Table 5), and increase in cell volume (Fig. 5) suggest that this quinolone derivative does not interfere with mitogen triggering of resting cells to activation (G_0-G_1 phase) but, rather, blocks the entry of activated cells into S phase (DNA synthesis).

The mechanism(s) by which A-56619 and A-56620 interfere with DNA synthesis is not known. In cell-free systems,

TABLE 4. Effects of A-56619 and A-56620 on protein synthesis[a]

Mitogen	Addition (25 μg/ml)	Incorporation (cpm ± SD) of:	
		[^{3}H]leucine	[^{3}H]thymidine
None	None	4,495 ± 525	1,099 ± 280
ConA	None	15,559 ± 1,967	35,825 ± 2,410
ConA	A-56619	15,549 ± 3,918	9,881 ± 844
ConA	A-56620	18,107 ± 2,591	11,091 ± 108

[a] MNC cultures were stimulated with 5 μg of ConA per ml, either alone or in the presence of indicated concentrations of A 56619 or A 56620. [^{3}H]leucine uptakes were assessed on day 2, and [^{3}H]thmidine uptakes were assessed on day 3. The results represent the mean of triplicate values with the standard deviation.

TABLE 5. Effect of A-56619 on OKT3-induced IL-2 receptor expression[a]

Additive	% Tac-positive cells	[^{3}H]thymidine incorporation (cpm)
Medium	2	560
OKT3	16.5	18,750
OKT3 + A-56619	15.0	1,610

[a] MNCs were activated for 24 h by OKT3 MAb with or without A-56619. The cells were divided into aliquots, with one portion used for IL-2 receptor expression and the remaining portion cultured for an additional 48 h for cell proliferation.

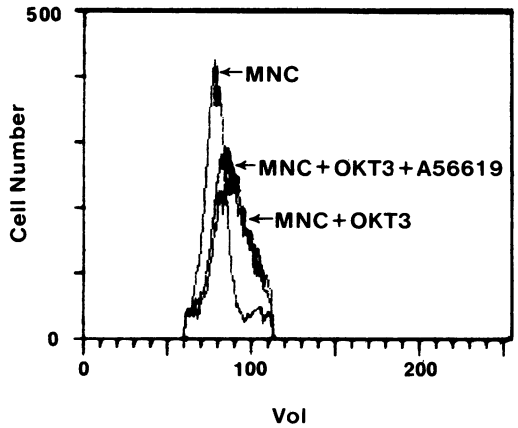

FIG. 5. Effect of A-56619 on OKT3 MAb-induced cell volume (size) increase. A-56619 (25 μg/ml) was added at culture initiation. Cell volume was determined with a FACS analyzer.

quinolone derivatives are capable of inhibiting the activity of topoisomerase II derived from liver cells (7). Recently, it has been shown that topoisomerase II activity increases in regenerating liver cells (7) and in the mitogen-stimulated lymphocytes (source quoted in reference 19). This increase in activity appears to be associated with commitment of cells to proliferate, and this topoisomerase might be the site of A-56619- and A-56620-induced inhibition. Recently, it has been reported that quinolone derivatives inhibit eucaryotic alpha and beta DNA polymerases (17). Thus, it is also possible that A-56619 and A-56620 diminish proliferation by preventing the activity of enzymes involved in DNA replication.

Currently, no information is available on the achievable levels in serum of A-56619 and A-56620 in humans. In mice, after a single dose (100 mg/kg) given orally or subcutaneously, the levels of A-56619 in serum are in the range of 18.5 to 22.5 μg/ml, and the levels of A-56620 in serum are in the range of 4.5 to 28.9 μg/ml (8). We have shown that DNA synthesis in mitogen-stimulated MNCs is diminished with concentrations of A-56619 and A-56620 that closely correspond to the levels in serum in mice. Whether or not such levels can be achieved in humans after therapeutic administration of A-56619 and A-56620 is unknown.

The in vivo consequences of in vitro inhibition of MNC proliferation by A-56619 and A-56620 on host defense against intracellular pathogens remain to be elucidated.

LITERATURE CITED

1. **Banck, G., and A. Forsgren.** 1979. Antibiotics and suppression of lymphocyte function in vitro. Antimicrob. Agents Chemother. **16**:554–560.
2. **Bettens, F., F. Kristensen, and A. L. deWeck.** 1982. Effect of macrophages on the G_0-G_1 and G_1-S transition of thymocytes. Immunology **45**:199–205.
3. **Bettens, F., F. Kristensen, C. Walker, and A. L. deWeck.** 1982. Human lymphocyte proliferation. II. Formation of activated G1 Cells. Eur. J. Immunol. **12**:948–952.
4. **Boyum, A.** 1968. Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab Invest. Suppl. **97**:77–88.
5. **Cantrell, D. A., and K. A. Smith.** 1984. The interleukin 2: a new cell growth model. Science **224**:1312–1316.
6. **Chaperon, E. A., and W. E. Sanders, Jr.** 1978. Suppression of lymphocyte responses by cephalosporins. Infect. Immun. **19**:378–384.
7. **Duget, M., C. Lavendt, F. Harper, G. Mirambean, and A. M. DeRecondo.** 1983. DNA topoisomerases from rat liver. Physiological variations. Nucleic Acids Res. **11**:1059–1075.
8. **Fernandes, P. B., D. T. W. Chu, R. R. Bower, K. P. Jarvis, N. R. Ramer, and N. Shipkowitz.** 1986. In vivo evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:201–208.
9. **Gollapudi, S. V. S., R. H. Prabhala, and H. Thadepalli.** 1986. Effect of ciprofloxacin on mitogen-stimulated lymphocyte proliferation. Antimicrob. Agents Chemother. **29**:337–338.
10. **Gollapudi, S. V. S., M. Ramanadham, and M. Kern.** 1984. Non-responsiveness of immature B-lymphocytes to anti-immunoglobulin is reversed by pronase. Biochem. Biophys. Res. Commun. **119**:1–4.
11. **Gunther, G. R., J. L. Wang, and G. M. Edelman.** 1974. The kinetics of cellular commitment during stimulation of lymphocytes by lectins. J. Cell Biol. **62**:366–377.
12. **Hauser, W. E., and J. S. Remington.** 1982. Effect of antibiotics on the immune response. Am. J. Med. **72**:711–715.
13. **Herzenberg, L. A., and L. A. Herzenberg.** 1978. Analysis and separation using the fluorescence activated cell sorter (FACS). *In* D. M. Weir (ed.), Handbook of experimental immunology. Blackwell Scientific Publications, Ltd., Oxford.
14. **Larsson, E. L., A. Coutinho, and P. Martinez.** 1980. A suggested mechanism for lymphocyte activation: implications on the acquisition of functional reactivities. Immunol. Rev. **51**:61–91.
15. **Manzella, J. P., and J. K. Clark.** 1983. Effects of moxalactam and cefuroxime on mitogen-stimulated human mononuclear lymphocytes. Antimicrob. Agents Chemother. **23**:360–363.
16. **Potts, R. C., H. A. A. Hassan, R. A. Brown, A. MacConnachie, J. H. Gibbs, A. J. Robertson, and J. S. Beck.** 1983. *In vitro* effects of doxycycline and tetracycline on mitogen stimulated lymphocyte growth. Clin. Exp. Immunol. **53**:458–464.
17. **Rusquet, R., M. Bonhomet, and J. C. David.** 1984. Quinolone antibiotics inhibit eukaryotic DNA polymerase alpha and beta and terminal deoxynucleotidyl transferase, but not DNA ligase. Biochem. Biophys. Res. Commun. **121**:762–769.
18. **Thong, Y. H., and A. Ferrante.** 1979. Inhibition of mitogen induced human lymphocyte proliferative response by tetracycline analogues. Clin. Exp. Immunol. **35**:443–446.
19. **Tricoli, J. V., B. M. Sahai, P. J. McCormick, S. J. Jarlinski, J. S. Bertram, and D. Kowalski.** 1985. DNA topoisomerase I and II activities during cell proliferation and the cell cycle in cultured mouse embryo fibroblast (C3H 10T 1/2) cells. Exp. Cell. Res. **158**:1–14.
20. **Wolfson, J. S., and D. C. Hooper.** 1985. The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrob. Agents Chemother. **28**:581–586.