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DEFECTIVE LEUKOTAXIS IN PATIENTS WITH LEPROMATOUS LEPROSY

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The inability of many patients with lepromatous leprosy to respond to antigens with a delayed-type hypersensitivity reaction in the skin is well documented. This defect is functionally defined by a greatly diminished inflammatory reaction at the site of a lepromin skin test. Since there is good evidence that inflammatory reactions of cell mediated immunity are induced by products ("lymphokines") from stimulated lymphoid cells, considerable attention has been paid in leprosy to the functional integrity of these cells and to the numbers thereof in the blood and lymphoid organs.

In previous studies, we found that among patients with lepromatous leprosy, there is a quantitative defect in the capacity of some individuals to mobilize inflammatory cells into special chambers applied to sites of skin abrasion (1).

Especially evident among these patients is a defect in the ability to accumulate neutrophils during the course of the inflammatory reaction to "non-specific" tissue injury induced by dermal abrasions. These results have suggested that a defect involving delayed inflammatory reactions in leprosy patients may be due to something more than impaired production of lymphocyte mediators. In this study we describe a chemotactic (leukotactic) inhibitor in the serum of patients with lepromatous leprosy that irreversibly inhibits a variety of chemotactic factors. This inhibitor appears to be similar to a serum factor recently termed the chemotactic factor inactivator (CFI) (2). Its presence in high concentration generally correlates with defective skin reactivity in leprosy.

MATERIALS AND METHODS

Clinical Classification of Patients

Patients were classified according to the criteria of Ridley and Waters (3). All patients had lepromatous leprosy and were classified as having LL, LI, or BL disease. Most patients were receiving diaminodiphenylsulfone (DDS), 125 to 500 mg weekly. Seven had been under therapy for <12 months, while the remainder had received chemotherapy for periods up to 85 months. Three patients were new and had received no therapy at the time of study. None were receiving steroids or other anti-inflammatory drugs.

Skin Testing

All patients were skin tested intradermally with the following antigens: Lepromin (Dharmendra preparation), Trichophytin, Candida in intermediate strength purified protein derivative (PPD) from tuberculoprotein, and mumps antigen. Some patients were only tested with four antigens, in which cases the mumps skin test was omitted. Skin tests were read as positive if the measurable induration was 5 mm or greater.

Leukotactic Assays

Sera from normal individuals as well as leprosy patients were collected and frozen at -70°C until assay. Assays for CFI were usually performed on all specimens simultaneously, with at least two replicate assays at subsequent intervals. For assessment of inhibitor, 50 µl of the bacterial chemotactic factor derived from Escherichia coli (2) was incubated with 50 µl of a test serum for 1/2 hr. at 37°C, followed by dilution to 1.0 ml with medium 199. The chemotactic assay was then performed using micropore filters of 5 micron porosity in modified Boyden chambers (2). Neutrophils from normal human donors served as indicator cells and were obtained by dextran sedimentation of red cells. They were suspended in 1.0 ml of medium 199 containing 10% autologous serum. The chemotactic inhibitor activity was expressed as percent inhibition of chemotactic activity. In the presence of normal serum, bacterial factor gave a chemotactic value of 260 (with a blank of 40). The percentage of this value obtained in the presence of a test serum subtracted from 100% gave the percent inhibition of chemotactic activity. Incubation of the bacterial chemotactic factor with normal human serum resulted in <15% reduction of the chemotactic activity. In some experiments the serum being tested for inhibitor activity was heated at 56°C for 1 hr. either before or after incubation with the bacterial chemotactic factor, which is completely heat stable under these conditions.

In some experiments the C3 and C5 leukotactic fragments were used. These were obtained by activation of normal human serum with inulin or zymosan in the presence of 0.5 M epsilonaminocaproic acid (4). Twenty-five µl of these chemotactic factor preparations, containing abundant chemotactic activity, were used.

RESULTS

Correlation of Serum Inhibitor with Depressed Skin Reactivity

The data presented indicate the extent to which inhibitory activity for the bacterial chemotactic factor was found in sera

from leprosy patients. These experiments were performed by incubating 50 µl bacterial chemotactic factor with 50 µl serum for 30 min at 37°C, then diluting the sample to 1.0 ml and testing for residual chemotactic factor activity. Incubation with normal human serum results in less than 15% loss of chemotactic activity (2). Also presented are the data pertinent to the results of skin testing and the clinical classification of 19 patients. In Group A, 12 patients had inhibitor activity in their sera ranging from 35 to 94% suppression of the chemotactic activity. With a few notable exceptions, skin testing revealed a uniform lack of responsiveness in 11 patients to the antigens. Patients 2, 7 and 8 responded positively to a single antigen, PPD. Each of these patients had active pulmonary tuberculosis and was being treated with appropriate chemotherapy. Patient 6 was a new and untreated case of leprosy who did not return for skin test studies and treatment; however, he did demonstrate a high level of chemotactic inhibitor in the serum (79% inhibition of chemotactic activity).

A second group (B) of two patients (13, 14) had relatively high levels of chemotactic inhibitor but still responded in skin tests to three or four of the antigens. In Group C (Patients 15, 16, 17) no inhibitor was found in two sera while a third showed 23% inhibition of the chemotactic factor. Despite the low level or absence of inhibitor, all three patients failed to react to antigens in skin testing, with the exception of patient 17 who had arrested pulmonary tuberculosis and responded to PPD.

Group D consisted of two patients whose sera had relatively low levels of inhibitor activity (9 and 23% inhibition of the chemotactic factor). They responded positively to 2 or 3 of the 4 or 5 skin tests and were considered as having relatively intact skin responsiveness in the presence of low levels of inhibitors.

Although exceptions do occur, the findings in this study do suggest a correlation between defective skin responsiveness in lepromatous leprosy and the presence in serum of a leukotactic inhibitor.

Characterization of the Chemotactic Inhibitor

The findings do not demonstrate the nature of the chemotactic inhibitor, that is, whether it was acting on indicator leukocytes or on the chemotactic factor itself. Therefore, sera from 6 different patients with lepromatous leprosy were further analyzed. Advantage was taken of the fact that CFI is heat labile while the bacterial chemotactic factor is heat stable. Three different manipulations were employed: (a) bacterial factor was incubated with fresh serum without further treatment; (b) bacterial factor was incubated with serum which had been preheated at 56°C for

1 hr. (a manipulation designed to inactivate CFI); (c) bacterial factor was incubated with serum and then the mixture was heat inactivated (56°C, 1 hr) so as to destroy any further CFI activity. Heated chemotactic factor had a chemotactic value of 260, compared with unheated factor having a value of 255. The results demonstrate that the chemotactic inhibitor is heat labile, that it acts directly on the chemotactic factor and that this inhibition is irreversible since heat inactivation of the inhibitor after it had first reacted with the chemotactic factor failed to restore the chemotactic activity. These data indicate that the chemotactic inhibitor is functionally indistinguishable from the CFI present in low concentration in normal human serum (2).

Lack of Inhibition of Chemotaxis by DDS

Since most of the leprosy patients were receiving DDS chemotherapy, consideration was given to the possibility that the chemotactic inhibitor might be due to a direct effect of the drug on chemotaxis. To investigate this possibility, DDS in a final concentration of 1.0 µg/ml was added to each of five normal sera and the effects on chemotaxis assessed. In these experiments 50 µl bacterial chemotactic factor was added to five sera containing, or lacking, DDS. A comparison of chemotaxis in each of the five normal sera containing or lacking the drug shows that no inhibitory effect of DDS was found. These results indicate that DDS does not directly inhibit the chemotactic response.

Spectrum of Chemotactic Inhibitor Activity by Leprosy Sera

Ten of the leprosy sera with CFI activity were studied for their spectrum of reactivity, using the C3 and C5 chemotactic fragments as well as the bacterial chemotactic factor. Five normal sera were used as controls.

The first subgroup of leprosy sera (numbers 3, 4, 10, 11) showed inhibitory activity for all three factors, with little evidence for selectivity of action. A second subgroup of sera (numbers 2, 6, 8, 16) also showed inhibitory activity for all three chemotactic factors, but more inhibition of the C3 fragment was found in relation to the C5 fragment. A third subgroup (numbers 1, 6) showed no inhibitory activity for the C3 fragment, some inhibitory activity for the C5 fragment and high levels of inhibition for the bacterial chemotactic factor. The five normal sera showed no inhibitory activity for any of the three chemotactic factors. These data indicate a diverse pattern of inhibitor activity, but with a tendency towards inhibition of all three chemotactic factors. The differences in levels of inactivator activities in various sera may be related to the fact that human CFI is heterogenous, existing in serum as two different chemotactic-factor inactivators each with a distinct specificity for chemotactic factors. One inhibits the C3

factor, the other inhibits the C5 factor, but both have the ability to inhibit the bacterial chemotactic factor (5).

DISCUSSION

The defective expression of cell mediated immune reactions in leprosy patients may be quantitative, qualitative, or both. The expression of delayed type allergy in the skin depends upon the infiltration of mononuclear cells (lymphocytes and monocytes) and, perhaps, the elaboration of lymphokines from antigen-triggered lymphoid cells. Preceding this, of course, many other steps in the reaction sequence take place, especially when the reaction involves de novo induction of sensitization, as in contact hypersensitivity with DNCB and picryl chloride. The precise point at which impairment of these reactions occurs in leprosy is not known. Defective skin reactions may thus reflect problems proximate to or far removed from the terminal events recognized clinically as inflammation.

Several theories have been offered to explain defective cellular immunity in leprosy. Firstly, it has been suggested that leprosy patients who exhibit these defects have an underlying genetic defect in the immune response. A second explanation includes the impairment of thymus dependent lymphocyte function, possibly because of an intrinsic defect. Alternatively, the infiltration of lymphoid organs by histiocytes may, in effect, remove lymphoid tissue from function (6) or interfere with T-lymphocyte circulation as we have demonstrated in experimental murine leprosy (7). A third possibility is the presence in serum of inhibitors that can interfere with lymphocyte function or otherwise block the infiltration of mononuclear cells into sites of antigen deposition. Indeed, there are several reports dealing with leprosy sera in which inhibitors have been found that block the ability of lymphocytes to respond to mitogenic stimuli (following contact with antigens or plant lectins) (8,9).

The inhibitor described in the present paper reacts directly and irreversibly with chemotactic factors to render them biologically inactive. On the basis of its functional features, the inhibitor resembles CFI, a protein present in normal human serum in concentrations that are too low to be detected except by special techniques of concentration or fractionation (2, 5). The only other condition, besides leprosy, in which the CFI has been detected in unfractionated or unconcentrated serum is Hodgkin's disease where CFI levels are elevated (>5 fold above normal) in nearly 50% of cases (10). There have been no studies of Hodgkin's disease to determine if a correlation exists between depressed skin reactivity and elevations in serum levels of CFI. However, it was shown that CFI activity in the serum of Hodgkin's patients was inhibitory to the chemotaxis of mononuclear as well as poly-

morphonuclear cells. Furthermore, there is good evidence that in patients with Hodgkin's disease, inflammatory reactions involving both neutrophils and monocyte cells are blunted, with many fewer cells appearing in Rebuck skin windows as contrasted with normal controls (11).

In patients with lepromatous leprosy we have described a quantitative defect in the ability to accumulate inflammatory cells at skin sites. These patients, in contrast to the normal controls or those with tuberculoid leprosy, accumulated only half the number of cells in special chambers placed over sites of dermal inflammatory reactions (1). Such findings suggest that in lepromatous leprosy, defects in the expression of inflammatory reactions may be entirely restricted to the reactions of cell mediated immunity but may be of a more general nature. Indeed, the finding of increased CFI in sera of patients with lepromatous leprosy could explain the diminished accumulation of cells at sites of skin abrasion if it is assumed that dermal inflammatory reactions are mediated by leukotactic factors.

Van Epps and Williams have recently described an inhibitor in human serum that is similar to CFI (12). Like CFI, this inhibitor interferes with the response of leukocytes to several chemotactic factors by a mechanism that involves a direct effect on the chemotactic stimuli. Furthermore, the presence in serum of this inhibitor has been correlated with deficient skin test responses of patients tested with six different antigens. Thus, elevations in the serum level of inhibitors of leukotaxis correlates with depressed skin responses in reactions involving cell mediated immunity as well as with the inflammatory reactions to "non-specific" tissue injury.

SUMMARY

Sera from patients with lepromatous leprosy contain a leukotactic (chemotactic) inhibitor that irreversibly inhibits a variety of chemotactic factors. The presence of this inhibitor correlates with lack of skin reactivity to a variety of antigens. The inhibitor appears to be similar to a serum factor recently termed the chemotactic factor inactivator. The presence in leprosy sera of this inhibitor may be responsible for some of the defects of cellular inflammatory responses found in patients with lepromatous leprosy.

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