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Polymorphonuclear leucocyte function and previous yersinia arthritis: correlation of enhanced superoxide production with late manifestations

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SUMMARY Polymorphonuclear leucocyte (PMN) functions (migration in vitro, chemiluminescence, O_2^- production, and aggregation) were studied in 32 patients with previous yersinia arthritis (YA). PMNs of 11 HLA-B27 positive patients who had chronic or recurrent inflammatory symptoms showed O_2^- production significantly higher than that of PMNs of 11 HLA-B27 positive patients without late manifestations. Also, PMNs of both HLA-B27 positive and negative patients tended to show chemotactic and chemokinetic migration rates higher than those of control cells of healthy HLA-B27 negative subjects. These functional aberrations may play a part in the development of the patients' inflammatory symptoms.

Key words: neutrophil, chemotaxis, superoxide, HLA-B27, chemiluminescence.

The association of HLA-B27 with ankylosing spondylitis and other seronegative arthropathies, such as reactive arthritides, after enteritis caused by salmonella, shigella, campylobacter, or *Yersinia enterocolitica* and urogenital infection caused by chlamydia is well established. The pathogenic mechanisms of these diseases may involve aberrant immune response[1–4] or inappropriately strong inflammatory reactivity,[5] or both. The assumption of strong inflammatory reactivity is supported by the finding that polymorphonuclear leucocytes (PMNs) of HLA-B27 positive subjects show high chemotaxis in vitro[6 7] and in vivo.[8] Also, after treatment with zymosan, an activator of the alternative pathway of complement, serum samples from HLA-B27 positive subjects stimulate more PMN migration[9] and contain complement derived oligopeptides C3a desArg and C5a desArg in higher amounts[10] than do such sera from HLA-B27 negative subjects.

In our last study PMNs of HLA-B27 positive patients whose acute yersinia arthritis (YA) had been severe showed chemokinetic and chemiluminescent responses higher than PMNs of those with a mild disease.[11] The purpose of the present work was to study whether there are differences in PMN function between HLA-B27 positive patients with YA and chronic/recurrent inflammatory manifestations and those without late manifestations. In previous studies PMNs of HLA-B27 negative patients with ankylosing spondylitis,[7] but not those with previous YA,[6] had shown enhanced migration in vitro; therefore we also studied groups of patients and controls who were HLA-B27 negative.

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Subjects and methods

SUBJECTS

Twenty two HLA-B27 positive patients with previous YA from a follow up study[12] were assigned to two groups on the basis of the presence (group I, Table 1) or absence (group II) of chronic or recurrent inflammatory symptoms. In group I one of the patients had definite ankylosing spondylitis at the time of acute YA, two patients developed it during the follow up period, and the rest had at least one of the inflammatory symptoms presented in Table 1. The patients in group II had recovered completely from YA and had no late manifestations

(Table 1). Two other groups were included in the present study: HLA-B27 negative patients with YA but without late manifestations (group III, Table 1) and HLA-B27 negative healthy volunteers from laboratory and hospital staff (group IV). The subjects had not taken non-steroidal anti-inflammatory drugs during a 72 hour period before bleeding, with the exception of one patient in group I who used indomethacin 50–150 mg daily.

Cells of one subject from each of the four groups were tested on a given day. The patients in groups I and II were matched for age and sex. Serum samples were stored at −20°C.

Table 1 *Clinical characteristics of the subject groups*

Characteristics	*I**	*II**	*III**	*IV**
General characteristics				
Number of subjects	11	11	10	11
Sex: men/women	6/5	6/5	2/8	1/10
Age at time of present study; mean (range), years	37·0 (21–51)	37·3 (26–51)	49·3 (42–59)	41·4 (34–46)
Follow up period; mean (range), years	8·9 (5–13)	9·5 (7–14)	11·0 (9–14)	—
Chronic or recurrent inflammatory symptoms†				
Ankylosing spondylitis	3	0	0	0
Radiological sacroiliitis	6	0	0	0
Recurrent arthritis	2	0	0	0
Recurrent iritis	2	0	0	0
Enthesopathy	1	0	0	0
Use of non-steroidal anti-inflammatory drugs†				
Regular	1	0	0	0
Irregular	3	1	0	0

*I=HLA-B27 positive patients with chronic or recurrent inflammatory symptoms; II=HLA-B27 positive patients without chronic sequelae; III=HLA-B27 negative patients without sequelae; IV=HLA-B27 negative healthy subjects.
†Values show number of subjects.

CELLS

Buffy coat cells for the migration assays were separated from heparinised peripheral blood by dextran sedimentation. PMNs (over 95% pure) for other cell function assays were separated from the buffy coat cells by Ficoll-Isopaque density gradient centrifugation followed by hypotonic lysis of the erythrocytes.

NEUTROPHIL STIMULATING AGENTS

Zymosan treated serum (ZTS) and opsonised zymosan particles were prepared as described previously.[11] *N*-Formyl-methionyl-leucyl-phenylalanine (fMLP) was purchased from Sigma Chemical Co, St Louis, Missouri. The concentrations of fMLP were 10^{-6} mol/l in the study of O_2^- production, 10^{-8} mol/l and 10^{-9} mol/l in the study of chemokinesis, 10^{-8} mol/l and 5×10^{-7} mol/l in the study of

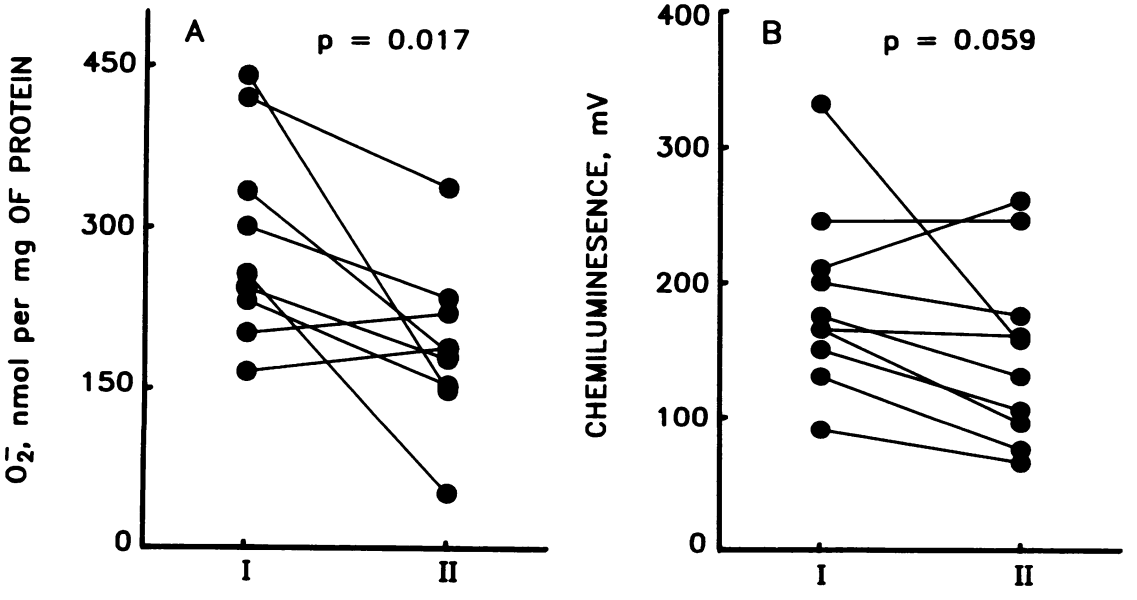

Fig. 1 *(A) O_2^- production in response to zymosan treated serum and (B) chemiluminescent responses to opsonised zymosan. Group I (sequelae); group II (recovered). All subjects were HLA-B27 positive. Each line indicates results obtained on a given day.*

chemotaxis in the filter and under agarose respectively, and 2×10^{-7} mol/l in the study of aggregation.

PHAGOCYTE FUNCTION ASSAYS

Testing of PMN function was carried out as described in detail previously.[11] The production of O_2^- induced by fMLP or 2% ZTS was determined by the method of Pick and Mizel[13]; the incubation period of the dishes at 37°C was 60 minutes and the results are expressed as nmol of O_2^-/mg of PMN protein. Luminol enhanced chemiluminescence induced by opsonised zymosan particles was determined as described earlier[11]; maximum heights (millivolts, mV) of the response curves are presented. The rates of chemotactic, chemokinetic, and spontaneous migration of PMNs were determined by the concurrent use of the leading front modification[14] of the Boyden chamber technique and the agarose assay.[11] After treatment with zymosan the chemokinetic effects of 6% serum samples of each patient and control on PMNs of a healthy HLA-B27 negative donor were tested by the leading front method. Aggregation assay was carried out according to Craddock[15]; maximum heights (cm) of the response curves are presented.

STATISTICAL METHODS

Significance of the difference between the groups was evaluated by Student's *t* test. Comparison of the results between groups I and II was performed with paired sample *t* test.

Results

COMPARISON OF HLA-B27 POSITIVE PATIENTS WITH AND WITHOUT SEQUELAE

Spontaneous and stimulated production of O_2^- were both increased in group I PMNs (Table 2), but the difference was significant only with ZTS (Fig. 1A). In nine out of 10 pairs group I PMNs displayed

Table 2 *PMN function in subject groups I-IV*†

Function‡	*I*§	*II*§	*III*§	*IV*§
O_2^- (nmol/mg PMN protein)	n=9	n=9	n=7	n=9
Spontaneous production	142 (79)‖	96 (51)	109 (64)	101 (55)
10^{-6} M fMLP	233 (102)	177 (52)	200 (115)	165 (87)
ZTS	287 (94)*	187 (77)	219 (100)	217 (107)
Chemiluminescence, maximum (mV)	n=10	n=10	n=8	n=10
	186 (67)	147 (67)	133 (45)	164 (85)
Migration in filter (μm)	n=11	n=11	n=10	n=11
Spontaneous migration in HBSS	17 (3)	18 (2)	18 (2)	17 (2)
Chemokinesis in: 0·2% HSA	53 (7)	56 (4)	57 (7)	53 (7)
12% ZTS	71 (10)	73 (12)	68 (14)	64 (19)
10^{-8} M fMLP	56 (10)	55 (8)	55 (9)	49 (6)**
10^{-9} M fMLP	54 (8)	58 (7)	58 (6)	55 (6)
Chemotaxis toward: 12% ZTS	64 (11)	63 (11)	68 (11)	62 (14)
10^{-8} M fMLP	80 (18)	80 (12)	81 (15)	74 (9)
Migration under agarose (mm)				
Spontaneous migration	1·1 (0·2)	1·1 (0·2)	1·1 (0·2)	1·2 (0·2)
Chemokinesis in: 12% ZTS	1·5 (0·3)	1·6 (0·2)	1·5 (0·2)	1·4 (0·2)
10^{-8} M fMLP	2·2 (0·3)	2·3 (0·3)	2·2 (0·4)	2·1 (0·3)
10^{-9} M fMLP	1·1 (0·2)	1·2 (0·1)	1·1 (0·2)	1·2 (0·1)
Chemotaxis toward: ZTS	2·1 (0·2)	2·1 (0·3)	2·1 (0·4)	2·0 (0·2)
5×10^{-7} M fMLP	2·3 (0·9)	2·6 (0·6)	2·6 (0·8)	2·2 (0·6)
Aggregation, maximum (cm)	n=11	n=11	n=10	n=11
	28·8 (4·3)	28·8 (4·5)	28·6 (4·6)	27·8 (2·1)
Chemokinetic activity of sera¶ (μm)	n=11	n=11	n=10	n=11
	103 (25)	108 (27)	107 (23)	117 (13)

*Significantly different from group II, $p<0.02$; **significantly different from patients (groups I–III, n=32), $p<0.05$.
†In some series of experiments all functions of PMNs could not be studied because of a low yield of cells.
‡HBSS=Hanks's balanced salt solution; HSA=human serum albumin; ZTS=zymosan treated normal human serum; fMLP=*N*-formyl-methionyl-leucyl-phenylalanine.
§I=HLA-B27 positive patients with chronic or recurrent inflammatory symptoms; II=HLA-B27 positive patients without chronic sequelae; III=HLA-B27 negative patients without sequelae; IV=HLA-B27 negative healthy subjects.
‖Values are mean (SD).
¶Chemokinetic activity of serum samples from patients and controls after treatment of the serum samples with zymosan.

chemiluminescent responses equal to or higher than those of group II cells (Table 2; Fig. 1B); the curves (not shown) were of uniform shape.

In the membrane filter the rates of spontaneous and chemotactic migration of group I PMNs were similar to those of group II cells (Table 2). Group II PMNs showed higher chemokinetic migration rates, but the difference was not statistically significant. In the agarose assay the rates of spontaneous migration and the rates of ZTS induced chemotactic and chemokinetic migration were very similar in the two groups (Table 2), whereas fMLP induced migration rates tended to be higher in group II.

In the aggregation test the responses of PMNs of groups I and II were much the same, as were the chemokinetic activities of the zymosan treated serum samples (Table 2).

COMPARISON OF PATIENTS AND CONTROLS

PMNs of both HLA-B27 positive patients (groups I and II) and HLA-B27 negative patients (group III) tended to show higher chemotactic and chemokinetic migration rates than group IV cells (Table 2).

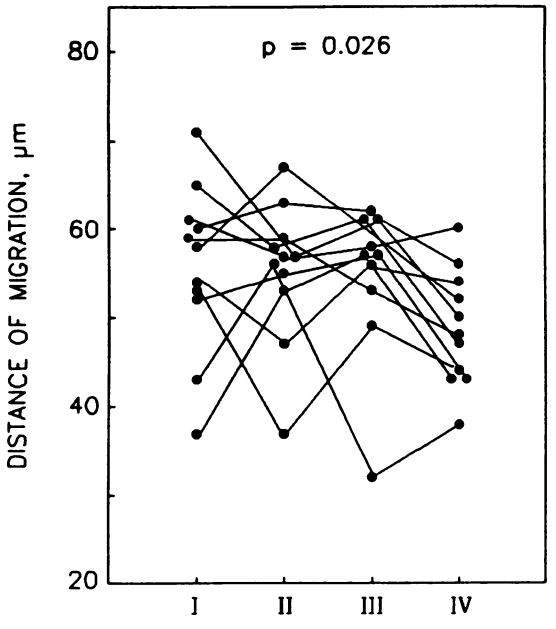

Fig. 2 *Chemokinetic migration in the membrane filter in 1×10^{-8} M N-formyl-methionyl-leucyl-phenylalanine. (I) HLA-B27 positive patients with sequelae and (II) without sequelae; (III) HLA-B27 negative patients without sequelae; (IV) HLA-B27 negative healthy controls. The p value indicates the significance of the difference between patients (groups I–III, n=32) and controls (group IV, n=11). The lines indicate results obtained on a given day.*

In the filter assay the difference between the patients (n=32) and controls (n=11) was significant in the study of chemokinesis in 10^{-8} M fMLP (Table 2; Fig. 2) and close to significance in the study of chemokinesis in 12% ZTS (Table 2; p=0·07). The corresponding differences between patients and controls in chemiluminescent responses, O_2^- production, rates of aggregation, and chemokinetic activity of serum samples were not significant (Table 2).

Discussion

The results show that oxy radical production, as determined by reduction of ferricytochrome *c* and by generation of luminol enhanced chemiluminescence, tends to be increased in the PMNs of those HLA-B27 positive patients with previous YA who have recurrent or chronic inflammatory symptoms. Thus the enhanced oxy radical production was associated with sequelae but not with HLA-B27 or previous YA themselves. This is in accordance with our previous study showing that O_2^- production by PMNs of HLA-B27 positive and negative patients with previous YA was similar to that by PMNs of healthy controls.[16] Indomethacin depresses O_2^- production induced by fMLP but enhances that induced by opsonised zymosan.[17] As only one patient had taken indomethacin during a 72 hour period before bleeding, however, the use of drugs does not explain the difference between groups I and II.

The enhanced oxy radical production by cells of the patients with sequelae (group I) may be due to an active disease i.e., a consequence of in vivo priming of the cells. Priming has been demonstrated in patients with severe bacterial infections[18] and may result from liberation of inflammatory mediators, such as chemotactic factors. PMNs can also be primed in vitro by treating them with chemotactic factors; such cells show enhanced oxy radical production,[19] increased adherence both to nylon fibres[20] and to plastic surfaces,[21] and depressed migration.[22]

Three patients in group I in the present study had ankylosing spondylitis and six had sacroiliitis. It has been suggested that turnover of complement is increased in ankylosing spondylitis.[23] This can result in liberation of inflammatory agents and subsequent priming of the patients' cells. The inflammatory agents may also activate eosinophil granulocytes.[24] A slight tendency to decreased migration could be seen in group I, but no differences were found in the adherent properties of the cells as determined by the aggregation test. Although the enhanced free radical production may be secondarily acquired, the possibility cannot be excluded that it is genetically

determined and would thereby render the patients susceptible to chronic and recurrent inflammatory manifestations.

In our present study PMNs of both HLA-B27 positive and negative patients showed increased chemotactic and chemokinetic migration, which agrees with the results in the study of patients with ankylosing spondylitis.[7] The finding that migration of the PMNs of HLA-B27 negative patients (group III, Fig. 2) also tended to be increased differs from our previous results, which suggested that migration of such PMNs is not increased.[6] In our previous study, however, cells of HLA-B27 positive and negative patients were not tested concurrently.[6] Furthermore, only one method, the agarose assay, was used. This method may be less sensitive than the filter assay[25]; also, in the present study a more clear cut difference was obtained between patients and controls by the filter assay. It is also noteworthy that HLA-B27 negative patients with ankylosing spondylitis have a chronic disease whereas most of the patients with YA, particularly those who are HLA-B27 negative, recover completely.[12]

Evidence has accumulated to show that disease expression in HLA-B27 associated disorders is likely to be multifactorial[26]; bacterial cell envelope structures resembling[1 4 27] or possibly modifying[2 28] HLA-B27 antigen(s) with subsequent autoimmune response can contribute to the triggering of a host cell injury but are not necessarily themselves sufficient to explain the varying clinical picture of HLA-B27 associated diseases. Evidently, additional factors play a part and these may involve different aspects of inflammation, such as efficiency of complement activation[10] and quality of PMN function.

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