Does reduced erythrocyte C3b receptor (CR1) activity contribute to the pathogenesis of yersinia triggered reactive arthritis?

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Abstract

Erythrocyte C3b receptor (CR1) activity was measured in 27 patients with yersinia triggered reactive arthritis and in 151 control subjects, including 36 patients with uncomplicated yersiniosis and 115 healthy subjects. CR1 was measured by the immune adherence haemagglutination method. Patients with yersinia triggered reactive arthritis had reduced levels of CR1 compared with the controls. This difference was mainly due to the finding that five out of six HLA B27 negative patients with arthritis had decreased CR1 activity. Such a quantitative difference may contribute to the pathogenesis of reactive arthritis by affecting the clearance of immune complexes.

Erythrocytes play an important role in the removal of immune complexes from the circulation via their immune adherence receptors (C3b receptors, CR1). Approximately 90% of the human CR1 in peripheral circulation are found on erythrocytes.¹ The binding of immune complexes to erythrocyte CR1 and their subsequent transport to the liver is thought to prevent the potentially harmful consequences of immune complexes. In normal subjects, a wide variation in the number of CR1 is due to CR1 genomic polymorphism^{2,3}; the number of CR1 sites per erythrocyte is a stable characteristic.⁴

Reduced levels of CR1 have been reported in several rheumatic diseases, including systemic lupus erythematosus (SLE),^{5,6} primary Sjögren's syndrome,⁷ and rheumatoid arthritis.^{6,8} Whether the reduction in erythrocyte CR1 is inherited or acquired is a matter of controversy. The latter argument is supported by the findings of Moldenhauer *et al.*,⁹ who used restriction fragment length polymorphism analysis to compare erythrocyte CR1 in patients with SLE with that in normal control subjects. The study revealed the same gene frequency of the alleles encoding the 6.9 and 7.4 kb bands, which correlate with low and high numbers of erythrocyte CR1, respectively. Similar results were reported by Cohen *et al.*¹⁰ In contrast, Wilson *et al.*¹¹ found an increase in the frequency of the 6.9 kb band coding for reduced erythrocyte CR1, suggesting an inheritance mechanism.

Reactive arthritis triggered by yersinia is characterised by a strong and persisting IgA response.^{11,12} Increased levels of circulating immune complexes that contain yersinia antigens are found in patients with yersinia triggered reactive arthritis, compared with controls with uncomplicated yersiniosis.¹³ Yersinia specific

immune complexes are also present in the synovial fluid of some patients.¹⁴ In some patients yersinia specific immune complexes are found in the circulation for several months after the onset of the disease.¹³ As erythrocyte CR1 is known to be an important mechanism for the clearance of immune complexes, we compared their occurrence in patients with yersinia triggered reactive arthritis to that in control subjects, including those with uncomplicated yersinia infection and healthy subjects.

Patients and methods

PATIENTS AND CONTROLS

Blood samples from 63 patients with a history of yersinia infection were studied. The diagnosis was based on clinical features, significantly increased antibody levels, and, in 32 subjects, on the positive isolation of yersinia from stools.¹⁵ Twenty seven patients (mean (SD) age 35 (11) years) developed reactive arthritis as a post-infection complication, and 36 patients (age 36 (12) years) recovered from yersiniosis with no subsequent sequelae. The HLA B27 marker was found in 21 patients with reactive arthritis and in six without this complication. With few exceptions, the samples were taken when the patients had clinically recovered from infection and joint symptoms. The mean time (SD) from the onset of the infection was 23 (17) months for the patients with reactive arthritis and 17 (17) months for the patients with uncomplicated yersiniosis. Samples from 115 healthy subjects, including blood donors, medical students, and laboratory staff, were studied as normal controls. Among these were 11 staff from our laboratory with the HLA B27 tissue antigen.

IMMUNE ADHERENCE HAEMAGGLUTINATION

Blood samples were placed in a tube containing the same volume of sterile Alsever's solution. The erythrocyte CR1 activity was measured by the immune adherence haemagglutination method.⁵ Briefly, serial two fold dilutions of heat aggregated human IgG were used to activate the complement. The C3b binding sites thus generated were protected from decay by adding dithiothreitol. At the final step erythrocytes isolated from human samples were incubated with the mixture of activated complement and IgG, and the haemagglutination pattern was observed. The C3b receptor activity was expressed as the titre, i.e. the highest dilution of aggregated human IgG (2⁻ⁿ), that induced haemagglutination. Additionally, the degree of haemagglutination was estimated and ranked

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from 0 to 4+; with 0 indicating a lack of, and 4 the highest degree of CR1 activity.

STATISTICS

The χ^2 test (table), Wilcoxon rank sum test (fig 1) and linear discriminant analysis (fig 2) were used to compare patients with and without reactive arthritis.

Results

CR1 ACTIVITY IN PATIENTS WITH REACTIVE ARTHRITIS

The CR1 activity was decreased in patients with

Erythrocyte CR1 activity in HLA B27 positive and negative patients with yersinia triggered reactive arthritis (YeA+), in controls with uncomplicated yersiniosis (YeA-), and in healthy controls. Values are the number of patients; numbers in parentheses are percentages

Patients and controls	CR1 activity*				Total
	-	+	++	+++	
YeA+	9 (33)	7 (26)	7 (26)	4 (15)	27
B27+	5	6	6	4	21
B27-	4	1	1	0	6
YeA-	6 (19)	5 (16)	8 (22)	17 (47)	36
B27+	2	1	1	2	6
B27-	4	4	6	13	27
ND†			1	2	3
Healthy controls	11 (10)	21 (18)	28 (24)	55 (47)	115
B27+	1	0	9	1	11
B27 ND	10	21	19	54	104

*Degree of haemagglutination.
†ND=not determined.

Figure 1 Erythrocyte C3b receptor (CR1) activity in patients with yersinia triggered reactive arthritis (A+), in patients with uncomplicated yersiniosis (A-), and in healthy subjects (C). The results are expressed as titres (2⁻ⁿ).

Figure 2 Erythrocyte C3b receptor (CR1) activity in patients with yersinia triggered reactive arthritis (○) and with uncomplicated yersiniosis (●) as expressed both in titre (2⁻ⁿ) and degree of haemagglutination.

yersinia triggered reactive arthritis, compared with controls with uncomplicated yersiniosis or with normal subjects. This difference between patient groups was observed when the results were expressed as titres ($p<0.005$, fig 1). A low degree of haemagglutination (-or+) was also more often found in patients with reactive arthritis than in those without arthritis ($p<0.02$; table). The difference between patient groups is still valid when the two parameters are taken into account (fig 2; $p<0.05$). The lack of CR1 activity was not significantly more common among patients with yersinia triggered reactive arthritis than in other groups.

There was no difference in the distribution of CR1 activity in normal controls compared with subjects with uncomplicated yersiniosis ($p=0.2992$; fig 1, table).

CR1 ACTIVITY ACCORDING TO HLA-B27 STATUS

An analysis of CR1 activity according to the HLA B27 status showed that four out of six patients with arthritis negative for HLA B27 lacked CR1 activity and one had only faint (+) activity (table). Although the numbers are low, this is a significantly different distribution than that observed in 104 healthy blood donors (with HLA undetermined, normal frequency of HLA B27 14% in the Finnish population) or among HLA B27 negative yersiniosis patients without arthritis (table). Among 11 healthy HLA B27 positive subjects the CR1 activity was in the same range as in the healthy blood donors with undetermined HLA (table).

STABILITY OF ERYTHROCYTE CR1 ACTIVITY

Serial samples (taken at two to eight different times) were taken from 11 patients within the 17 (3) months. The CR1 activity in these patients remained stable with time. This was true also for the five patients who lacked or had a low CR1 activity in the early stages of the disease (data not shown).

Discussion

Patients with yersinia triggered reactive arthritis were found to have reduced erythrocyte CR1

activity compared with controls and the difference between the patient groups was quantitative. An interesting finding was that five out of six patients with HLA B27 negative reactive arthritis had either a lack of (four patients) or a low (one patient) erythrocyte CR1 activity. In only one of these patients was a higher degree of CR1 activity (2+) found. If the CR1 activity had been solely an acquired feature, a similar distribution of CR1 activity would have been expected in both HLA B27 positive and negative patients with reactive arthritis. As reactive arthritis rarely develops in HLA B27 negative patients, this group of patients was small in this study. However, a low CR1 activity was found in these patients, who do not have the well known predisposing factor of the HLA B27 antigen. Therefore, it is reasonable to speculate that the decreased erythrocyte CR1 activity may contribute to the pathogenesis of arthritis, particularly in this group of patients.

In a previous study, HLA B27 positive patients with juvenile rheumatoid arthritis were seen to have a decreased CR1 activity compared with those with the same disease and lacking HLA B27.¹⁶ Likewise, in another study, based on 150 healthy blood donors, Thomsen *et al*¹⁷ have suggested that an association between HLA B27 and decreased CR1 activity exists, especially in subjects positive for Cw2. The present findings reveal no specific role of HLA B27 in determining erythrocyte CR1 activity, as reduced activity was also found in HLA B27 negative patients with reactive arthritis. Furthermore, in our limited population of HLA B27 positive subjects, there was a lack of correlation of HLA B27 to low CR1 activity.

The present results suggest several possibilities about decreased CR1 activity, which can be discussed in the context of acquisition or inheritance. Most of the test samples were taken at a stage when clinical signs of the disease were no longer seen. Our earlier studies showed that patients with yersinia triggered reactive arthritis have a higher level of immune complexes containing yersinia antigens than those without postinfection complications.¹³ This quantitative difference was seen at the early stages of the disease after onset of the infection, although yersinia specific immune complexes could still be seen in certain subjects over eight months after the onset of infection. There was also a significant decrease of circulating immune complexes in patients with reactive arthritis early during the follow up, as measured by methods not specific to a particular antigen.¹⁸ On this basis, it would be unlikely that at the time when the samples were taken, the decreased CR1 activity would have been due only to the circulating immune complexes either occupying CR1 or consuming them through the transport system and interaction with hepatic Kupffer cells.¹⁹

In this study, we observed that CR1 activity did not change with time. In the few samples taken early after the onset of infection, low and high values were found. The follow up showed that the degree of CR1 activity was a stable characteristic for each subject; even in the five patients with low CR1 activity at the onset of

the disease, the erythrocyte CR1 activity remained low throughout the follow up period. These findings suggest that decreased CR1 activity may have, at least partly, a genetic basis in patients with yersinia triggered reactive arthritis.

Patients with yersinia triggered reactive arthritis are characterised by a strong and persisting IgA anti-yersinia response, suggesting the continued presence of the bacterial antigens.^{11 12} The prolonged occurrence of yersinia specific immune complexes¹³ and the presence of yersinia antigens in joints, both in the form of immune complexes in the synovial fluid¹⁴ and as intracellularly phagocytosed antigen in synovial fluid,²⁰ support bacterial persistence. Reduced erythrocyte CR1 activity in patients with yersinia triggered reactive arthritis may contribute to the increased occurrence of yersinia specific immune complexes and antigen persistence in such patients. Therefore, reduced CR1 activity may be one of the factors important in the pathogenesis of reactive arthritis.

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