Clin. exp. Immunol. (1992) **87**, 20–23

HLA-associated susceptibility to HIV-1 infection

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(Accepted for publication 23 July 1991)

SUMMARY

We studied HLA antigen distribution of 50 heterosexual partners of HIV$^+$ drug abusers with more than 1 year of sexual exposure to HIV, 36 children born to seropositive mothers and 61 haemophiliac patients exposed to presumably infectious clotting factor concentrates. B52 and B44 antigens were associated with HIV resistance while B51 was associated with HIV susceptibility. Forty-nine HIV$^+$ drug abusers, spouses of heterosexual partners studied and 25 HIV$^+$ mothers of the children were also typed. DR11 phenotype was associated with infectiousness of HIV$^+$ subjects. Our data suggest that the HLA region controls susceptibility to infection with HIV and infectiousness of HIV$^+$ subjects in different risk groups.

Keywords HIV-1 susceptibility to infection HLA

INTRODUCTION

Although considerable information about the pathogenesis of HIV-1 infection is available, there is still uncertainty about the factors that actually influence the great interindividual variability of susceptibility to infection and/or progression of the disease [1,2]. These may be due in part to the considerable variability of the immunological and biological responses of HIV-exposed individuals that results from both the environmental and genetic background of the subjects.

At present recent data suggest that several HLA alleles influence both the rapidity and the type of HIV-1 infection [3–12]. We have much less information about HLA-linked genetic control of HIV susceptibility. This is not surprising because there are major obstacles in this kind of study, one being that it is impossible to define exactly the population at risk, since the extent and the frequency of exposure to the virus are difficult to measure, even in a high risk and close population. Most of our information about the role of the HLA region in HIV transmission is derived from studies of HLA phenotype distribution in HIV$^+$ and HIV$^-$ subjects in a single risk group. The time when HIV$^+$ subjects seroconvert is not known and the extent of exposure of HIV$^-$ subjects is not defined.

Some information in this field has come from the study of HLA phenotype distributions of HIV$^+$ and HIV$^-$ haemophiliac patients exposed to a single batch of factor VIII contaminated with HIV [5] or exposed for the same period of time to comparable amounts and batches of presumably infectious clotting factor concentrates [6]. In the patients studied by Steel *et al.* [5], the HLA haplotype A1 B8 DR3 was weakly associated with an increased risk of seroconversion and our data for Italian population [6] suggested a possible role of class I HLA antigens in susceptibility to HIV infection.

In this study we tested the significance of the associations we found in haemophiliacs, in two other risk groups in which HIV$^+$ and HIV$^-$ subjects had similar levels of exposure to the virus: (i) HIV$^+$ and HIV$^-$ children of HIV infected mothers; (ii) HIV$^+$ and HIV$^-$ monogamous partners of HIV$^+$ intravenous drug abusers with more than 1 year of sexual exposure to HIV.

In this study we were able to confirm the significant associations between B52 and HIV resistance and to demonstrate the association between B51 and HIV susceptibility. Therefore, our results strongly support the role of the HLA region in susceptibility to HIV, as well as in progression of the disease, as previously shown [7,8].

SUBJECTS AND METHODS

Subjects

We studied 39 female and 11 male heterosexual partners of HIV$^+$ drug abusers with more than 1 year of sexual exposure to HIV. None of the HIV$^+$ drug abusers received treatment with AZT during the relationships. The median age was 30 years (range 21–50); two men and 15 women were HIV seropositive. Screening for HIV antibodies was carried out by the ELISA technique. Positive reactions were confirmed by Western blotting. We also studied 19 HIV seropositive (median age and range: 52, 25–90 months) and 17 seronegative (median age and

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Table 1. Distribution of the CDC classes for HIV$^+$ spouses of heterosexual partners during the relationship and of HIV$^+$ mothers at delivery

CDC classes during relationship or at delivery	HIV$^+$ spouses of				HIV$^+$ mothers of			
	HIV$^+$ partners ($n=17$)		HIV$^-$ partners ($n=33$)		HIV$^+$ children ($n=19$)		HIV$^-$ children ($n=17$)	
	n	%	*n*	%	*n*	%	*n*	%
CDC II	5	29·4	7	21·2	5	26·3	6	35·3
CDC III	10	58·8	21	63·6	12	63·2	8	47·1
CDC IV	2	11·8	5	15·2	2	10·5	3	17·6

range: 36, 20–80 months) children born to seropositive mothers. All the mothers were positive before pregnancy. The children were followed from birth for at least 20 months at I and IV Pediatric Departments of the University of Milan. Infection was defined by persistence of antibody (as measured by ELISA, confirmed by Western blot beyond the age of 15 months) or the presence of virus or p24 antigen. Children who lost antibody and whose peripheral blood mononuclear cells (PBMC) did not have HIV sequence when analysed by the polymerase chain reaction with *gag* and *env* primers were presumed not to be infected.

Table 1 shows the distribution of CDC classes, on the basis of the Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome [13], for HIV$^+$ spouses of heterosexual partners during the relationship and of HIV$^+$ mothers at delivery.

Forty-nine HIV$^+$ spouses of the partners studied and 25 HIV$^+$ mothers of the children were also typed for HLA A, B and C antigens; of these, 43 spouses and 17 mothers were typed for DR antigens.

Heterosexual couples have been selected from a larger pool of subjects of the Italian Study for HIV heterosexual transmission [14].

Sixty HLA antigens were studied: 15 A, 23 B, 7 C, 12 DR, and 3 DQ. HLA A, B, and C typing was carried out on peripheral blood lymphocytes separated by gradient centrifugation [15]. The standard two-stage microcytotoxicity test was used [16], with 165 selected monospecific or oligospecific antisera. HLA DR and DQ typing was carried out on peripheral blood lymphocytes depleted of T cells by rosetting with 2-aminoethyl-isothiouroniumbromide-treated sheep erythrocytes [17]. Fifty-four antisera were used in the same two-stage test but with a longer incubation time. Cytotoxic antisera were obtained from commercial sources (Bio Test, Dreieich, Germany; Behring, Marburg, Germany; Fresenius, Germany; Merieux, France) or were sera of multiparous women screened in our laboratory against a panel of 250 individuals for their specificity.

The frequencies of HLA antigens were compared by the Yates' χ^2-test corrected for continuity. Corrected *P* values (P_c) obtained by multiplying the original *P* values by the number of antigens tested [18], are considered significant when the probability value is $<0·05$; uncorrected *P* values are given when P_c values were not significant provided they had *P* values of $<0·05$. *P* values for deviations of HLA B52 were not corrected for the number of antigens tested, since the association of this antigen with susceptibility to HIV infection has been already reported [6]. When one or more of the expected numbers was less than five, we performed the significance testing by Fisher's exact method [19]. The degree of association between a single antigen and susceptibility to infection or to the probability of transmission of infection was expressed as relative risk (RR) and as etiological fraction (EF) when the RR was higher than 1 or as preventive fraction (PF) when RR was lower than 1 [20]. To analyse the combined population of the exposed subjects we also considered the χ^2 for heterogeneity [18], which would indicate whether or not the RR values for the three groups of subjects differed.

RESULTS

Previously reported data for haemophiliac patients [6] have been included in the following results. Before pooling the three groups of subjects we considered the χ^2 for heterogeneity and we found that the RR values for the three groups were completely similar.

Table 2 shows the distribution of relevant HLA antigens in HIV$^+$ and HIV$^-$ children born to HIV$^+$ mothers and in HIV$^+$ and HIV$^-$ monogamous heterosexual partners of HIV$^+$ spouses, together with the frequencies of the same antigens in HIV$^+$ and HIV$^-$ haemophiliacs and in overall HIV$^+$ and HIV$^-$ subjects. Ten of the 11 B52$^+$ patients of the pooled three groups are HIV$^-$ (90·9%). The greater B52 in HIV$^-$ subjects than in HIV$^+$ subjects was statistically significant ($\chi^2=7·7$, $P<0·01$, P_c NS) (Fisher's exact test: $P<0·02$); the relative risk of seroconversion (RR) was 0·11, with a PF value of 0·11. The comparison between HIV$^+$ and HIV$^-$ subjects of the three different groups shows a similar trend, but not statistically significant. We also observed a significant increase in B51 in the pooled group of HIV$^+$ subjects ($\chi^2=4·68$, $P<0·05$, P_c NS); 66·7% of B51$^+$ subjects had seroconverted (RR = 2·98, EF = 0·16). In this study we were not able to confirm the deviation of A2 that we demonstrated in haemophiliac patients. The negative association with B44 (RR = 0·42, PF = 0·13, $\chi^2=4·19$, $P<0·05$, P_c NS) is also statistically significant. The frequency of this antigen was lower in the pooled groups and 72% of B44$^+$ subjects remained seronegative even after a long exposure time. The frequency of DR5 was high in HIV$^+$ subjects, and significantly high ($\chi^2=4·39$, $P<0·05$, P_c NS, RR = 2·84, EF = 0·40) in haemophiliac patients and in children, but there was no difference in heterosexual partners. The increase of DR5 frequency is mainly due to the increase of one of its splits, DR12, in haemophiliacs and children ($\chi^2=4·87$, $P<0·05$, P_c NS).

Table 2. Distribution of relevant HLA antigens in children born to HIV+ mothers and in monogamous heterosexual partners of HIV+ spouses, together with the frequencies of the same antigens in exposed haemophiliacs and in overall subjects

	Haemophiliac patients				Partners				Children				All			
	HIV+ (30,28)*		HIV− (31,25)*		HIV+ (17,16)‡		HIV− (33,27)*		HIV+ (19,16)*		HIV− (17,14)*		HIV+ (66,65,60)†		HIV− (81,66)*	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
A2	16	(53·3)	7	(22·6)	8	(47·0)	16	(48·5)	5	(26·3)	3	(17·6)	29	(43·9)	26	(32·1)
B51	6	(20·0)	2	(6·4)	5	(31·2)	3	(9·1)	5	(26·3)	3	(17·6)	16	(24·6)	8	(9·9)
B52	0		4	(12·9)	1	(6·2)	5	(15·1)	0		1	(5·9)	1	(1·5)	10	(12·3)
B44	3	(10·0)	6	(19·3)	1	(6·2)	7	(21·2)	3	(15·8)	5	(29·4)	7	(10·8)	18	(22·2)
DR5 (11+12)	16	(57·1)	9	(36·0)	5	(31·2)	11	(40·7)	11	(68·7)	5	(35·7)	32	(53·3)	25	(37·9)
DR11	12	(42·8)	9	(36·0)	4	(25·0)	8	(29·6)	8	(50·0)	5	(35·7)	24	(40·0)	22	(33·4)
DR12	4	(14·2)	0		1	(6·2)	3	(11·1)	3	(18·7)		0	8	(13·3)	3	(4·5)

* Number tested for HLA A, B, C antigens, numbers tested for DR antigens.
† Number tested for HLA A and C antigens, number tested for B antigens, number tested for DR antigens.
‡ Number tested for HLA A and C antigens, number tested for B and DR antigens.

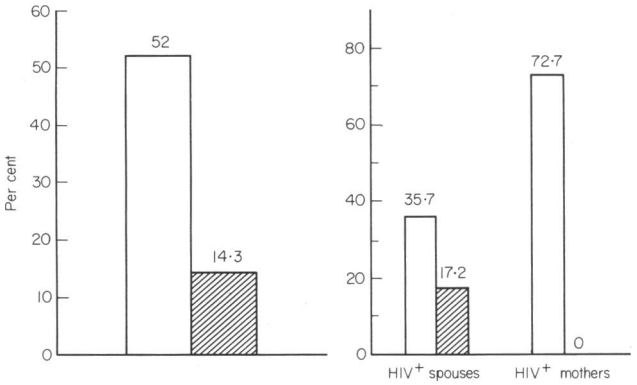

Fig. 1. Distribution of DR11 in HIV+ drug abusers, spouses of HIV+ and HIV− partners and in HIV+ mothers of HIV+ and HIV− children. HIV+ spouses and mothers: □, HIV+ partner ($n=14$) or child ($n=11$); ■, HIV− partner ($n=29$) or child ($n=6$).

The CDC class of the HIV+ cases does not affect their ability to transmit infection to the partner or the child. The infectiousness of HIV+ spouses and of the mothers seems (Fig. 1) however to be significantly associated with DR11 ($\chi^2=8{\cdot}16$, $P<0{\cdot}005$, P_cNS, RR = 6·5, EF = 0·44). The frequency of DR11 is higher in the mothers of HIV+ children than in mothers of HIV− children (72·7% *versus* 0%, $\chi^2=5{\cdot}58$, $P<0{\cdot}02$, P_c NS). For the heterosexual partners, the frequency of DR11 is still increased, but to a much lesser extent.

We did not find any effect of HLA antigen sharing within the heterosexual couples, while 6/8 mothers share DR11 with their HIV+ children.

DISCUSSION

The present data strengthen our previous observations in haemophiliac patients and confirm the role of B52 as a factor of resistance to HIV infection. Though the statistical significance of these findings for the pooled HIV seropositive and seronegative subjects is not high, the result is confirmed by its repeatability in three independent groups of patients, typed at different times and belonging to different risk groups [18]. B52 phenotype appears to be an absolute factor for resistance. Indeed, only one of the 11 B52+ subjects studied (9·1%) seroconverted. Nevertheless, the frequency of B52 in HIV− subjects is only 12%, meaning that B52 is only one of the factors related to individual resistance to HIV infection. This result was not unexpected, because in experimental animals and in man [21,22] the genetic control of susceptibility and resistance to virus infection is polygenic and multifactorial.

In exposed HIV− subjects we also observed a lower B51 frequency. B51 and B52 are 'splits' of B5, therefore one might argue that the results depend on a poor definition of the two antigens. However, the frequency deviations of B51 and B52 in exposed HIV seropositive and seronegative subjects are mirror images and are constant in all three different risk groups. We think it unlikely that technical errors would be so reproducible with time as to interfere with a single antigen definition in only a specific subgroup of patients.

Class I HLA molecules are involved in T cell-mediated cytotoxicity against virally infected cells as restriction molecules and it has been shown that not all class I antigens are recognized equally by cytotoxic T lymphocytes specific for different virus antigens [23,24]. These observations, explained by the molecular structure of class I HLA antigens [25] suggest that our findings are due to a different ability for antigen presentation by B51+ and B52+ lymphocytes. Similar reasoning could be applied to DR12 as a marker of susceptibility to HIV infection. Nevertheless, DR12 is a class II antigen, so it very probably acts at a different step of the anti-HIV immune response.

Our findings in HIV+ mothers of HIV+ and HIV− children and in HIV+ spouses of HIV+ and HIV− heterosexual partners suggest the existence of a genetic influence on infectiousness of HIV+ subjects. One hundred per cent of DR11+ mothers transmit the infection to their children, compared with 27% of DR11− ones. If this observation were to be confirmed in a larger group of patients, DR11 could become an important clinical prognostic marker. Obviously, mother and child very frequently share DR11, and therefore we are not able to exclude a role of DR11 sharing in transmission of HIV infection. However, DR11 is not by itself a marker of increased susceptibility, since

its frequency in HIV^+ and HIV^- children is not significantly different.

The association of DR11 phenotype with infectiousness of HIV^+ subjects is much more evident for HIV^+ mothers than for HIV^+ spouses of heterosexual couples. We have two possible explanations for this: the first is that pregnancy immunesuppression might amplify the biological effects of DR11 and the second is that HIV^+ mothers transmit to their children the HLA and non-HLA genes of HIV susceptibility, giving rise to a pair more homogeneous for susceptibility factors than the heterosexual couple.

We do not know at present the biological mechanism that underlies this association. Our previous observations [22] and studies in progress show a statistically significant association between DR11 and a low production of interferon-gamma (IFN-γ) by human alloactivated lymphocytes. If we consider that IFN-γ acts on the anti-viral immuneresponse and, particularly by directly inhibiting HIV replication [27], these data serve as a starting point to clarify the relationship between DR11 and the infectiousness of HIV^+ subjects.

ACKNOWLEDGMENTS

This work was supported by funds from Ministero della Sanita', grant AIDS to R. Scorza C.R. 420657 and C.R. 5206090.

REFERENCES

1 Weller IVD, Carne C, Sattentau Q, *et al.* HIV infection in the regular sexual partners of homosexual men with AIDS and PGL. J Med Virol 1987; **22**:91–5.

2 Peterman TA, Stoneburner RL, Allen JR *et al.* Risk of Human Immunodeficiency Virus transmission from heterosexual adults with transfusion-associated infection. JAMA 1988; **259**:55–8.

3 Enlow RW, Nunez Roland A, Lo Galbo P *et al.* Increased frequency of HLA DR 5 in lymphoadenopathy stage of AIDS. Lancet 1983; **ii**:51–2.

4 Kaslow RA, Dusquenoy R, VanRaden M, *et al.* A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection. Lancet 1990; **335**:927–30.

5 Steel CM, Ludlam CA, Beadson D, *et al.* HLA haplotype A1, B8, DR3 as a risk factor for HIV-related disease. Lancet 1988; **ii**:1185–8.

6 Fabio G, Scorza Smeraldi R, Gringeri A *et al.* Susceptibility to HIV infection and AIDS in Italian haemophiliacs is HLA associated. Brit J Haematol 1990; **75**:531–6.

7 Scorza Smeraldi R, Fabio G, Lazzarin A *et al.* HLA-associated susceptibility to acquired immunodeficiency syndrome in Italian patients with human-immunodeficiency-virus infection. Lancet 1986; **ii**:1187–9.

8 Scorza Smeraldi R, Fabio G, Lazzarin A *et al.* HLA associated susceptibility to AIDS: HLA B35 is a major risk factor for Italian HIV-infected intravenous drug addicts. Human Immunol. 1988; **22**:73–9.

9 Jeannet M, Sztajzel R, Carpentier N *et al.* HLA antigens are risk factors for development of AIDS. J AIDS 1989; **2**:28–32.

10 Mann DL, Murray C, Yarchoan R *et al.* HLA antigen frequencies in HIV-1 seropositive disease-free individuals and patients with AIDS. J. AIDS 1988; **1**:13–7.

11 Prince HE, Schroff RW, Ayoub G *et al.* HLA studies in acquired immunodeficiency syndrome patients with Kaposi's Sarcoma. J Clin Immunol 1984; **4**:242–5.

12 Raffoux C, David V, Couderc LD *et al.* HLA-A, B and DR antigen frequencies in patients with AIDS-related persistent generalised lymphadenopathy (PGL) and thrombocytopenia. Tissue Antigens 1987; **29**:60–2.

13 Revision of the CDC surveillance case definition for Acquired Immunodeficiency Syndrome. Morbid Mortal Week Rep 1987; **36**:1–15.

14 Lazzarin A, Saracco A, Musicco M *et al.* Man to woman sexual transmission of the Human Immunodeficiency Virus. Italian Study Group on HIV Heterosexual Transmission. Risk factors related to sexual behavior, man's infectiousness and woman's susceptibility. Arch Inter Med, December 1991. In press.

15 Boyum A. A simple method for separation of mononuclear cells on Ficoll–Hypaque. Scand J Clin Lab Invest 1968; **21**:97–101.

16 Mittal KK, Mickey MR, Singai DP *et al.* Serotyping for homotransplantation XVIII-refinement of microdroplet lymphocyte cytotoxicity test. Transplant 1968; **6**:913–27.

17 Pellegrino MA, Ferrone S. Isolation of human B and T lymphocytes with 2-amino-ethyl-isothiouroniumbromide (AET) treated sheep red blood cells. In: Dusquesnoy RJ, Fuiler C, eds. *The First HLA Workshop of the Americas.* Bethesda: Department of Health, Education and Welfare, 1975:176–8.

18 Svejgaard A, Jersild C, Staub Nielsen L *et al.* HLA antigens in disease. Statistical and genetical considerations. Tissue Antigens 1974; **4**:95–105.

19 Armitage P. Statistical methods in medical research. Oxford: Blackwell, 1971; 78–92.

20 Svejgaard A, Platz P, Ryder LP. HLA and disease 1982—A survey. Immunol Rev 1982; **70**:193–218.

21 Millich DR, Leroux-Roes GG, Louie RE *et al.* Genetic regulation of the immune response to HBsAg.IV. Distinct H-2 linked Ir genes control antibody responses to different HBsAg determinant on the same molecule and map to the I-A and I-C subregions. J Exp Med 1984; **159**:41–56.

22 Scorza Smeraldi R, Di Sapio M, Fabio G *et al.* Condizionamento genetico della risposta all'HBsAg. Il fegato 1986; **32**:63–9.

23 McMichael AJ, Gotch FM, Santos-Aguado J *et al.* Effect of mutations and variations of HLA-A2 on recognition of a virus peptide epitope by cytotoxic T lymphocytes. Proc Natl Sci 1988; **85**:9194–8.

24 Bodmer HC, Gotch FM, McMichael AJ. Class I cross-restricted cells reveal low responder allele due to processing of viral antigen. Nature 1989; **337**:653–5.

25 Bjorkman PJ, Saper MA, Samraoui B *et al.* Structure of the human class I histocompatibility antigens. Nature 1987; **329**:506–12.

26 Scorza R. Genetic conditioning and dynamic of viral infections. In: Aiuti F, Bonomo L, Danieli G, eds. *Topics in Immunology* IX European Immunology Meeting. Roma: Il Pensiero Scientifico Editore, 1988; 183–202.

27 Kornbluth RS, Oh PS, Munis JR *et al.* The role of interferons in the control of HIV replication in macrophages. Clin Immunol Immunopathol 1990; **54**: 200–19.