An inhibitor of leucocyte movement in the plasma of patients with rheumatoid arthritis

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SUMMARY Plasma from patients with rheumatoid arthritis shows inhibitory activity towards the random and stimulated movement of human polymorphonuclear leucocytes and peripheral phagocytic monocytes in vitro. The inhibitory activity is heat-labile, not present in plasma from patients with noninflammatory diseases, and appears to exert its inhibitory effects by occupying sites on the cell membrane from where it may be removed by washing. It is suggested that this factor(s) is responsible for the diminished accumulation of polymorphonuclear leucocytes into skin chambers which occurs in patients with rheumatoid arthritis.

It has been reported that a significant reduction in the accumulation of polymorphonuclear leucocytes (PMNs) in skin chambers occurs in patients with rheumatoid arthritis (RA) compared with controls who were either normal subjects or patients with osteoarthritis.¹ The depressed cellular response could be due to one or more of a variety of factors, but most attention has been given to possible alterations in PMN migration. It has been reported that both cellular and humoral defects are present in the circulation of patients with RA.^{1,2} In this communication we present evidence that a heat-labile factor which inhibits the movement of both PMNs and peripheral mononuclear phagocytes (MN) in vitro is present in the plasma of patients with RA.

Patients and methods

All the patients reported on in this study were attending rheumatology outpatient clinics at King's College Hospital and had either definite or classical rheumatoid arthritis (RA group) or were suffering from either osteoarthritis or low back pain (control group). All the RA group and a number of the control patients were being treated with non-steroidal anti-inflammatory drugs. In addition, penicillamine, and occasionally gold and steroids, were being administered to the patients with RA.

Accepted for publication 13 December 1979

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PLASMAS (ALL SUBJECTS)

Samples (20 ml) of blood were obtained by venepuncture and transferred to bottles containing EDTA. The plasmas were separated by centrifugation and used as unheated plasmas either immediately or after storage at -20°C for no longer than 2 weeks. Heated plasmas were portions of the same specimens which had been pretreated by heating at 56°C for 30 min before use.

CELLS (CONTROL GROUP)

PMN preparations (70-80%) were collected by dextran sedimentation of heparinised venous blood as described previously.¹ Purified PMN and MN populations were obtained by a 1-step Hypaque-Ficoll separation.³ All the leucocyte preparations were washed twice with 0.9% (w/v) saline and finally resuspended in MEM (Wellcome Reagents, Beckenham, Kent), buffered to pH 7.3 with 30 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulphonic acid (HEPES). In some experiments mixed leucocyte preparations (60-75% PMNs) were obtained from blood samples of patients in the control and RA groups by sedimentation under gravity, removal of most of the contaminating platelets by centrifugation, and resuspension of the leucocytes to the same concentration in buffered MEM, any further washing step with saline being omitted (unwashed leucocyte preparations).

CHEMOTAXINS

Synthetic FMet-Leu-Phe (FMLP) was used as the

chemotaxin in experiments with PMNs only. In the experiments with the unwashed leucocytes and in all those with the MNs, the chemotaxin was plasma, from one of the control group of patients, which was activated by zymosan as described previously.¹

CELL MIGRATION STUDIES

The terminology of cell migration used in this communication is that of Keller *et al.*⁴

Agarose microdroplet assay. Details of the methodology have been published elsewhere.⁵ Basically, equal volumes of leucocyte suspensions in MEM and of agarose containing MEM and 10% heated human serum were mixed, and samples (2 μ l) of the diluted cell suspension (4×10^5 PMN or 2×10^6 MN) were pipetted into each well of cooled 96-well microtitre plates (Sterilin Ltd., Teddington, Middlesex). After solidification of the droplet (3–5 min), 5 μ l of plasma and either 0.1 ml of MEM or of FMLP (2.5×10^{-9} M) for 4 h PMN stimulated migration studies, or double quantities of MEM for PMN and MN random migration experiments left for 22 h, were added to the wells. In each experiment samples were tested in 4 to 6 wells, the first and last series of wells on each plate being MEM controls. The areas of cell migration were measured by planimetry after incubation in a humid atmosphere and expressed as percentage increases in the initial areas of the agarose droplet.

Agarose plates. The agarose plate technique⁶ was used as previously described⁷ to study the effects of plasmas on the directed movement of both PMN and MN towards an attractant. Samples (5 μ l) of cell suspension (2.5×10^5 PMN or 5×10^6 MN) were placed in the centre well and 2 μ l of control or RA plasma was added to the cells. FMLP (10^{-6} M) and undiluted zymosan-activated plasma were used as attractants for the PMNs and MNs respectively. After 2 h (PMN) or 22 h (MN) incubation in a humid atmosphere at 37°C the plates were fixed with methanol, assessed as described previously, and the results expressed as the chemotactic differential, that is, the distance moved by directed migration.

Boyden chambers. The response of unwashed PMNs prepared from blood samples from the patients with RA and the control group, towards zymosan-activated plasma from subjects in the control group was studied in modified Boyden chambers (Neuroprobe Inc., Maryland, USA). The cells were prepared as described above and suspended in MEM to a standard concentration of 2.5×10^6 /ml and placed in the upper compartment of chambers fitted with 5 μ m Millipore filters. The activated plasma diluted (5% v/v) in MEM was placed in the lower chamber. After 90 min incubation at 37°C in a humid atmosphere the

filters were removed, stained, and the numbers of cells reaching the lower surface of the filters per high-power field ($\times 400$) were counted as described previously.¹

Results

The effects of the plasmas on aspects of leucocyte movement in vitro are shown in Tables 1–3. A consistent finding was that only untreated plasma from patients with RA significantly inhibited the random migration of both PMNs and MNs (Table 1), the chemokinetic response of PMNs to FMLP (Table 2), and the directed migration of PMNs to FMLP and MNs to zymosan-activated plasma (Table 3). A heat-labile factor which interfered

Table 1 Effects of plasmas from control and RA group on random migration of PMNs and MNs by the agarose microdroplet method

Group	Pretreatment	Percentage increase in migration area ($\pm$ SEM)	
		PMNs	MNs
Medium (MEM) alone		40 $\pm$ 3	64 $\pm$ 4
Control (n=10)	Unheated	99 $\pm$ 3	104 $\pm$ 6
	Heated	101 $\pm$ 5	94 $\pm$ 5
RA (n=10)	Unheated	52 $\pm$ 1*	44 $\pm$ 5*
	Heated	117 $\pm$ 7	97 $\pm$ 4

*P<0.001 from results of corresponding heated RA and unheated control subgroups after 22 hr incubation.

PMN=polymorphonuclear leucocyte. MN=peripheral mononuclear phagocyte.

Table 2 Effects of plasmas from control and RA groups on chemokinetic response of PMNs to FMLP using the agarose microdroplet method

Group	Pretreatment of plasma	Percentage increase in migration area ($\pm$ SEM)
Control (n=20)	Unheated	188 $\pm$ 12.1
	Heated	189 $\pm$ 7.2
RA (n=32)	Unheated	119 $\pm$ 6.3*
	Heated	196 $\pm$ 9.2

Percentage increase in area to peptide alone in the absence of plasma = 182 $\pm$ 10.5 after 4 h incubation.

*Result significantly different from other groups, P<0.001.

Table 3 Effects of plasmas from control and RA groups on the directed migration of PMNs to FMLP and MNs to zymosan-activated plasma by the agarose plate method

Group	Pretreatment of plasma	Directed migration (μ m) ($\pm$ SEM)	
		PMNs	MNs
Control (n=10)	Unheated	1536 $\pm$ 34	317 $\pm$ 24
	Heated	1605 $\pm$ 19	327 $\pm$ 24
RA (n=10)	Unheated	1276 $\pm$ 26*	67 $\pm$ 22*
	Heated	1501 $\pm$ 34	286 $\pm$ 36

*P<0.001 from results of corresponding heated RA and unheated control sub-group.

Table 4 Response of unwashed leucocyte preparations from control and RA group to zymosan-activated plasma in Boyden chambers

Group	Number of cells per high power field ($\pm$ SEM)
Control (n=9)	86 $\pm$ 4
RA (n=11)	47 $\pm$ 5*

*P<0.001 from results of control group; each experiment was performed in triplicate.

with the locomotion of both PMNs and MNs in vitro was therefore present in the circulation of patients with RA.

The results given in Table 4 show that unwashed preparations of leucocytes, prepared from blood samples from patients with RA responded to a significantly lesser degree to zymosan-activated plasma than corresponding preparations of leucocytes from control patients. The period of the experiment (90 min) was such that only PMN movement was measured.

Discussion

The results of the present work show the presence of a factor in the plasma of patients with RA which inhibits the random movement and stimulated migration, both random and directed, of normal PMNs and MNs in vitro. This factor is heat-labile, being destroyed by heating at 56°C for 30 min, exerts a direct action on the cells, and is absent from the plasma of patients with either osteoarthritis or back pain, that is, suffering from noninflammatory conditions. The presence of this material in the circulation of patients with RA could explain the reduced accumulation of PMNs found to occur in skin chambers in such patients.¹ As in our previous study, there was no apparent relationship between the inhibitory activity observed in any one patient's or group of patients' plasmas and drug therapy.

Its relation to other reported defects of leucocyte movement in patients with RA is of interest. Mowat and Baum,² using a filter assay, found that PMNs from the blood of patients with RA were significantly less responsive to chemotaxins than were the corresponding cells from normal subjects. This effect was not observed by Walker *et al.*,^{1,7} but the experimental conditions used by both groups of workers differed with respect to the extent of washing of the isolated leucocyte preparations. It has recently been demonstrated⁸ that when PMN preparations are washed with saline or buffers there is a progressive removal of proteins, including activators of cell migration. In the present work the minimum of washing during the preparation procedures produced a leucocyte preparation from the blood of

patients with RA which was significantly less responsive to chemotactic stimuli than leucocytes from control patients (Table 4). It is therefore suggested that the heat-labile inhibitor of leucocyte movement found in the plasma of patients with RA is weakly bound to leucocytes in the circulation. Its presence would explain the experimental findings of Mowat and Baum,² who did not wash their leucocyte preparations and its removal in vitro would explain the results of Walker *et al.*,⁷ who washed their leucocyte suspensions several times before use.

An inhibitory material described by Walker *et al.*¹ differs from the present factor in being heat-stable and not formed in the plasma from patients with RA until the plasma has been activated by zymosan. The heat-stable inhibitor also affects the migration of MNs and occurs in some of the RA patients in which the heat-labile factor is absent (unpublished data). The 2 factors cannot be identical, but they may both be produced during the development of chronic inflammatory diseases such as RA.

We thank Drs E B. D. Hamilton, C. J. Goodwill, and H. Berry for allowing us access to patients under their care and Mrs N. Henderson for excellent technical assistance. J. R. Walker was the holder of the Cowburn scholarship at King's College Hospital Medical School during the course of these studies.

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