INHIBITION OF THE INTRADERMAL HYPOSENSITIVITY REACTION OF DELAYED TYPE BY PATIENTS' SERUM FRACTION CONTAINING LEUKOCYTE MIGRATION INHIBITION FACTOR

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The blood serum of patients with active pulmonary tuberculosis and with chronic pneumonia inhibited migration of donors' leukocytes and of peritoneal exudate macrophages of guinea pigs compared with bovine serum or donors' serum. After chromatography of the patients' sera on columns with Sephadex G-100, fractions containing leukocyte migration inhibition factor (LMIF) inhibited the intradermal reaction to tuberculin or even abolished it completely in man and guinea pigs sensitized with BCG. A role for LMIF in the regulation of the hypersensitivity reaction of delayed type is postulated.

KEY WORDS: hypersensitivity of delayed type; migration inhibition factor; intradermal reaction.

After stimulation in vitro of sensitized lymphocytes by specific antigen, lymphokines appear in the supernatant [7], including a migration inhibition factor [1, 5], which can activate macrophates [8, 9]. This factor has been found in the serum of patients with certain lymphoproliferative diseases [6], but its role in vivo is not clear.

In the investigation described below the effect of fractions of the blood serum of patients with tuberculosis and chronic pneumonia, containing a leukocyte migration inhibition factor (LMIF), on the intradermal reaction of hypersensitivity of delayed type (HDT), developing in man and guinea pigs after injection of antigen, was studied.

EXPERIMENTAL METHOD

LMIF was discovered in the blood serum of patients with active tuberculosis and chronic pneumonia by the test of inhibition of migration of leukocytes from healthy persons or macrophages from guinea pigs from glass capillary tubes [2] during culture in medium 199 containing the test serum, compared with migration of the the same leukocytes in medium with bovine serum for healthy human serum. The indicator leukocytes were isolated as described previously [3] from heparinized donors' blood and suspended in medium 199 (5-10 million/ml). The test and control sera were added in a volume of 20% to equal portions (0.4 ml) of these leukocytes. With each portion of leukocyte five capillary tubes were filled, and these were lowered into walls in microtitrator slabs [3, 4] containing 0.05 ml medium 199 with the same serum as in the capillary tubes. The slabs were incubated for 24 h at 37°C, after which the number of cells which had migrated from the capillary tubes into the wells containing medium were counted and the index of inhibition of migration (IIM) was calculated (in %):

$$\text{IIM} = 1 - \frac{\text{Number of cells with test serum in wells}}{\text{Number of cells with control serum in wells}} \times 100\%.$$

The significance of differences was determined by Student's method.

The patients' blood sera, inhibiting leukocyte migration, and the control sera were subjected to gel-filtration on columns filled with Sephadex G-100 in Tris-buffer or with phosphate buffer, pH 6.8. The column was calibrated with blue dextran (mol. wt. 2,000,000), alcohol dehydrogenase (mol. wt. 150,000), egg albumin (mol.

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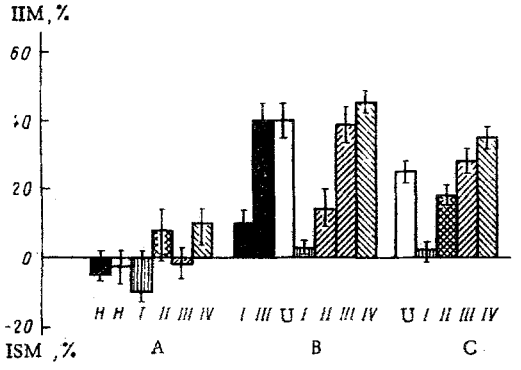

Fig. 1

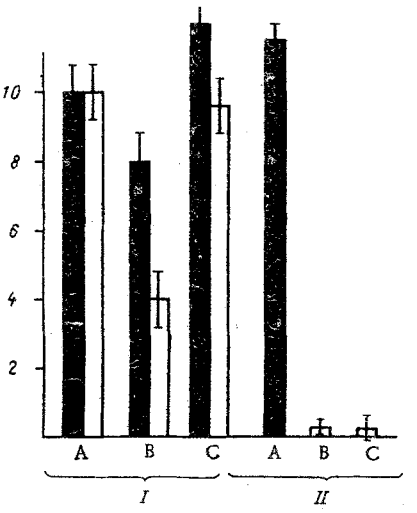

Fig. 2

Fig. 1. Effect of blood serum of patients with pulmonary tuberculosis and chronic pneumonia on migration of donors' leukocytes and of peritoneal exudate macrophages of guinea pigs. Ordinate, index of inhibition (stimulation) of migration – IIM (ISM) of leukocytes, in %. Confidence intervals shown for P = 0.05. A) Blood sera of healthy donors (n = 9); B) blood sera of patients with active pulmonary tuberculosis (n = 10); C) blood sera of patients with chronic pneumonia (n = 6). U) Unfractionated sera; I-IV) their various fractions. Black columns – peritoneal exudate macrophages of guinea pigs used as test cells; otherwise donors' leukocytes used.

Fig. 2. Inhibition of intradermal reaction to tuberculin in guinea pigs sensitized with BCG by third and fourth fractions of patients' serum containing LMIF. Ordinate, size of area of infiltration (in mm). A) Fractions of serum from healthy donors (n = 6); B) fractions of blood serum of patients with pulmonary tuberculosis (n = 4); C) fractions of blood serum of patients with chronic pneumonia (n = 6). I) Experiments of series I; II) experiments of series II.

TABLE 1. Inhibition of Intradermal Tuberculin Tests in Healthy Subjects by Fractions of Patients' Sera Containing LMIF

Donors of 3rd and 4th fractions of blood serum	Number of sera tested	IIM ($M \pm m$)	Number of people tested	Mean diameter of areas of infiltration (M ± m)	
Healthy subjects	4	1,04±0,065	15	$\frac{9\pm0,89}{9\pm0,92}$	(0)
Patients with pulmonary tuberculosis	3	0,70±0,077	10	$\frac{3\pm1,43}{12\pm1,78}$	(–75)
Patients with chronic pneumonia	3	0,62±0,062	6	$\frac{3\pm0,95}{7\pm1,26}$	(–57)

Legend. Numerator gives size (in mm) of intradermal infiltration in response to tuberculin injected with test fraction; denominator – the same without it. Percentage inhibition of intradermal reaction 48 h after testing, given by size of infiltration in experimental series

$$\frac{\text{size of infiltration in experimental series (in mm)} \times 100\%}{\text{size of infiltration in control (in mm)}} - 100\%$$ shown in parentheses.

wt. 45,000), trypsin inhibitor (mol. wt. 21,500), and ribonuclease (mol. wt. 13,500). Fractions of blood serum sterilized by filtration through a millipore filter (pore diameter 0.30 μ) were restored to physiological concentration with dry Eagle's medium and their effect was tested in various dilutions (1.0-0.001 mg/ml protein) on migration of indicator cells. The cytotoxicity of the fractions against healthy human leukocytes also was determined in the test with trypan blue.

The active and control fractions of serum were mixed with tuberculin, diluted in physiological saline so that the protein concentration of the fractions was 0.2-0.5 mg/ml, and the tuberculin dilution 1×10^{-4} (Mantoux test), and the mixture was injected intradermally in a dose of 0.1 ml into healthy subjects.

Tuberculin was injected intradermally in a dilution of 1×10^{-3} and a dose of 0.1 ml, mixed with the fractions of patients' or healthy donors' serum, into guinea pigs sensitized 2 weeks before the experiment with 1 mg BCG. Each mixture was injected at 2 or 3 points in the dorsal region of the guinea pigs after preparation of the skin. The reaction was read, just as in man, after 12, 24, 48, 72, and 96 h.

EXPERIMENTAL RESULTS

The blood serum of patients with active tuberculosis and chronic pneumonia inhibited migration of donors' leukocytes and peritoneal exudate macrophages of guinea pigs compared with migration of the same cells in medium with bovine or donors' serum (Fig. 1). Mainly fractions 3 and 4 (mol. wt. 10,000-40,000), obtained by gel-filtration of patients' sera on Sephadex G-100, possessed the ability to inhibit leukocyte migration. Consequently, with respect to molecular weight, the active fraction of the blood serum corresponded more to the factor inhibiting migration of guinea pig macrophages than to the factor inhibiting migration of polymorphs found in the supernatant of stimulated lymphocytes [10]. LMIF present in the blood serum of different patients differed somewhat in molecular weight within the above limits, i.e., it was a heterogeneous class of molecules. Only 2 of the 15 sera had weak leukocyte migration inhibiting activity in fraction 1. This activity was evidently due to the antigen–antibody complex, which can inhibit leukocyte migration [4]. The blood serum fraction had no cytotoxic action on leukocytes, as shown by the trypan blue test. Consequently, the blood serum of patients with chronic inflammatory diseases of the lungs accompanied by strong activation of lymphoid tissue, LMIF is present.

The test fractions of blood serum mixed with tuberculin were injected intradermally into guinea pigs sensitized with BCG. The active fraction containing LMIF reduced the size of the area of infiltration of the skin in response to tuberculin (Fig. 2). Even greater inhibition of the intradermal reaction to tuberculin was observed in guinea pigs into which the same active fraction was injected intraperitoneally in a dose of 1-3 ml/mg protein 2 h before the intradermal test (Fig. 2, series II).

Fractions of patients' blood serum containing LMIF, if injected intradermally into healthy subjects mixed with tuberculin, also considerably inhibited their intradermal reaction (Table 1). However, the degree of its inhibition by fractions of different blood sera in different tested individuals varied, although strong reactive fractions inhibited the intradermal reaction considerably in all subjects, whereas weakly active fractions did so only in some subjects.

Fraction 1 of the patients' serum, tested in the same dose of protein as fractions 3 and 4, not only did not inhibit the intradermal reaction, but could actually potentiate it, possibly because of the presence of antigen–antibody complex, stimulating the inflammatory reaction. In some cases fraction 3 of some clinically healthy subjects also inhibited the intradermal reaction. Evidently either these fractions also contained LMIF in low doses in connection with some pathological process, or this inhibition was due not to it, but to some other substance present in these fractions. However, substances in normal plasma inhibiting the inflammatory reaction have a molecular weight of under 1000 [11] and they must have been removed in the present experiments during dialysis of the fractions.

Fractions of patients' serum containing LMIF thus possessed the ability to inhibit the intradermal reaction of HDT nonspecifically in guinea pigs and man, i.e., in xenogeneic and allogeneic systems. However, whereas in guinea pigs this inhibition was caused by patients' blood serum, in man the fractions of blood serum of some healthy subjects also occasionally possessed this property. There is evidence that the supernatant of stimulated guinea pig lymphocytes, containing a factor inhibiting migration of their macrophages, can inhibit their intradermal HDT [12]. The presence of LMIF in the patients' blood serum and the ability of the fraction containing it to inhibit the intradermal reaction, discovered in these experiments, point to the participation of this factor in the regulation of HDT, possibly by a mechanism of feedback type: contact between sensitized lymphocytes and antigen leads to their activation and to the liberation of LMIF, which acts on the receptors of lymphocytes and other leukocytes and inhibits HDT.

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