Complement activation and HLA-B27

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SUMMARY The efficiency of complement activation was studied in sera from HLA-B27 positive and negative subjects (27 with previous yersinia arthritis and 35 controls). Activation of complement with zymosan induced higher mean levels of the anaphylatoxin C3a in HLA-B27 positive sera (mean (SD) 7.40 (1.66) mg/l) than in HLA-B27 negative sera (6.41 (1.79) mg/l). Similarly, higher levels of C3d,g, another C3 breakdown fragment, were obtained in HLA-B27 positive sera after *Escherichia coli* 0111:B4 lipopolysaccharide treatment (17.6 (3.7)% v 15.0 (3.8)%). The differences occurred irrespective of previous arthritis, complement C4 or B2 microphenotype, or variation in background complement levels. The findings suggest that an increased responsiveness to complement activators may contribute to the pathogenesis of HLA-B27 associated inflammatory diseases.

Key words: yersinia arthritis, chemotaxis, C4 alleles.

Ankylosing spondylitis^{1 2} and reactive arthritis after enteric or urogenital infections³ are both associated with HLA-B27. Although the mechanism by which the HLA-B27 allele predisposes to disease is unknown, it is possible that HLA-B27 positive subjects are unduly susceptible to mediators of inflammation or they generate them in excess in response to a phlogistic stimulus.⁴ This proposal is supported by the findings that polymorphonuclear leucocytes from HLA-B27 positive patients with reactive yersinia arthritis or ankylosing spondylitis, and from healthy subjects with HLA-B27, show increased chemotaxis in vitro⁵ and in vivo.⁶ It is also in agreement with the clinical observation that inflammatory symptoms are more severe in HLA-B27 positive patients with yersinia arthritis than in HLA-B27 negative patients.^{7 8} The reason for the aberrant polymorphonuclear leucocyte migration is unknown. The finding that after treatment with zymosan polymorphonuclear leucocyte migration in vitro is stimulated more by HLA-B27 positive sera than by HLA-B27 negative sera^{9 10} suggests that complement derived chemotaxins, such as C5a, may

play a part. This prompted us to study whether activation of complement is, in itself, aberrant in sera from patients with previous yersinia arthritis and healthy subjects with HLA-B27.

Subjects and methods

SUBJECTS

Thirty HLA-B27 positive subjects (20 women, 10 men), aged 23–55 (mean 40.5 years) and 32 HLA-B27 negative individuals (28 women, four men), aged 30–59 (mean 44.3 years) were studied. Sixteen (nine women and seven men) of the HLA-B27 positive subjects and 11 (nine women and two men) of the HLA-B27 negative subjects had had clinically¹¹ and serologically⁸ recorded yersinia arthritis six to 15 (mean 10) years earlier. The HLA-B27 positive (11 women and three men) and negative (19 women and two men) controls were healthy laboratory staff and hospital personnel. The patients were followed up at the outpatient department of the University Central Hospital of Helsinki. At the time of venipuncture no patient had arthritis though four complained of mild arthralgia of peripheral joints. Seven patients had occasionally experienced low back pain, and one of 20 patients had radiological evidence for sacroiliitis. The mean erythrocyte sedimentation rate was 11 mm/h (range

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2–28). C reactive protein levels were normal (<8 mg/l) in all patients.

SAMPLES

Fresh serum and ethylenediaminetetra-acetate (EDTA) (0.01 M) plasma samples were obtained from all subjects and stored in aliquots at -70°C for no more than three weeks before anaphylatoxin studies and for about two months before C3d,g assays.

HLA AND COMPLEMENT TYPING

HLA typing was performed using several antisera to 30 HLA A, B and C antigens. C4 and Bf allotypes were determined by immunofixation electrophoresis of EDTA plasma samples.^{12 13} Nomenclature was used only for well defined C4 allotypes.^{14 15}

COMPLEMENT DETERMINATIONS

Serum C3, C4, and factor B levels were assayed by radial immunodiffusion (Behringwerke, Marburg, FRG) and the haemolytic complement titre (CH_{50}) by Mayer's tube haemolysis assay.¹⁶ Complement activation products, C3a and C5a, were assayed as C3 desArg and C5a desArg by a competitive radioimmunoassay developed by Hugli and Chenoweth¹⁷ (Upjohn Diagnostics, Kalamazoo, Michigan, USA). C3d,g levels were determined by radial immunodiffusion after precipitation with polyethylene glycol,¹⁸ and the results are expressed as a percentage of a maximally zymosan activated (5 mg/ml, six hours at 37°C) standard. To activate complement in sample sera equal volumes (200 μl) of serum and zymosan in phosphate buffered saline (PBS, 0.01 mg/ml for C3a and 0.3 mg/ml for C5a), or *E coli* 0111:B4 lipopolysaccharide (Difco Laboratories, Detroit, Michigan, USA) 2 mg/ml, or

a suspension of *Yersinia enterocolitica* 0:3 isolate (approximately 10^{10} bacteria/ml), were mixed and incubated for 30 min at 37°C . The reaction was stopped by addition of 200 μl of ice cold EDTA (0.02 M) in PBS. To remove natural antizymosan antibodies from sample sera a zymosan absorption procedure was carried out by incubating sera at 4°C overnight with 2 mg/ml (final concentration) of zymosan. After centrifugation supernatants were activated as above, and the anaphylatoxin levels were determined.

STATISTICAL ANALYSIS

Statistical comparison of mean values was by Student's *t* test. The results were analysed to determine HLA specificities other than HLA-B27, and corrected *p* values were determined by multiplying each *p* value by 30. The equality of variances was tested with the *F* test, and, when necessary, the Welch's correction was used.

Results

GENERATION OF COMPLEMENT ANAPHYLATOXINS

Activation of complement in sample sera with zymosan led to about a 100-fold increase in levels of C3a desArg. The mean level of C3a desArg was higher ($p<0.05$) in HLA-B27 positive activated sera than in HLA-B27 negative sera (Table 1). The corresponding difference between healthy subjects and patients with previous yersinia arthritis was not significant (6.69 (1.51) v 7.13 (2.05) mg/l respectively, $p>0.05$). In fresh non-activated sera the levels of C3a desArg did not differ between the groups and the levels of C5a desArg were below the detection limit of the assay. In zymosan activated absorbed (with zymosan) sera C5a desArg values

Table 1 Complement anaphylatoxin levels in sera from HLA-B27 positive and negative subjects with previous yersinia arthritis and healthy controls after activation with zymosan*

Parameter	All subjects		Subjects with previous yersinia arthritis		Healthy controls	
	B27+ (n=30)	B27- (n=32)	B27+ (n=16)	B27- (n=11)	B27+ (n=14)	B27- (n=21)
C3a desArg in						
Non-activated sera ($\mu\text{g/l}$)	74.1 (16.9)	72.8 (19.4)	72.2 (17.5)	80.5 (16.1)	71.4 (16.5)	68.8 (21.1)
Zymosan activated sera (mg/l)	7.40 (1.66)†	6.41 (1.79)	7.38 (1.77)	6.78 (2.40)	7.39 (1.64)†	6.22 (1.41)
C5a desArg ($\mu\text{g/l}$) in						
Zymosan activated sera	701 (166)	641 (131)	731 (135)†	616 (125)	671 (185)	653 (134)
Zymosan activated absorbed sera	329 (246)†	202 (113)	339 (259)	250 (164)	321 (235)‡	177 (87)

*Results are given as mean (SD).

† $p<0.05$, comparison with respective B27- groups.

‡ $p<0.01$.

Table 2 Complement C3d,g levels in sera from HLA-B27 positive and negative subjects with previous yersinia arthritis and healthy controls after activation with *E coli* 0111:B4 lipopolysaccharide (LPS) or *Yersinia enterocolitica* 0:3

Parameter	All subjects		Subjects with previous yersinia arthritis		Healthy controls	
	B27 ⁺ (n=30)	B27 ⁻ (n=32)	B27 ⁺ (n=16)	B27 ⁻ (n=11)	B27 ⁺ (n=14)	B27 ⁻ (n=21)
C3d,g (%) in sera activated with						
<i>E coli</i> 0111:B4 LPS	17.6 (3.7)‡	15.0 (3.8)	18.4 (3.7)‡	13.6 (2.4)	17.0 (3.7)	15.8 (4.5)
<i>Y enterocolitica</i> 0:3	18.8 (3.1)†	16.8 (2.9)	20.3 (3.5)	18.0 (2.4)	17.5 (2.8)	16.1 (3.1)

*Mean (SD). Values are expressed as a percentage of standard (maximally activated normal serum).

†p<0.05.

‡p<0.01.

were higher in HLA-B27 positive sera (Table 1). In non-absorbed sera a difference in C5a desArg levels was also seen, but it was not statistically significant.

GENERATION OF C3d,g

In light of the potential significance of the above results the experiments were repeated with C3d,g as an index of complement activation and *E coli* lipopolysaccharide and cells of *Y enterocolitica* as complement activators. Thus when patients and controls were considered together significant differences in C3d,g levels were observed between HLA-B27 positive and negative groups after activation with either of the two activators (Table 2). After activation with *E coli* lipopolysaccharide no significant difference was seen between patients with previous yersinia arthritis and healthy subjects, whereas after activation with *Y enterocolitica* higher C3d,g levels were observed in the former group (19.3 (3.0)% v 16.7 (3.0)% respectively, p<0.05).

COMPLEMENT LEVELS

A comparison of HLA-B27 positive and negative fresh sera before activation showed no significant difference in the levels of C3 (863 (120) v 865 (131) mg/l, mean (SD)), C4 (364 (92) v 357 (105) mg/l), factor B (172 (45) v 170 (46) mg/l) or CH₅₀ activity (37.3 (9.5) v 36.8 (7.1) IU/ml). Moreover, the distribution of C4 and factor B alleles did not differ between these groups (data not shown).

HLA-B8

Analysis of the results in respect of class I major histocompatibility antigens other than HLA-B27 showed that HLA-B8 positive subjects (n=12) had a lower mean level of C3a desArg in activated sera than HLA-B8 negative subjects (n=35) (5.59 (1.11) v 7.25 (2.10) mg/l, corrected p<0.05); nine of the

HLA-B8 positive subjects did not have HLA-B27, and their C3a desArg level was 5.26 (1.20) mg/l (corrected p<0.05).

C4 PHENOTYPES

Analysis of the C4 phenotypes with respect to disease indicated that the prevalence of the C4AQO ('null') allele was 29% (6/21) in patients with previous yersinia arthritis and 40% (4/10) in those patients who were HLA-B27 negative compared with 19% (5/27) in healthy controls. In a group of 254 healthy Finnish blood donors prevalence of the C4A null allele was 22%.

Discussion

The results of the present study show that activation of complement with zymosan, *E coli* lipopolysaccharide, or *Y enterocolitica* tends to lead to higher levels of complement derived activation products in HLA-B27 positive sera than in HLA-B27 negative sera. No major complement abnormality was associated with HLA-B27 despite the genetic linkage of the HLA-B locus to the major histocompatibility complex class III complement genes ('complement activation locus'). A slightly increased prevalence of the C4A null allele (40% v 20% in general population) was observed in HLA-B27 negative subjects with a history of reactive arthritis.

The reasons for the differences in the complement activation responses between HLA-B27 positive and negative sera are unknown. Increased levels of complement components, and consequently of the activation fragments, may occur during the 'acute phase response'. This is, however, an unlikely explanation because the levels of C3, C4, factor B and C reactive protein were not increased in HLA-B27 positive sera. During an actual inflamma-

tory process the situation could be different as changes in complement activity possibly reflect the degree of inflammation.

Specific IgG class antibodies have been shown to augment formation of the alternative pathway C3 convertase and thereby to enhance complement activation.¹⁹ Higher levels of such antibodies in HLA-B27 positive sera could lead to increased complement activation. The fact that after absorption of these antibodies significantly higher C5a desArg levels could still be generated in HLA-B27 positive sera than in HLA-B27 negative sera, makes this explanation unlikely. In the case of complement activation by cells of *Y enterocolitica* (which may be inhibited by the expression of a virulence plasmid²⁰) the higher C3d,g response in the patient group is possibly due to enhancement by antibody mediated complement activation as the patient sera apparently contained anti-yersinia antibodies more frequently than did the control sera. Still, this does not explain the observation that C3d,g levels were higher also in HLA-B27 positive subjects.

The presence of different amounts of complement activating factors, such as endotoxin, immune complexes, increased proteolytic or C3 convertase, stabilising activity in sample sera may provide an explanation. In fresh EDTA plasmas, however, there was no significant difference in the background C3a desArg levels, suggesting that complement activation may not have occurred in vivo. Finally, it may be that an aberration in the regulation of complement activation exists in HLA-B27 positive sera. This could lead to an exaggerated function of the alternative or classical pathway C3 convertases in the presence of a suitable target.

An increased tendency to generate the oligopeptides C3a and C5a may play an important part in HLA-B27 associated diseases and provides a possible explanation for the previously observed increased neutrophil migration^{6,9} and inflammatory symptoms⁸ in HLA-B27 positive patients with yersinia arthritis. C3a increases vascular permeability by releasing histamine from mast cells.²¹ Binding of C5a by specific cell surface receptors²² is responsible for several events in neutrophils such as adherence,²³ chemotactic and chemokinetic migration,²⁴ activation of the respiratory burst,²⁵ release of lysosomal enzymes,²⁶ and production of leucotrienes with chemotactic or microvascular permeability (or both) increasing activities.²⁷

The prevalence of HLA-B8 is decreased in patients with ankylosing spondylitis.²⁸ We found low levels of C3a desArg in HLA-B8 positive, zymosan treated sera. It is tempting to propose that a low anaphylatoxin response protects against the development of ankylosing spondylitis, whereas

enhanced efficiency of complement activation, e.g., in response to endotoxin released from the gut or persisting in tissues, contributes to the development of ankylosing spondylitis or reactive arthritis.

Increased efficiency of complement activation may thus be one of the factors affecting disease expression and severity of inflammation in patients with HLA-B27 associated diseases.

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