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# The Journal of Infectious Diseases

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## Effects of Tetracycline on Leukotaxis

R. Russell Martin, Glenn A. Warr, Robert B. Couch,  
Henry Yeager, and Vernon Knight

From the Departments of Medicine and of  
Microbiology and Immunology,  
Baylor College of Medicine, Houston, Texas

Tetracycline, in concentrations common during therapy, markedly depressed migration of human leukocytes in vitro. Sera from 12 of 13 volunteers who received oral tetracycline after infection with *Mycoplasma pneumoniae* inhibited leukotaxis of normal leukocytes. Random migration and chemotaxis of leukocytes from two additional subjects were depressed for up to 24 hr after a single 1-g dose of tetracycline. When tetracycline was tested over a wide range of concentrations, leukotaxis was depressed by lower concentrations (0.01–10 µg/ml) but was stimulated by higher concentrations (30–300 µg/ml) of the antibiotic. Metabolic studies revealed that production of leukocyte lactate was elevated significantly in the presence of a high level of tetracycline. The mechanisms by which tetracycline affects leukotaxis are not known.

Leukotaxis (the migration of leukocytes toward an attracting stimulus) is one of the fundamental responses of polymorphonuclear leukocytes. This property has been examined in vitro with various modifications of the micropore filter chamber developed by Boyden [1]. Numerous substances have been shown to attract leukocytes, including bacterial culture filtrates [2, 3], extracts from leukocytes themselves [4], components of the serum complement system [5, 6], and tissues infected with virus [7, 8].

The effects on polymorphonuclear leukocytes of drugs used in treatment of infections are usually monitored by the observation of changes in counts of total and differential leukocytes in the peripheral blood. The possibility that therapy with drugs may alter some of the functions of peripheral blood leukocytes has not been extensively investigated. However, Ward [9] and Peters et al. [10] have described the effects of corticosteroids on leukotaxis in vitro and in vivo, respectively.

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Please address requests for reprints to Dr. R. Russell Martin, Department of Medicine, Baylor College of Medicine, 1200 Moursund Avenue, Houston, Texas 77025.

We became interested in the effects of tetracycline on leukotaxis when we observed reduced migration of leukocytes in a group of volunteers receiving this antibiotic [11]. The present studies extend these observations by means of a systematic evaluation of the influence of tetracycline on leukotaxis, measured in vitro.

### Materials and Methods

Experimental infection with *Mycoplasma pneumoniae* was produced in normal volunteers by intranasal and nasopharyngeal inoculation of 2 ml of broth containing  $1 \times 10^6$  cfu of *M. pneumoniae*. These volunteers had initial low ( $\leq 1:2$ ) or negative titers of growth-inhibiting antibody to *M. pneumoniae* in serum, and all volunteers became infected, as evidenced by a fourfold rise in antibody titer in serum and by repeated isolation of *M. pneumoniae* from throat swab specimens.

Leukocytes were collected after sedimentation of anticoagulated blood in dextran, as previously described [12]. A portion of the separately collected serum was used on the day obtained, and the rest was stored at  $-70^\circ\text{C}$  for subsequent tests.

The system used for leukotaxis has been described in detail [11]. Briefly, the modified Boyden chambers used were similar to those described by Cornely [4] and consisted of two compartments, separated by a 13-mm diameter Millipore® filter (SSWP 01300) with a mean pore size of 3 µm. The upper chamber held a volume of 0.35 ml containing  $1.3 \times 10^6$  leukocytes, and the lower

chamber contained a total volume of 1.0 ml. To test migration of leukocytes toward an antigen,  $1.3 \times 10^6$  leukocytes in 0.35 ml of fluid were added to the upper chamber, and 0.1 ml of antigen was placed in the lower chamber. For tests with mycoplasma antigen and influenza vaccine, the basic medium was Hanks' balanced salt solution with 1% gelatin (Hanks'-gel). The influenza antigen used was zonally purified, dialyzed monovalent influenza vaccine (A<sub>2</sub>/Hong Kong/Aichi/68, Eli Lilly, Indianapolis, Ind.) with a potency of 1,600 chick cell-agglutinating units/ml. In tests with serum, a final concentration in serum of 10% was used in both upper and lower chambers.

Preliminary studies of the time-course of migration of leukocytes in the chambers revealed that leukocytes counted on the lower surface of the filters increased linearly between 60 and 90 min, then reached a plateau until 240 min, after which a decrease was noted. Incubations were conducted for 2 hr, with temperature maintained at 37 C by circulating water at a constant temperature. Filters were processed for microscopy, and for each filter, leukocytes on the bottom surface were counted in 10 random microscopic fields at a magnification of  $\times 400$  [11]. Leukotaxis was expressed as the number of leukocytes migrating completely through to the bottom surface of the filter. Thus, for each condition of incubation for each subject, counts were made of a total of 30 random microscopic fields. Under the conditions described, virtually all of the cells counted were polymorphonuclear leukocytes.

In incubations with tetracycline, tetracycline HCl (USP, kindly supplied by the Upjohn Co., Kalamazoo, Mich.) was used in final concentrations of 0.01–300  $\mu\text{g/ml}$ . Each of the two volunteers received an oral 1.0-g dose of tetracycline (TETREX<sup>®</sup> *bid*CAPS<sup>®</sup>, Bristol Laboratories, Syracuse, N.Y.). The volunteers with infection due to mycoplasma received 250 mg of tetracycline four times daily on days 22–28 after inoculation. Levels of tetracycline in serum were measured by the agar diffusion method of Bennett et al. [13].

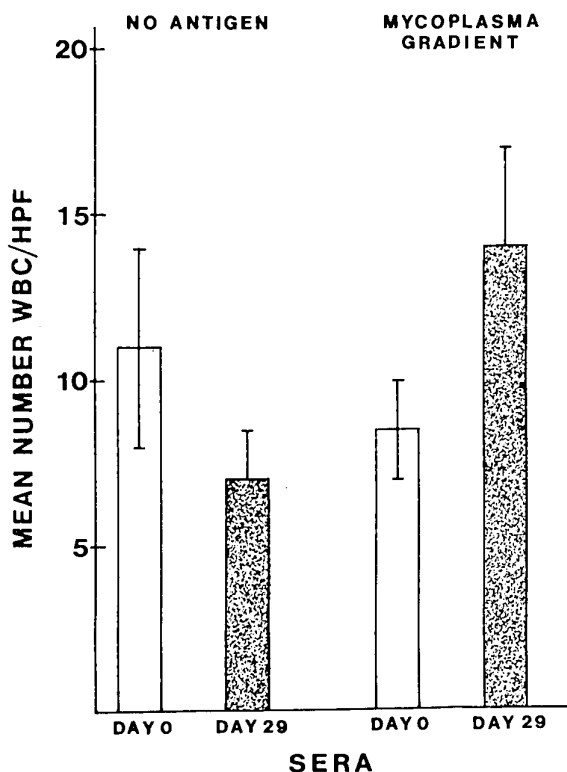
*Metabolic studies of leukocytes.* Determinations were made on three separate occasions with leukocytes from the same individual. Leukocytes were placed in Hanks'-gel solution with 10% autologous serum and 0.050 ml of D-glucose-U-14C (final specific activity, 0.023–0.064  $\mu\text{Ci/mol}$ , New

England Nuclear, Boston, Mass.). Triplicate incubations were performed in 25-ml flasks with plastic wells (Kontes Glass, Vineland, N.J.) under three experimental conditions: with no added antibiotic, with 1  $\mu\text{g}$  of tetracycline phosphate/ml, and with 300  $\mu\text{g}$  of tetracycline phosphate/ml. Additional controls included flasks without leukocytes and flasks containing acid-treated leukocytes. The mixtures were kept at 4 C in the interval between the addition of radioisotope and the beginning of incubation. The flasks were gassed for 10 min with 95% O<sub>2</sub>–5% CO<sub>2</sub>, then incubated for 2 hr at 37 C at 68 oscillations/min in a Dubnoff metabolic shaker. After incubation, 1.0 ml was withdrawn for determination of glucose. Hydroxide of hyamine, 0.4 ml (Packard Instrument Co., Downers Grove, Ill.), was added to the plastic wells to trap <sup>14</sup>CO<sub>2</sub>, which was measured by pipetting 0.100 ml of hyamine solution into 15 ml of toluene containing 1.0 mg of 2,5 diphenzloxazole/ml and 0.25 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene/ml. Samples were counted in a Packard Tri-Carb model 3375 liquid scintillation counter and were corrected for quenching by the standard external technique.

The glucose remaining after incubation was determined by measurement of glucose enzymatically (Sigma Chemical, St. Louis, Mo.) in flasks with and without cells; production of lactic acid was measured enzymatically (Sigma Chemical) as the difference between content of lactic acid in supernatant fluid of flasks with tissue that had been incubated and that in those that had been initially killed with acid.

## Results

The inhibitory effects of tetracycline on migration of leukocytes were discovered during a serial study of leukotaxis in which white blood cells and sera from 13 adult volunteers with experimental *M. pneumoniae* infection were used [11]. In the final week of that study, on days 22–28 after infection, 13 volunteers received 1.0 g of tetracycline each day in an attempt to reduce carriage of *M. pneumoniae*. The random migration of leukocytes incubated in autologous serum obtained on day 29 (one day after completion of therapy with oral tetracycline) was 36% lower ( $\chi^2$ ,  $P < 0.01$ ) than random migration of the same leukocytes in control serum collected before infection (figure 1).



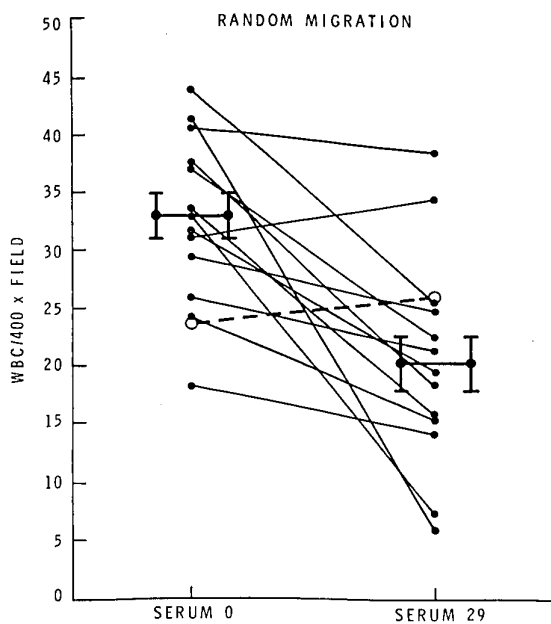
**Figure 1.** Random migration (no chemotactic agent in lower chamber) and chemotaxis (mycoplasma antigen in lower chamber) of autologous leukocytes collected from 13 volunteers on day 29 after infection with mycoplasmas. Clear bars show mean  $\pm$  SE of migration in serum before infection (day 0), while dark bars show migration in serum taken on day 29.

Although chemotaxis of leukocytes toward mycoplasma was maintained in sera from day 29 (migration was greater with mycoplasma than with no antigen present,  $\chi^2$ ,  $P < 0.01$ ), migration was lower in magnitude than expected, based on other observations [11]. The possibility that sera from day 29 might interfere with leukotaxis was considered.

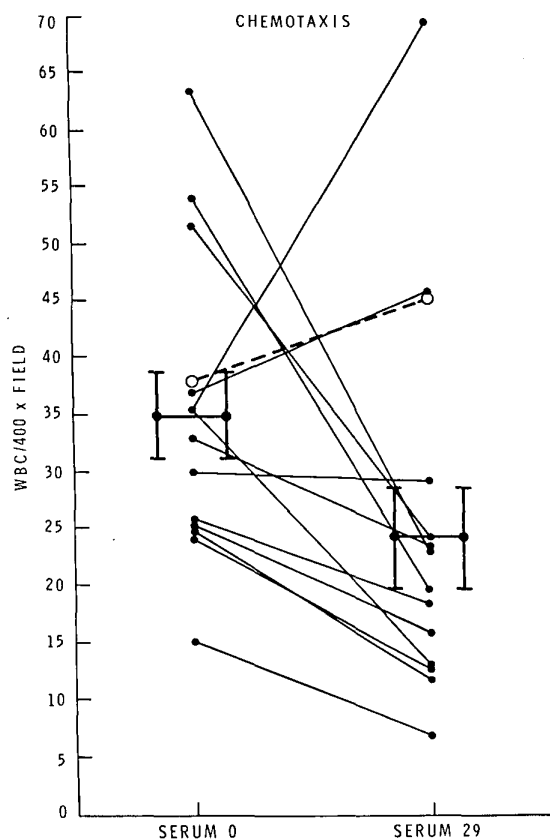
The effects of these sera on migration of homologous leukocytes were then studied. Leukocytes from a single normal donor were tested simultaneously with sera obtained from the 13 volunteers at the start of the study (day 0) and at the completion of therapy with tetracycline (day 29). Levels of tetracycline in the sera from day 29 were  $\leq 5$   $\mu\text{g/ml}$ . The random migration of leukocytes (no chemotactic agent in the lower compartment of the Boyden chamber) was a mean value

of 33 leukocytes per high-power field in serum from day 0, decreasing to 21 leukocytes per high-power field in serum from day 29 (figure 2). In 12 of the 13 individual serum pairs, migration of the homologous leukocytes was lower in serum from day 29 than in serum from day 0 from the same volunteer (paired  $t$ -test,  $P < 0.01$ ). That this was not a result of the handling and storage of the sera was demonstrated by the unchanged values for migration of leukocytes in the serum from a normal control subject who did not receive tetracycline, but whose serum was collected and similarly processed.

There was a similar suppressive effect by sera from day 29 on chemotaxis toward mycoplasma antigen of homologous leukocytes from the same donor (figure 3). In 10 of the 13 serum pairs, chemotaxis was reduced in serum from day 29 compared with migration in serum from day 0 (paired  $t$ -test,  $P < 0.05$ ). In one instance migration of leukocytes was the same in both sera, while chemotaxis of leukocytes was higher in sera from day 29 in two instances. Leukotaxis was reduced



**Figure 2.** Random migration of homologous leukocytes from a normal donor in sera collected before illness (day 0) and on day 29 after infection with mycoplasmas. Migration in individual serum pairs is represented by  $\bullet$ — $\bullet$ . Migration in a normal control serum is represented by  $\circ$ — $\circ$ . Brackets show mean  $\pm$  SE.



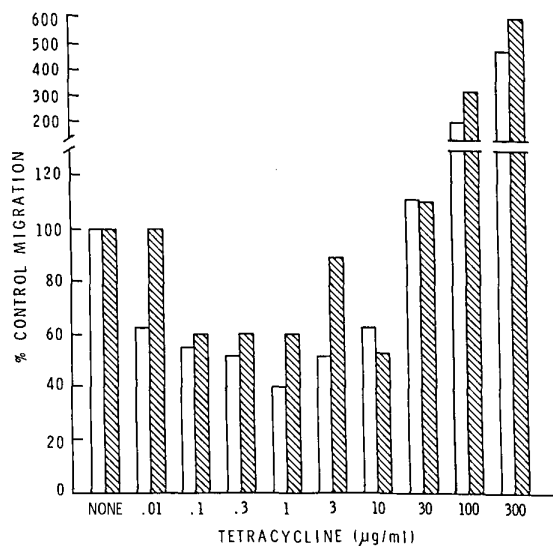
**Figure 3.** Chemotaxis toward mycoplasma antigen of homologous leukocytes from a normal donor, incubated in 10% sera collected on day 0 and day 29 after infection with mycoplasmas. Migration of leukocytes in individual serum pairs is represented by ●—●. Chemotaxis in serum from an untreated control is represented by ○—○. Brackets show mean  $\pm$  SE.

from a mean of 35 leukocytes per high-power field in sera taken before illness to 24 in sera from day 29. Leukotaxis was not significantly different in serum from the control subject taken on day 0 or day 29, collected and processed in the same way as the patient sera.

To investigate further the inhibition of leukotaxis by serum containing tetracycline, serial dilutions of tetracycline were incubated with 10% serum and autologous leukocytes from a single normal donor. Random migration of leukocytes and chemotaxis of leukocytes toward mycoplasma were tested as before. The effects of tetracycline were dependent on the concentration of antibiotic. In the range from 10 ng to 10  $\mu$ g/ml, both ran-

dom migration ( $t$ -test,  $P < 0.001$ ) and chemotaxis of leukocytes toward mycoplasma ( $t$ -test,  $P < 0.01$ ) were depressed (figure 4). Leukotaxis was reduced by as much as 60% compared with control migration without the presence of tetracycline. With concentrations of tetracycline ranging from 30 to 300  $\mu$ g/ml, migration of leukocytes was increased to as much as 600% of control levels ( $t$ -test,  $P < 0.001$ ). Thus leukotaxis was depressed when concentrations were in the range found in the serum during therapy but was stimulated by higher concentrations of tetracycline. Although not shown here, leukotaxis was similarly affected in incubations in which serum was omitted.

To confirm the original observation that oral administration of tetracycline might affect leukotaxis, two volunteers received 1 g of tetracycline as a single oral dose, and sera and leukocytes were collected serially for quantitation of leukotaxis. In the first volunteer, random migration of leukocytes and chemotaxis toward two different chemotactic stimuli (0.1% casein and *M. pneumoniae*) were measured (table 1). Chemotaxis toward 0.1% casein and mycoplasma was reduced to 50% or less of control by 2 hr and to 10% or less of control by 4 hr after administration of



**Figure 4.** Migration of normal leukocytes in 10% serum containing various concentrations of tetracycline. Values for random migration (□) and chemotaxis (■) toward mycoplasma are expressed as a percentage of migration in incubations without tetracycline (mean of two experiments).

**Table 1.** Effects of oral tetracycline (TC) on in vitro leukotaxis.

Subject no.	Time after TC (hr)	Level of TC in serum ( $\mu\text{g/ml}$ )	Random migration: no antigen	Chemotaxis		
				0.1% casein	<i>Mycoplasma pneumoniae</i>	A <sub>2</sub> influenza virus
1	0	0	16* (100)	58* (100)	48* (100)	...
	2	6.2	22 (137)	29 (50)	16 (33)	...
	4	10.2	16 (100)	6 (10)	4 (8)	...
	24	1.7	4 (25)	3 (5)	7 (14)	...
2	0	0	40 (100)	180 (100)	...	108 (100)
	2	4.0	15 (37)	91 (50)	...	50 (46)
	4	7.6	20 (50)	26 (14)	...	43 (40)
	24	2.5	38 (95)	165 (92)	...	52 (48)
	48	< 0.3	48 (120)	192 (107)	...	158 (146)

\* Number of leukocytes per  $\times 400$  microscopic field (percentage of migration in serum before tetracycline).

tetracycline, at which time a peak level of tetracycline in serum of 10.2  $\mu\text{g/ml}$  was present. Leukotaxis remained depressed 24 hr after the single dose of tetracycline.

In a second volunteer, observations of leukotaxis were extended through 48 hr after the oral ingestion of tetracycline. Preliminary studies had shown that this volunteer's leukocytes were not responsive to mycoplasma antigen; thus, A<sub>2</sub> influenza virus, to which the leukocytes were responsive, was used as a chemotaxin. Casein was used as a second chemotactic agent. The degree of decrease in chemotaxis after administration of tetracycline was similar to that observed in the first volunteer. Maximal depression of leukotaxis occurred at 4 hr, when the serum contained 7.6  $\mu\text{g}$  of tetracycline/ml. Random migration of leukocytes and chemotaxis of leukocytes toward casein returned to levels found before administration of tetracycline between 4 and 24 hr. In this subject, leukotaxis did not return to normal until 24–48 hr after the administration of oral tetracycline.

To see whether tetracycline could induce changes in metabolism of leukocyte glucose that might be related to the observed changes in leukotaxis, we incubated groups of leukocytes from a single donor with D-glucose-14C with no antibiotic, and with 1.0 g of tetracycline/ml and 300 g of tetracycline/ml. No significant change in uptake of glucose or production of  $^{14}\text{CO}_2$  was measured at either concentration of tetracycline, but a  $44\% \pm 11\%$  increase ( $P < 0.05$ ) in production of lactic acid occurred at 300  $\mu\text{g}$  of tetracycline/ml, suggesting that increased use of glucose did occur at higher levels of tetracycline.

## Discussion

These studies have shown that the antimicrobial agent, tetracycline, in concentrations usually achieved during therapy, inhibits leukotaxis. When sera from volunteers convalescing from infection with mycoplasma were first noted to depress leukotaxis, several different possibilities were considered as explanations of this observation. Circumstantial evidence implicated the tetracycline therapy as the cause of the observed reduction in leukotaxis. The additional studies documented that tetracycline can have this effect, even in low concentrations such as those ( $\leq 5 \mu\text{g/ml}$ ) in sera of these treated volunteers.

The in vitro addition of tetracycline over a range of concentrations commonly encountered in therapy (0.1–10  $\mu\text{g/ml}$ ) depressed both random migration and chemotaxis of leukocytes in either the presence or the absence of serum. The marked stimulation of leukotaxis at higher concentrations of tetracycline (30–300  $\mu\text{g/ml}$ ) was not anticipated. The basis of this stimulation is not known; however, the increased production of lactic acid by leukocytes in the presence of 300  $\mu\text{g}$  of tetracycline/ml may be a manifestation of a metabolic effect of the drug.

The mechanisms by which lower concentrations of tetracycline inhibit leukotaxis have not been delineated. Leukocytes in blood have a high rate of aerobic glycolysis [14], and several studies have indicated that glycolytic energy is necessary for leukotaxis [15, 16]. Uptake of glucose was not affected measurably by tetracycline, an observation which agrees with the studies of McCurrach et al. [17]. Other cellular pathways, such as the

synthesis of cytochrome oxidase, may be depressed by tetracycline [18]. Another possible mechanism for the alterations of leukotaxis by tetracycline is the prolonged effect on membranes of leukocytes exposed to this drug [19].

Leukocytes can take up and store tetracycline, resulting in intracellular concentrations that are considerably higher than concentrations in the surrounding medium [20]. The phenomenon of uptake and concentration of tetracycline may explain the effects on leukotaxis of the in vitro addition of very low levels of the antibiotic. The prolonged depression of leukotaxis observed with leukocytes collected from volunteers long after the peak levels in serum had passed may also be related to intracellular retention of the drug.

The accumulation of leukocytes in inflamed tissue is one of the fundamental responses to infection. Depressed migration of leukocytes has been demonstrated in a number of conditions associated with an increased incidence of bacterial infections, including poorly controlled diabetes [21], cirrhosis [22], rheumatoid arthritis [23], and deficiencies in the complement system [24, 25].

Even though tetracycline is a bacteriostatic antibiotic, and therefore requires a participation by the host defenses to eradicate infection, it is likely that the reduction of leukotaxis by tetracycline does not critically impair defenses in most patients who are otherwise normal. However, in patients with other complicating conditions (especially those known to be associated with altered function of leukocytes), the additional effects of depression of leukotaxis by tetracycline could be of clinical importance.

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