Neutrophil chemotaxis in juvenile chronic arthritis

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SUMMARY   The leucocyte infiltration observed in histological lesions from patients with juvenile chronic arthritis (JCA) suggests the possibility of an abnormal leucochemotaxis. A group of 21 patients with JCA which fulfilled the Eular criteria (Oslo Symposium 77) were investigated with a paired group of 21 children. The chemotactic assay used was a microscopical direct observation technique. The chemotaxis of the patient's leucocytes was in the normal range, as was the chemotactic activity of their serum. However, their serum had an enhancing effect on the chemotaxis of normal leucocytes. An attempt to characterise this chemotactic enhancing factor was undertaken. It was not dialysable, heat stable, destroyed at 80°C, nor precipitated by ammonium sulphate at 45%; it could be migrated on PAGE with albumin, and, by precipitation with a goat antihuman albumin antiserum, seemed to be bound to the albumin fraction. The function of this factor in the regulation of the inflammatory process is discussed.

One of the characteristics of the anatomical lesion observed in juvenile chronic arthritis (JCA) is an infiltration of inflammatory cells. The chemotaxis of polymorphonuclear and mononuclear cells towards the inflammatory site is considered to be an important mechanism leading to the formation of the lesion.[1] Mowat and Baum have found, using the Boyden chamber's system, that the polymorphonuclear (PMN) leucocytes from 8 children with JCA have a significant decrease of chemotaxis.[2][3] It was suggested that this impairment was due to phagocytosis of immune complexes by the patient's leucocytes.

Our object was to study chemotactic activity in a group of 21 children with JCA using a direct microscopic observation method,[4][5] which allowed us to evaluate 3 different parameters: (1) the chemotaxis of the PMN leucocytes; (2) the chemotactic factors generated in the patients' serum in the presence of immune complexes; (3) the effect of the patients' or normal serum on the chemotactic response of normal PMN leucocytes previously incubated in the tested serum. We found that the chemotaxis of patient PMN and the generation of chemotactic substances in the patient's sera in the presence of immune complexes were comparable to that of the controls. However, the patient's sera enhanced the chemotactic response of normal PMN after a previous incubation with abnormal sera, suggesting the presence of an enhancing factor of leucochemotaxis in JCA sera.

Material and methods

Group of patients. Twenty-one children (17 girls and 4 boys) who fulfilled the criteria of JCA established at the Oslo Symposium[6] were investigated. The 21 patients, regularly followed up in our unit, were classified as follows: 11 systemic, 6 polyarticular, and 4 pauciarticular forms of JCA. Laboratory investigations showed inflammatory symptoms in most of them (18 out of 21). At the time of the investigation 16 patients were under cortiosteroid therapy, 4 were on salycilate, 1 was on gold salts, and 1 was treated with chlorambucil. The control group consisted of 21 children with upper respiratory infections without feverish symptoms. There was no significant difference between the ages of the patients with JCA and the patients in the control group (respectively $11 \cdot 09 \pm 3 \cdot 37$ and $10 \cdot 60 \pm 7 \cdot 43$; Student's t test, P=0·27). Three children with systemic lupus erythematosus (SLE) were also investigated, 2 of them being under steroid therapy, the other before he had received treatment.

Chemotaxis assay. Each patient was investigated in comparison with a normal control. 5 ml of blood was withdrawn in a sterile syringe with 50 IU of preservative-free heparin (Liquemine) and was allowed to sediment in a plastic tube at room temperature for about 30 min. The buffy coat was collected and washed twice in 199 medium (Gibco Biocult), then▼resuspended at a concentration of 5×10^3/ml. The chemotaxis index of the leucocytes

was determined by a direct microscopic method previously described. [4] [5] We studied the chemotactic responses of the patients' leucocytes using as chemotactic factors normal human serum (pool of AB Rh+ sera) activated with immune complexes BSA-rabbit anti-BSA (BSA-aBSA). The generation of chemotactic factors in the patients' serum after activation with immune complexes BSA-aBSA was tested by the chemotactic responses of normal leucocytes. Chemotactic responses of normal leucocytes preincubated in the patients' serum (37°C for 30 min) were tested with normal human serum activated with BSA-aBSA complexes. Leucocytes after incubation were washed twice in 199 medium, and the leucocyte viability was always greater than 95% by the trypan blue exclusion test. All sera were stored at −70°C whenever they were not tested immediately.

Characterisation of serum chemotactic enhancing factor (CEF). The enhancing capacity of the chemotactic responses of normal leucocytes was tested after preincubation (37°C for 30 min) in the presence of sera from 4 different patients: after an overnight dialysis against PBS; after heating at 56°C for 30 min without dilution; and after heating at 80°C for 30 min and a 1/5 (v/v) dilution. The patients' sera were precipitated in the presence of a 45% ammonium sulphate solution (v/v), which resulted in a precipitated fraction (PF) and a soluble fraction (SF). Redissolved PF in 0·5 v distilled water and SF were dialysed against PBS and reconstituted to the initial volume.

Each fraction was tested for its enhancing capacity on leucochemotaxis. Chemotactic enhancing activity was found only in SF. Subsequent analysis of the SF by immune electrophoresis and polyacrylamide gel electrophoresis (PAGE) showed that SF contained mostly albumin and few contaminants of β motility. SF was then precipitated in the presence of a goat antialbumin serum (Meloy Lab. lot 77642) and the supernatant tested for chemotactic enhancing activity.

Results

Our results are shown on Fig. 1. Chemotactic responses of 18 out of 21 JCA patients' leucocytes

Chemotactic responses of leucocytes	Normal leucocytes		JCA patient leucocytes	Normal leucocytes					
Preincubation	No	No	No	Normal serum	Miscellanous patient serum	JCA patient serum	PF	SF	SLE serum
No. tested	45	21	21	12	21	21	4	4	3
Chemotactic index	①	②	③	④	⑤	⑥	⑦	⑧	⑨
Serum used for generation of chemotactic factor	Normal	JCA patient serum	Normal serum						
Mean ± SD	1.41 0.064	1.4209 0.187	1.458 0.133	1.405 0.074	1.476 0.232	1.783 0.231	1.33 0.05	1.647 0.137	1.25 0.05
t test	①-③ 2.80 NS	①-② 2.73 NS		④-⑥ 4.08 p<0.001		⑤-⑥ 4.84 p<0.001		⑦-⑧ 4.16 p<0.02	④-⑨ 0.7 NS

Fig. 1   *Chemotactic index was the mean of 3 assays for each sample. The figure shows: (1) Chemotactic index of leucocytes from normal control ▲ (col. 1) and JCA leucocytes ■ (col. 3) in the presence of chemotactic factors generated by normal serum. (2) Chemotactic index of normal leucocytes in the presence of chemotactic factors generated by JCA sera ▲ (col. 2). (3) Chemotactic index of normal leucocytes tested after preincubation with normal serum ● (col. 4) with miscellaneous patient serum (control group) ● (col. 5), with JCA patients' serum ● (col. 6), with precipitated fraction PF ● (col. 7) and supernatant fraction SF ● (col. 8). (4) Chemotactic index of normal leucocytes tested after preincubation with systemic lupus erythematosus (SLE) serum ● (col. 9).*

(col. 3) to chemotactic factor generated by normal human serum in the presence of BSA-aBSA were identical to responses of normal leucocytes studied in the same condition (col. 1). Generation of chemotactic factors by JCA patients' sera activated by BSA-aBSA (col. 2) did not differ from the chemotactic activity obtained with normal human sera. However, while the preincubation of normal leucocytes in the presence of normal human serum (col. 4) and noninflammatory sera (col. 5) had no effect, the preincubation of normal leucocytes in 19 out of 21 JCA patients' sera (col. 6) significantly enhanced their chemotactic responses to chemotactic factor generated by normal human serum activated by BSA-aBSA complexes ($P<0\cdot001$). Incubation of normal leucocytes in SLE sera did not affect their chemotactic responses (col. 9).

Chemotactic enhancing factors appeared to be not dialysable and resistant at 56°C, but their effect was abolished by heating to 80°C. Storage of patients' sera at −70°C did not modify their enhancing effect. The effect was not present in ammonium sulphate precipitated fractions (PF) and recovered in supernatants (SF) (cols. 7 and 8). Elimination of the majority of albumin in SF by a goat antihuman albumin antiserum abolished the chemotactic enhancing activity. A possible enhancing chemotactic effect of albumin itself was eliminated, since chemotactic experiments performed with normal human or bovine albumin and ovalbumin did not modify the leucochemotaxis.

Discussion

In the present study we observed that the large majority of JCA patients' leucocytes responded normally to chemotactic factors, in contrast to previous studies performed in 8 JCA patients by Mowat *et al.* [2][3] We showed that the generation of chemotactic factors was identical in serum obtained from JCA patients and normal human serum activated by immune complexes. However, incubation of normal leucocytes in the presence of JCA serum resulted in a significant increase of their chemotactic responses. This finding suggests that JCA serum contains chemotactic enhancing factors (CEF) not present in normal serum, in sera from 21 patients with noninflammatory diseases, and in sera from 3 SLE patients.

In an attempt to characterise CEF present in JCA sera we found that the activity was not dialysable and not detected in ammonium sulphate precipitate but was recovered in supernatant. This result indicates that the effect was not due to antibodies or immune complexes. The enhancing activity was not due either to complement derivative

such as C3a or C5a, since these components with molecular weights of 7200[7] and 8500[8] are dialysable. The fact that CEF activity was abolished by precipitation of albumin fraction suggested that CEF was associated with albumin. Albumin fraction from normal human serum or from other species did not exert a chemotactic enhancing effect. Thus it is likely that CEF may be bound to albumin fractions.

Chemotactic enhancing factor (CEF) was detected in the majority of JCA patients tested in the present study. This activity could not be related to the various clinical aspects of the disease, since it was observed both in systemic onset cases and in cases of polyarticular or pauciarticular onset. Similarly, we did not find any correlation with the presence of acute-phase reactants, which more or less varied in intensity from one patient to another. Therapeutic agents (gold salts, salicylate, and corticosteroids) did not seem to affect the chemotactic enhancing activity. However, in 1 patient who received chlorambucil at the time of the study no CEF was found.

Hyperleucocytosis, with high rate of PMN, raised the question of a possible role of this serum CEF on the appearance of the hyperleucocytosis. The mechanism by which this enhancing capacity is generated and the role of this factor in the inflammatory process remain a matter of speculation. Two hypotheses may be suggested—increased production of the CEF itself or dysregulation of the chemotactic factor inactivator usually found in normal human serum.[9][10]

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