The Journal of Infectious Diseases

JIDIAQ 129 93–232 (1974)

Volume 129 Number 2 February 1974

Medical Microbiology / Clinical Immunology / Epidemiology

Official publication of the Infectious Diseases Society of America Published by the University of Chicago Press

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Communications for the editors and manuscripts should be addressed to The Editor, The Journal of Infectious Diseases, Channing Laboratory, Boston City Hospital, Boston, Massachusetts 02118.

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Cover design by Albert Alcalay.

Published by the University of Chicago Press, Chicago, Illinois 60637; The University of Chicago Press, London, S. W. 1.

ISSN0022-1899

THE JOURNAL OF INFECTIOUS DISEASES • VOL. 129, No. 2 • FEBRUARY 1974

Effects of Tetracycline on Leukotaxis

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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.

Received for publication March 5, 1973, and in revised form August 20, 1973.

This investigation was supported by grants from the AMA-ERF Committee for Research on Tobacco and Health, by the National Tuberculosis and Respiratory Diseases Association, by contract no. NAS 9-13139 P/1 from the National Aeronautics and Space Administration, and by grants no. RR-00350, PH 43-NIAID-68-963, and 1 T01 AI00446-01 from the U.S. Public Health Service.

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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 1×10^6 cfu of *M. pneumoniae*. These volunteers had initial low ($\leqslant 1:2$) 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 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 1.3×10^6 leukocytes, and the lower

chamber contained a total volume of 1.0 ml. To test migration of leukocytes toward an antigen, 1.3×10^6 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_2/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 ×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 μg/ml. Each of the two volunteers received an oral 1.0-g dose of tetracycline (TETREX® *bid*CAPS®, 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 (f[illegible] activity 0.023–0.064 μCi/mμ[illegible] 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 μg of tetracycline phosphate/ml, and with 300 μg 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_2–5% CO_2, 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 $^{14}CO_2$, 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 (χ^2, $P < 0.01$) than random migration of the same leukocytes in con[illegible]

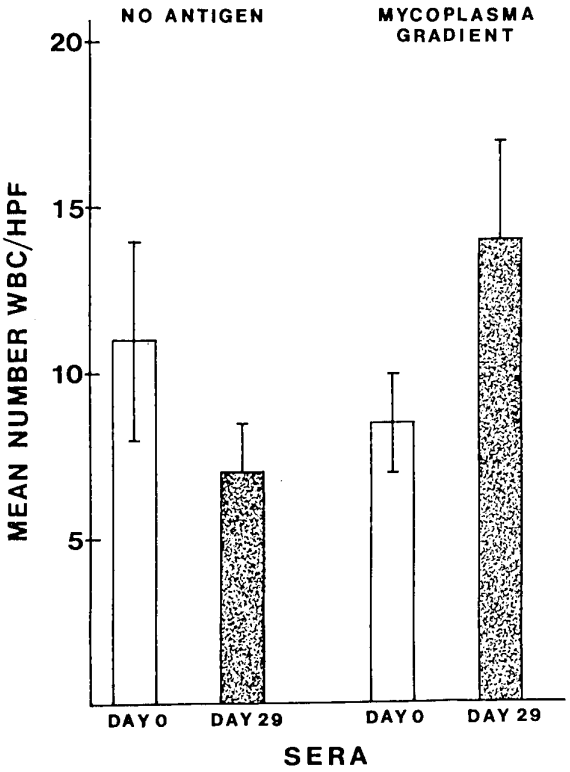

Figure 1. Random migration (no chemotactic agent in lower chamber) and chemotaxis (mycoplasma antigen in lower chamber) of autologous leukocytes collected from 13 volunteers on day 29 after infection with mycoplasmas. Clear bars show mean ± SE of migration in serum before infection (day 0), while dark 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, χ^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 $\leqslant 5$ μg/ml. The random migration of leukocytes (no chemotactic agent in the lower compart-

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

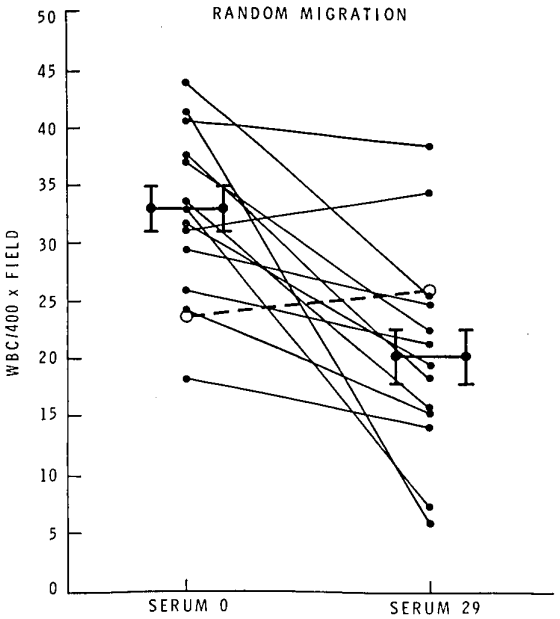

Figure 2. Random migration of homologous leukocytes from a normal donor in sera collected before illness (day 0) and on day 29 after infection with mycoplasmas. Migration in individual serum pairs is represented by ●——●. Migration in a normal control serum is represented by ○——○. Brackets show

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