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Influence of C4 null genes on infection with human immunodeficiency virus

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Abstract

The hypothesis that complement is important in the host response to human immunodeficiency virus (HIV) was tested. Complement C4 and Bf allotypes were determined in 26 patients who fulfilled the diagnostic criteria for persistent generalised lymphadenopathy due to HIV, 72 homosexuals who were negative for antibody to HIV, and 185 control subjects drawn from the local population. HLA-A, B, and DR were also typed and the phenotypes examined for the presence of supratypes and C4BQ0. Eleven patients (42%) had C4B null alleles compared with only 13 (18%) homosexuals who were negative for antibody and 28 (15%) controls. From estimates of gene frequencies the difference between the patients with lymphadenopathy and the controls was significant after conservative correction. In the patients only a minority (six) of the C4B null alleles were contained within ancestral haplotypes. Together with the fact that C4 null alleles result in partial deficiency of C4, this finding suggests that products of complement genes are important in infection with HIV or its consequences, or both. A role is proposed for complement and Fc receptors.

Introduction

The serological response to human immunodeficiency virus (HIV) varies substantially among subjects. Several studies have shown that lower titres of antibody to HIV are associated with a higher concentration of virus and a more severe outcome,[1 2] but whether the titre falls as a consequence of failing immune function or whether some patients have an inherently defective response and therefore a more rapid course is unclear. Our work suggests that infection of the follicular dendritic cell and the consequent destruction of the germinal centre is an important factor in the failure of specific immune responses, including synthesis of antibody to HIV.[2] We therefore postulated that infection of the follicular dendritic cell may depend on antibody, complement, and receptors for the Fc moiety of antibody and complement and proposed that genes controlling the components of complement may be important in determining susceptibility to infection with HIV. Accordingly, we undertook allotyping for the class III genes (C4 and Bf) within the major histocompatibility complex as well as those in class I (HLA-A, B, and C) and class II (HLA-DR and DQ).

Patients and methods

We compared two groups of homosexual subjects attending the immunology clinic at this hospital. All were white residents of Western Australia. They were tested for antibodies to HIV by commercial enzyme immunoassays (Abbott or Genetic Systems) and were typed for HLA-A, B, C, DR, and DQ with a standard microcytotoxicity assay. The fourth component of complement (C4) and the factor B (Bf) component of the alternate pathway were typed by methods described previously.[3] In the method for C4 the bands are assessed by densitometry and null alleles can be assigned with confidence. Null alleles at the C4B locus were assigned if fewer than two alleles had been identified and the densitometric ratio of C4A to C4B was $\geq 1{\cdot}5$.

One of the groups studied comprised 26 patients positive for antibody to HIV who fulfilled the standard criteria for persistent generalised lymphadenopathy; the other comprised 72 subjects who did not have symptoms, who were negative for antibody, but who considered themselves at risk of infection with HIV. The frequencies of the alleles in the two groups were compared with those in a control population of 185 white subjects drawn at random from the town of Busselton. The frequencies of alleles and phenotypes were analysed by the χ^2 test or Fisher's exact test. Probability values were multiplied by the number of complement alleles detected in these populations (20).

Serum C4 concentrations were determined by automated rate nephelometry (Beckman ICS) and the results compared by Student's *t* test.

Results

The patients who were negative for HIV antibody and the controls did not differ significantly in the frequency of any alleles, including C4. The figure shows, however, that 11 patients (42%) who were positive for antibody to HIV and had persistent generalised lymphadenopathy had C4B null alleles (C4BQ0), compared with 13 (18%) of the homosexuals who did not have antibodies (data not shown) and 28 (15%) of the controls. In the patients the increased frequency of C4BQ0 corresponded to a decrease in other C4B alleles ($p<0{\cdot}005$); no such changes were found in alleles at the C4A locus. As five of the 26 patients who had antibodies to HIV were homozygous for C4B null alleles we estimated gene frequencies by direct counting; they were

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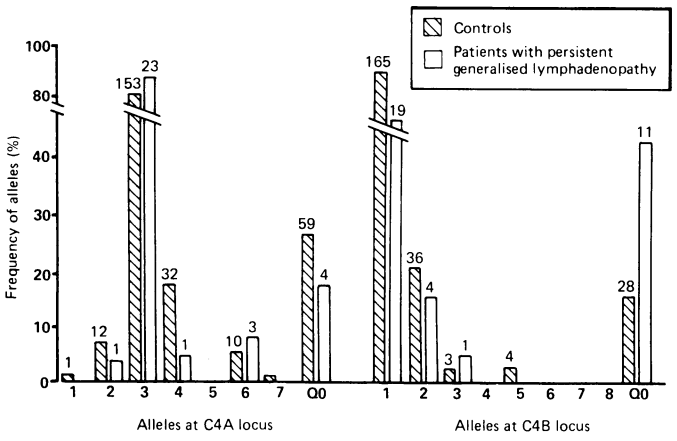

Frequency of alleles at C4A and C4B loci in patients positive for antibody to HIV with persistent generalised lymphadenopathy (n=26) and controls (n=185). Number of patients above each bar.

the major histocompatibility complex. We cannot exclude a contribution from genes that are tightly linked to the C4B locus. Interestingly, both HLA-B35 and B44 have been reported to be associated with progressive disease[6] and with Kaposi's sarcoma.[7] These alleles occur on ancestral haplotypes bearing C4BQ0, and an increase in their frequency may be due to linkage disequilibrium with C4BQ0. The observation that most of the C4BQ0 is orphan to ancestral haplotypes and the finding of reduced serum C4 concentrations argue that partial deficiency of C4 is important functionally. As C4B is the more haemolytically active component of C4 and is most active at the cell surface its absence may be particularly important with respect to infection with HIV. Impaired activation of complement may reduce clearance of the virus and contribute to an increased viral load. Further antibody responses might be impaired at an early stage as activation of complement by immune complexes is an absolute requirement both for follicular trapping and for priming memory B cells.[8] In this context we envisage the route of infection being shunted towards entry of the virus through Fc or complement receptors on follicular dendritic cells.

We are currently examining the C4 genes in other subgroups of patients with infection with HIV. Meanwhile our observations provide an explanation for previous but inconsistent reports of associations of human leucocyte antigens with infection with HIV[9]; more important, they suggest that complement may play a part in host defence against HIV.

Distribution of ancestral haplotypes in the two groups of patients

Case No	A	B	Bf	C4A	C4B	DR	A	B	Bf	C4A	C4B	DR
					Patients with persistent generalised lymphadenopathy							
1	2	44	S	3	Q0	5	3	44	F	3	1	7
2	2*	44*	S*	3*	Q0*	4*	2	17	S	3	Q0	1
3	26*	44*	S*	3*	Q0*	4*	1	8	S	Q0	1	3
4	11	35	S	3	Q0	5	30	41	S	3	Q0	6
5	3*	35*	F*	3/2*	Q0*	1*	2*	18*	F1*	3*	Q0*	3*
6	3*	35*	F*	3/2*	Q0*	1*	11*	35*	S*	3*	Q0*	1*
7	3	35	S	3	Q0	3	28	35	F	1	Q0	6
8	24	62	F	3	Q0	4	1	62	F	3	1	6
9	24	62	F	3	Q0	7	1	17	S	6	1	7
10	26	60	F	3	Q0	7	1	8	F	3	1	6
11	11	51	S	2	Q0	5	2	51	S	2	1	2
					Patients negative for antibody to HIV							
12	24*	44*	S*	3*	Q0*	4*	28	17	S	6	1	7
13	3*	44*	S*	3*	Q0*	4*	1	17	S	6	1	6
14	9*	44*	S*	3*	Q0*	4*	9	7	S	3	1	2
15	2	44	S	3	Q0	5	32	44	S	3	1	6
16	2	44	S	3	Q0	1	2	51	F	3	1	6
17	28	40	S	3	Q0	4	3	40	F1	3	Q0	7
18	2	44	F	3	Q0	6	2	27	F1	3	Q0	3
19	10	40	S	2	Q0	2	2	7	S	6	1	2
20	28	27	S	4	Q0	3	11	53	S	4	2	1
21	2	27	F	4	Q0	6	3	7	F	4	2	4

*Ancestral haplotypes with CB4Q0 (present in only six of the haplotypes with C4BQ0).

0·31, 0·10, and 0·11, respectively. Because a total of 20 alleles had been analysed at the C4 and Bf loci the p values were multiplied by 20; the differences between the patients positive for antibody to HIV and the controls remained significant ($\chi^2=18·6$, $p_c<0·01$).

We have previously identified commonly occurring combinations of alleles designated extended haplotypes, supratypes,[4] or ancestral haplotypes. From the data for each subject we examined the HLA phenotype to determine the proportion of the C4BQ0 alleles that were contained within these ancestral haplotypes. Several haplotypes bearing C4BQ0 have been identified in four patients with lymphadenopathy (table). Examples of the ancestral haplotypes B18, BfF1, C4A3, C4BQ0, DR3 (one case), B35, BfF, C4A3+2, C4BQ0, DR1 (two), B35, BfS, C4A3, C4BQ0, DR1 (one), and B44, BfS, C4A3, C4BQ0, DR4 (two) were identified, but 10 of the 16 haplotypes were orphan in that the C4BQ0 occurred outside an ancestral haplotype. These findings show that lymphadenopathy related to HIV is associated with C4BQ0 rather than a specific C4BQ0 bearing ancestral haplotype.

Serum C4 concentrations were determined in all subjects. As expected,[5] they were lower in subjects homozygous for C4BQ0 (n=8, mean 0·17 (SD 0·05)) than in those without C4BQ0 (n=33, mean 0·22 (0·07), p=0·05).

Discussion

Our data show that null alleles at the C4B locus are significantly increased in patients with persistent generalised lymphadenopathy compared with subjects negative for antibody to HIV. Our analysis suggests that this allele per se is more important than other genes of

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