Effects of Sulfasalazine on Selected Lymphocyte Subpopulations in Vivo and in Vitro

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Sulfasalazine has proven to be an effective agent in the therapy of inflammatory bowel disease (IBD). Despite long and widespread usage, the mechanism of action of this drug is still not understood. Several investigators have suggested that the drug might act as an immunosuppressant. To examine this possibility, an in vivo study was undertaken to ascertain any quantitative change in the circulating T cells, Ig-bearing B cells, and complement receptor-bearing lymphocytes (CRL) of patients before and during therapy with sulfasalazine. Concomitant responses to skin test antigens were also evaluated. In vitro studies with control cells were performed to determine the influence of sulfasalazine and its components (sulfapyridine or 5-aminosalicylic acid) on the extent of antibody-dependent cellular cytotoxicity (ADCC), as well as on the number of T cells and CRL. Results indicate that neither sulfasalazine nor either of its components quantitatively alters those subpopulations of circulating mononuclear cells studied in vivo or in vitro—nor are these compounds responsible for any functional inhibition of ADCC.

Although sulfasalazine has been used in the therapy of inflammatory bowel disease (IBD) since 1942 (1), its mechanism of action is as yet undefined. Despite its being a sulfonamide, its therapeutic effect appears unrelated to antibacterial activity (2, 3). This belief has not been fully confirmed, however (4). High concentrations of the drug, which exhibits an affinity for collagen, are found in the subepithelial connective tissue of the intestine (5).

Holm and Perlmann have demonstrated that sulfasalazine inhibits phytohemagglutinin (PHA)-stimulated lymphocyte cytotoxicity in vitro (6), while Campbell reported that skin grafts are prolonged in sulfasalazine-treated mice and that the symptoms of experimental allergic encephalitis are significantly abated in guinea pigs treated with the drug (7). Since such manifestations of immunosuppression may ensue from either a reduction in lymphocyte number with a concomitant decline in activity or from a direct inhibition of lymphocyte function, this present study was initiated to determine if the drug exercises any in vivo or in vitro effects on selected mononuclear cells.

MATERIALS AND METHODS

In Vivo Studies

White Blood Cells. Twenty-one individuals were studied prior to and again two weeks or more after commencing treatment with sulfasalazine (3-4 g/day). Twelve individuals had ulcerative colitis (UC), six individuals had Crohn's disease (CD), and three individuals...
were non-IBD controls. Individuals who had been on sulphasalazine before this study were withdrawn from treatment for two weeks prior to the initiation of the testing. No medication other than psyllium seed preparation was permitted while the individuals were participating in the study.

Thirty cubic centimeters of blood were collected by venipuncture into a heparinized syringe (300 units/0.3 ml heparin). A standard white blood cell count was obtained using a Coulter’s counter, and a differential was determined by counting at least 100 white cells in a Wright-stained smear. After the mononuclear cells were purified by a procedure described fully in an earlier paper (8), they were assayed for the number of T cells, defined by their ability to rosette sheep red blood cells (SRBC), according to a modification of the method described by Yu et al (9). The B cells were enumerated by two techniques: their ability to bind fluorescein-conjugated goat anti-human gammaglobulin (anti-IgG, -lgM, and -lgA) (10) and their ability to rosette SRBC coated with 19S anti-SRBC antibodies and mouse complement (11). A description of these methods is also detailed in the earlier publication (8).

Standard control values used are those determined for a group of 35 healthy laboratory personnel. Statistical analysis of untreated populations was carried out using the Student’s t test.

Skin Tests. The following five skin test antigens were used before treatment with sulphasalazine: Candida (Dermatophytin “O”*, Hollister-Stier Laboratories, Spokane, Washington), Mumps (Eli Lilly and Company, Indianapolis, Indiana), PPD (Tubersol®*, Connaught Laboratories, Ltd., Toronto, Canada), streptokinase-streptodornase (SK-SD) (Varidase®, Lederle Laboratories, Pearl River, New York), and trichophytin (Dermatophytin®, Hollister-Stier Laboratories, Spokane, Washington). All skin tests were injected in 0.1-ml amounts in the volar aspect of the forearm. If an initial skin test was negative, a second strength dilution was administered. The results were recorded in millimeters along the longest axis by an independent observer. Those skin tests which elicited a positive response prior to treatment were repeated two to three weeks after initiation of drug therapy. The results obtained before and during therapy were then compared and analyzed using the paired Student’s t test.

In Vitro Studies

One hundred cubic centimeters of blood were collected from 22 healthy laboratory personnel by venipuncture into two 50-cc heparinized syringes (500 units/0.5 ml heparin in each). Mononuclear cells were separated as described above. The separated mononuclear cells were suspended in RPMI-1640 supplemented with 20% fetal bovine serum (FBS) and 10 units penicillin/10 μg streptomycin/ml (PS). (Unless otherwise stated, all reagents were obtained from Grand Island Biological Co., Grand Island, New York.) The mononuclear cells were counted and adjusted to 5 x 10⁶ cells/ml for antibody-dependent cellular cytotoxicity (ADCC) assays and to 3 x 10⁶ cells/ml for rosette assays. Immunoglobulin-bearing B cells were not quantitated in these experiments.

ADCC. One-half milliliter of RPMI/FBS/PS containing 5 x 10⁶ mononuclear cells/ml was added to each of four sterile 125 x 16-mm glass culture tubes (Mercer Glass Works, Inc., New York) fitted with stainless-steel closures (Bellco Glass, Inc., Vineland, New Jersey) for each time study. A response curve, establishing an optimum ratio of mononuclear cells to CRBC antiserum concentration was defined prior to starting this phase of our study. We found that with 5 x 10⁶ mononuclear cells/ml, an antiserum dilution of 3 x 10⁻⁶ assured effective cytotoxicity, yet precluded spontaneous lysis of controls. A stock of chicken red blood cell (CRBC) antiserum (3 x 10⁻⁶) was diluted 1/100 with RPMI-1640 medium containing either (1) sulfasalazine, (2) 5-aminosalicylic acid, or (3) sulfapyridine at a concentration of 500 μg/ml. Control antiserum was diluted 1/100 with RPMI only. To the first tube in each set was added 1 ml of sulfasalazine/CRBC antiserum; to the second tube, 1 ml of 5-aminosalicylic acid/CRBC antiserum; to the third tube, 1 ml of sulfapyridine/CRBC antiserum; and to the fourth, 1 ml of RPMI/CRBC antiserum. To all tubes was added 0.5 ml of RPMI containing 1 x 10⁶ CRBC which had been tagged with ⁵¹Cr according to the procedure outlined by Perlmann et al (13). Controls, where unlabelled CRBC (2 x 10⁶ cells/ml) was substituted for mononuclear cells, were simultaneously cultured. After each set of four tubes and their controls were incubated (5% CO₂/95% air; 37°C) for a period of time ranging from 0 to 24 hr, cultures were centrifuged (50g) for 10 min. One milliliter of the supernate in each tube was removed to another glass tube. Both supernate (S) and pellet (P) tubes were counted in a Packard Auto-Gamma Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, Illinois). Percent lysis was determined by the following formula:

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\text{Lysis (\%)} = \frac{2S}{S + P} \times 100
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Rosette Assays. One milliliter of RPMI/FBS/PS containing 3 x 10⁶ mononuclear cells was added to each of four sterile glass culture tubes for each time study. To the first tube in each set was added 1 ml of stock sulfasalazine (500 μg/ml); to the second, 1 ml of stock 5-aminosalicylic acid (500 μg/ml); to the third, 1 ml of stock sulfapyridine (500 μg/ml); and to the fourth, 1 ml of RPMI. After each set of four tubes was incubated (5% CO₂/95% air; 37°C) for a predetermined period of time, cultures were transferred to 12 x 75-mm polystyrene tubes (#2058, Falcon Plastics, Oxnard, California). Cells were then washed three times with phosphate-buffered saline (PBS) at 425g for 10 min. Washed cells were resuspended in 0.25 ml PBS, counted, and adjusted to 3 x 10⁶ mononuclear cells/ml. Viability was determined by trypan blue exclusion; since more than 95% of the cells were viable in all cases, this laborious step was eliminated after 30 time studies. Mononuclear cells in each tube were then assayed for the number of T cells and complement receptor-bearing lymphocytes (CRL) as described earlier (8).

All statistical analyses for in vitro studies were performed using the analysis of variance (ANOVA).