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Prolonged clinically asymptomatic evolution after HIV-1 infection is marked by the absence of complement C4 null alleles at the MHC

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SUMMARY

The length of time after which persons infected with HIV-1 progress to AIDS is variable. Certain alleles at the MHC have been shown to influence negatively the clinical outcome of HIV-1-infected persons and to be associated with special clinical manifestations. We investigated the MHC class I, class II and class III antigens in 54 Caucasian HIV-1-infected persons. The MHC profile of individuals with a prolonged period before AIDS is marked by a lower frequency of C4 null alleles.

Keywords HIV-1 asymptomatic period C4 null alleles

INTRODUCTION

Acute HIV-1 infection is followed by a clinically asymptomatic period, which, sometimes after a phase of generalized persistent lymphadenopathies, leads to AIDS. The clinical progression down the HIV-1 pathway is best correlated to the progressive decline in CD4 helper lymphocytes, the main target cells of HIV-1 infection. In general, signs of major immune deficiency start when the count of peripheral blood CD4 lymphocytes is at or below 200 cells/μl [1,2]. The duration of survival of patients in the AIDS phase has greatly improved upon prompt treatment of opportunistic infections but also by prophylactic antibiotic regimens and by anti-HIV-1 treatment. The duration of the asymptomatic or pauci-symptomatic period before AIDS is highly variable and ranges from a few years to more than 10. Besides external factors, such as co-infections or toxic hazards (drugs), the immunogenetic background of the infected persons very likely plays a major role in the disease progression and the nature of the clinical manifestations.

Several markers of the MHC were found to be associated with special clinical manifestations in AIDS. The DR5 allele was increased in patients with Kaposi's sarcoma [3], whereas in patients with opportunistic infections an increase of B8, DR3 [4,5] and B35 was found [6,7]. Certain HLA alleles were shown to be associated with disease progression. The DR1 antigen was associated with an increased risk of rapid progression from an asymptomatic state to AIDS [8]. Especially the Caucasian haplotype A1 B8 DR3 was consistently found to be associated with rapid progression to AIDS [9] and rapid decline in CD4 helper cells [10]. Complement C4 null alleles [11] and several ancestral haplotypes that carry them have also been shown to be associated with rapid progression to AIDS [12,13]. However, particular clinical manifestations have been found in association with certain HLA alleles, namely a Sjögren-like syndrome with CD8 lymphocytosis was strongly correlated to DR5 [14]. In this single-centre study we compared the MHC profile of HIV-1 long-term-infected asymptomatic persons with that of persons infected only for a short period of time, and of patients with AIDS. C4 null alleles were significantly decreased in long term (over 5 years) asymptomatic persons as compared with those with recent HIV infection.

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SUBJECTS AND METHODS

Patients and control subjects

During the period from January to March 1990, 54 Caucasian HIV-1-infected individuals were typed for different HLA markers. All the persons were regularly followed at the Referral Centre of the Department of Infectious Diseases for assessment or treatment of HIV infection or its complications. Thirty-five of the patients were homosexual men, 16 were male or female drug users or heterosexual partners of HIV-infected persons; and three were transfusion recipients. Their mean age was 38 years (range 22–64). Clinical staging was done according to the Centers for Disease Control (CDC) classification system for HIV infection: group II, asymptomatic infection; group III, persistent generalized lymphadenopathy; group IV.C, HIV-infected with opportunistic infections. To check for persons resistant to early deterioration and progression to AIDS, group II and group III patients were pooled, and divided into those with infection proven to be older than 5 years (tests first available) and those with infection documented for less than 5 years.

Screening for HIV antibodies was carried out by ELISA technique (Abbot). Positive reactions were confirmed by West-

Table 1. Number and frequency of selected HLA-A, -C, -B antigens in different HIV-1-infected groups and local controls

Antigen	Group II-III over 5 years ($n=9$) Antigens n	%	Group II-III less than 5 years ($n=24$) Antigens n	%	Group IV. C1 and C2 ($n=19$) Antigens n	%	Local controls ($n=126$) Antigens (%)
A1	1	11	3	12	5	26	21
A2	7	77	9	37	9	47	49
A3	2	22	7	29	7	36	33
A24	2	22	6	25	4	21	20
CW7	3	33	11	45	11	58	38
B51	2	22	3	12	3	16	13
B7	2	22	5	21	5	26	28
B8	0	0	5	21	2	10	13
B35	0	0	4	16	5	26	21
B44	2	22	7	29	4	21	23
BW62	4*	44	1*	4	1	5	12

*$P=0{\cdot}05$ Fisher's (not corrected for number of antigens tested).

ern blot. T cell subpopulations were quantified by flow cytometry using MoAbs to CD3, CD4 and CD8. The HLA control population consisted of 126 typed blood donors.

HLA typing
HLA-A,B,C typing was done on Ficoll-separated peripheral blood lymphocytes, by a modified two-colour NIH microcytotoxicity assay and read by an automated photometer [15]. HLA-DR and -DQ typing was done on peripheral blood B lymphocytes separated by immuno-bead technique; after double-staining technique, reading was done by photometer and checked by eye. A large battery of highly selected sera was used recognizing 63 HLA antigens: 14 A, 27 B, seven C, 12 DR and three DQ.

Class II oligonucleotide typing
Two sets of reagents were used to perform class II oligonucleotide typing. The Eurotransplant oligonucleotide typing set [16] was used to define 12 DR alleles. For confirmation and further dissection of DR subspecificities, but also definition of DQA and DQB alleles, the reagents of the XI International Histocompatibility Workshop [17] were employed. In three situations where the oligotyping of the DR genes and the serological typing of the DR antigens differed, the typing was based on the oligo results of the Workshop reagents.

The oligotyping results of the DRB1 gene have not been given separately but were expressed as the corresponding broad DR antigen specificity. In cases of DR typing where serology and oligotyping detected only one specificity, homozygosity was assumed and the specificity was counted twice in the calculation of antigen frequencies.

Complement allotyping
Complement C4 typing and properdin factor B typing were done by agarose electrophoresis followed by immunofixation with specific antisera (Atlantic AB) on EDTA plasma samples stored at $-70°C$. For C4 allotyping plasma samples were digested by carboxypeptidase B, before treatment by neuraminidase [18]. Assignment of C4 null alleles was done according to the methods of Zhang *et al.* [19] and Kramer *et al.* [20]. Briefly, null alleles were assigned when there was a complete absence of C4A or C4B (homozygous AQ0 or BQ0). Null alleles were also assigned when there were less than two alleles at C4A or C4B, and the C4A/C4B densitometric ratio was $>1{\cdot}4$ or $<0{\cdot}6$, implying a heterozygous C4BQ0 or C4AQ0 condition.

Statistical analysis
Antigen frequencies were compared using 2×2 contingency tables and Fisher's exact test. When necessary, the P values were adjusted for the number of antigens tested.

RESULTS

The antigen frequencies of the control population, 126 normal blood donors for ABC, and 104 normal blood donors for C4A, C4B, DR, corresponded to the antigen frequencies for caucasians as published by Baur *et al.* [21], except for an overall 5% decrease of A1 CW7 B8 DR3 (selection bias of the control population) and an increase of about 5% for A3, B7, DR5 and DR6.

The antigen counts and antigen frequencies of three groups of HIV-1-infected persons, namely disease-free for ≥ 5 years after infection; disease-free for <5 years after infection; and patients with AIDS (group IV.C), are given in Table 1 for selected HLA-A,C,B, alleles, and in Table 2 for selected C4 and HLA-DR alleles (broad specificities). The allele frequencies for the control population are also given.

In the group free of disease for more than 5 years, the antigens B8 and B35, known to be associated with rapid deterioration, are absent; however, with the sample studied this is not statistically significant, when compared with the other

Table 2. Number and frequency of complement C4 null alleles and selected DR antigens in different HIV-1-infected groups and local controls

C4 alleles	Group II-III over 5 years ($n=9$) Antigens		Group II-III less than 5 years ($n=22$) Antigens		Group IV. C1 and C2 ($n=19$) Antigens		Local controls ($n=104$)
Antigen	*n*	%	*n*	%	*n*	%	Antigens (%)
C4AQ0	0	0	5	22	3	16	19
C4BQ0	1	11	10	45	4	21	33
C4 null alleles	1*	11	15*	68	7	37	52
DR alleles	($n=9$)		($n=24$)		($n=19$)		($n=104$)
DR1	1	11	7	29	2	11	18
DR2	1	11	7	29	4	21	30
DR3	1	11	7	29	3	16	21
DR4	4	44	6	25	4	22	26
DR5	6	66	5	21	9	47	36
DR6	2	22	7	29	8	42	27
DR7	0	0	6	25	5	26	29

* $P=0{\cdot}005$ Fisher's exact test. Relative risk 17.

Table 3. Helper lymphocyte counts of the nine patients with disease-free follow up over 5 years (estimated time of infection)

Patient no.	CD4 count* at time of study	First CD4 count time/months	Decrease CD4/year	Estimated duration of infection (years)
1	563	619/36	18	13
2	490	560/48	18	> 13
3	663	937/51	64	⩾5†
4	403	525/24	61	6‡
5	463	673/53	47	7
6	637	957/68	56	⩾5†
7	897	1206/35	105§	⩾5†
8	251	378/36	43	12
9	281	590/57	65	8

* Cell count, number given per μl. The CD4 count of controls is 880+320.

† The time of the first positive serological test is considered; a retrospective estimation of the time of infection is not possible because the initial CD4 cell count is higher than the reference value (800) used for estimation.

‡ Transfusion.

§ The patient has 6439 CD8 cells.

groups. The antigen BW62 is increased in the group disease free for over 5 years, when compared with the group disease free for less than 5 years ($P=0{\cdot}05$ before correction for the number of antigens tested).

The frequency of C4 null alleles is significantly decreased in the group disease free for more than 5 years when compared with those disease free for less than 5 years ($P=0{\cdot}005$) confirming the *a priori* assumption that C4 null alleles have a negative effect on clinical outcome after HIV infection.

DISCUSSION

This single-centre study shows a significantly lower frequency of C4 null alleles in persons free of disease in the long term after HIV infection. The cut-off point of 5 years was chosen because at this time serological testing for HIV-1 infection became available. As these persons were regularly monitored for their CD4 helper cell count, it was possible to calculate the mean decrease of CD4 cells per year. It has recently been shown [22] that the CD4 helper cell counts in HIV-infected persons decline at different rates for different persons, but that the slope of decrease for each individual remains constant over time. Assuming an initial helper cell count of 800, we estimated (Table 3) the time of infection of each individual in the long-term disease-free group. The estimated time of infection ranged from 5 to 13 years.

The other HLA antigens found to be decreased in the long-term disease-free persons, as compared with the persons with a disease free follow up for less then 5 years, namely the HLA-B antigens B8, B35 and, less so, B44, the HLA-DR antigens DR1, DR3 and, less so, DR2 and DR7, are carried by the ancestral haplotypes A1,CW7,B8,C4AQ0,DR3; -X,CW4,B35,C4BQ0,DR1; -X, B44,C4BQ0, X. The common marker to all these haplotypes is the presence of a C4 null allele. In the group with disease-free follow up for less than 5 years (Table 4) these three ancestral haplotypes can be found four times each (55% of the persons); in the groups with disease-free survival for more than 5 years (Table 5) only one (B44,C4BQ0) haplotype can be found (10% of the persons), and it is carried by the person with the lowest CD4 count compared with the presumed evolution time.

Clinicians often consider the blood C4 level as one homogeneous entity; in fact, this is the total of four different proteins coded for by four different genes: two C4A genes and two C4B genes. Law *et al.* [24] showed that the C4B molecules are haemolytically more effective (covalent ester bond with hyd-

Table 4. Phenotype and probable ancestral haplotypes (AH) of the 22 HLA-A, B, C, DR and C4 typed persons with a disease-free follow up of less than 5 years

Patient	A		B		C4A		C4B		DR		10th Workshop cell line*	AH
10	*3*	32	*35*	38	*3*	*2*	1	*0*	*1*	13	9006	35.2
11	*2*	11	*44*	55	*3*	3	1	*0*	1	10	9090	44.1
12	3	25	18	57	3	3	1	2	1	15		
13	26	*32*	*38*	49	3	3	1	2	3	*11*	9035	
14	11	*29*	18	*44*	*3*	3	2	*0*	1	15	9027	44.
15	2	30	*8*	13	3	*0*	1	*1*	*3*	11	9022	8.1
16	*24*	26	*35*	38	*3*		*1*		*4*	7	9042	
17	24	32	*8*	44	3	*0*	*1*	1	*3*	15	9022	8.1
18	24	29	*51*	53	3	2	1	1	*11*	11	9044	
19	*2*	24	*44*	18	*3*		0	*0*	*7*	13	9090	44.
20	3	31	7	*44*	3	*3*	1	*0*	*4*	4	9027	44.1
21	*3*		*35*	62	*2*	3	3	*0*	*1*	4	9006	35.2
				—		—				—	9031	
22	2	*3*	*7*	52	*3*	(3)	*1*	(1)	*15*	15	9082	
				—		—		—		—	9011	
23	2	30	51	60	3		1		15	10		
24	*2*	*31*	*51*	52	3	3	1	0	*16*	9	9016	
				—						—	9073	
25	1	32	8	44	3		1		3	4	9022	
											9027	
26	24	33	14	*60*	2	*0*	*2*	1	1	*13*	9059	60.3
	—	—	—	—	—			—	—		9002	
27	1	*3*	13	35	*3*	*2*	1	*0*	*1*	7	9006	35.2
28	1	33	13	14	2	*0*	*1*	1	*3*	7	9022	8.1
29	2	24	*18*	44	3	*3*	1	*0*	*3*	11	9085	18.2
30	2	28	7	27	3		1		7	14		
31	2	3	7	*8*	3	*0*	1	*1*	*3*	4	9022	8.1

* When the phenotype of a patient includes all specificities, or at least two class III and one class I or II specificity, or two class I and one class II specificity of an ancestral haplotype as they have been defined on the lymphoblastoid cell lines of the 10th International Histocompatibility Workshop, the corresponding cell line number [23] is given, and the fitting antigens are italicized.

The ancestral haplotypes that carry a C4 null allele are given a denotation as defined by Cameron *et al.* [13]. Briefly, 8.1 stands for the haplotype A1,B8, C4AQ0, C4B1, DR3; 60.3 stands for A2,B60, C4AQ0,C4B2,DR13; 35.2 stands for A3,B35,C4A3(+2) C4BQ0, DR1; 44.1 stands for A2,B44,C4A3,C4BQ0,DR4; 18.2 means ancestral haplotype A30,B18,C4A3,C4BQ0,DR3.

roxyl groups) whereas C4A molecules react better with immune aggregates (covalent amide bonds). Recently, Gatenby *et al.* [25] have shown that C4A is more effective than C4B in binding immune complexes to the CR1 receptor of erythrocytes.

This immune adherence receptor is present on neutrophils, macrophages, dendritic cells and B cells, and it mediates endocytosis, phagocytosis and also activation. A consequence of the presence of one C4 null allele is that only half the amount of C4A or C4B is present, with about half the capacity to fulfil certain specific functions. This will obviously have an effect on the kinetics of the immune adherence and lytic activities of the complement system. This could be specially detrimental in situations where the immune system is under a major attack, as in HIV infection. These complement deficiencies would have consequences for the progression of the HIV infection itself, and in the later stages also for the resistance to opportunistic infections.

The finding that C4 null alleles are more frequent (Table 2) in recently infected persons than in normal controls, an observation already made by Cameron *et al.* [11,13], allows the intriguing question whether persons with C4 null alleles resist an infectious contact less well, and conversely whether persons with all C4 alleles expressed resist an infectious contact better. This again would mean that in certain people the presence of a normal set of C4 genes would prevent the spread of HIV after an infectious contact.

Although this single-centre study shows a significant association between a long-term disease-free period after HIV infection and the absence of C4 null alleles, other known MHC alleles seem to influence the clinical outcome after HIV-1 infection, for instance the presence of a HLA-DR5 allele is associated with Kaposi's sarcoma, or a Sjögren-like syndrome.

Likewise, it cannot be ruled out that other known genes such as the genes for tumour necrosis factors, the gene for heat shock protein 70, or some other still unknown genes that are located near the C4 genes on chromosome VI, are responsible for the clinical outcome, and that the association found with C4 null alleles is due to linkage dysequilibrium.

If, however, C4 null alleles are not just markers but are enhancers of rapid deterioration after HIV infection, this ma

Table 5. HLA-A, B, DR, complement C4 phenotypes and probable ancestral haplotypes (AH) of the nine patients with disease-free follow up over 5 years

Patient no.	A		B		C4A		C4B		DR		10th Workshop cell line*	AH
1	*2*	11	*27*	51	3	2	1	1	*1*	10	9004	
2	*2*	24	*7*	44	*3*		*1*		12	*15*	9081	
3	*2*	3	*51*	57	3		1		3	*11*	9045	
4	3	32	37	62	3	3	1	94	8	14		
5	24	32	*18*	62	3		1		*11*	11	9039	
6	2		13	57	3		1		4	11		
7	*2*	26	*41*	62	3		1		*4*	13	9024	
8	1	*2*	7	*18*	3		1		*1*	11	9004	
9	*2*		*44*	62	3	*3*	*0*	3	*4*	4	9090	44.1

* When the phenotype of a patient includes all specificities, or at least two class III and one class I or II specificity, or two class I and one class II specificity of an ancestral haplotype as they have been defined on the lymphoblastoid cell lines of the 10th International Histocompatibility Workshop, the corresponding cell line number [23] is given, and the fitting antigens are italicized.

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have consequences in the clinical management of the 50% of HIV-infected persons who have C4 null alleles. This possibility gains even more importance with the recent finding [26] that other factors of humoral immunity, namely high total IgM levels before HIV-1 infection and high total IgA levels after HIV-1 infection are associated with rapid disease progression in haemophiliac patients.

Very early anti-retroviral treatment might indeed become mandatory for HIV-1-infected patients having C4 null alleles, because their chance *a priori* of having a prolonged disease-free period is less than one-tenth of that of a person with C4 alleles completely expressed.

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