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Detection of HLA-DR Associated with Monocytotropic, Primary, and Plasma Isolates of Human Immunodeficiency Virus Type 1

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This study determined whether HLA-DR was incorporated into human immunodeficiency virus type 1 produced in vivo or by primary cultured cells. HLA-DR was associated with virions from primary isolates, macrophage cultures, and blood plasma. These results represent the first demonstration of major histocompatibility complex molecules associated with an in vivo source of virus.

During budding of certain enveloped viruses, host cell molecules are incorporated into the virion membrane. The lentiviruses human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) have been shown to incorporate major histocompatibility complex (MHC)-encoded class I and II molecules, as well as other host cell molecules (1).

Incorporation of MHC molecules by virus can have significant immunologic effects. For example, vaccination of macaques with inactivated SIV produced in human cells induces a strong immune response to human MHC molecules carried by virus. This immune response protects against challenge with live SIV produced in human cells (6). Additionally, studies by Rossio et al. indicate that HLA-DR (class II MHC) on cell line-derived HIV can bind the superantigen staphylococcal enterotoxin A and stimulate highly purified human T lymphocytes (16). Although this has not been demonstrated in vivo, this finding does suggest that class II MHC molecules on virus are functional. These and other findings have led to the hypothesis that MHC class II molecules on the surface of HIV could function in antigen presentation to $CD4^+$ T cells (1).

While the above findings are potentially important, to date, nearly all studies demonstrating incorporation of MHC class II molecules on HIV have used virus produced by transformed cell lines (1, 4, 5, 12, 16). The goal of this study was to determine whether HLA-DR was incorporated into several sources of HIV-1, including monocytotropic virus produced in macrophages and primary isolates produced by mitogen-stimulated peripheral blood cells, since viruses from these sources are more likely to be similar to viruses found in vivo (8, 13). Plasma virus obtained from infected individuals was also analyzed. To achieve this aim, a sensitive immunocapture method was utilized.

Production and isolation of viruses. The HIV_{MN}, $HIV_{Ada\text{-}M}$, and $HIV_{Ba\text{-}L}$ isolates and the AA-2 B-lymphoblastoid cell line were obtained from the AIDS Research and Reference Program (National Institutes of Health, Rockville, Md.). The HIV_{MN} isolate was used to infect AA-2 B-lymphoblastoid cells, which express high levels of HLA-DR (18). Supernatant was collected between 4 and 12 days postinfection and filtered (0.45-μm pore size). Virus was concentrated and then purified by ultracentrifugation over a sucrose gradient (25 to 55%) (2).

Monocytotropic viruses Ada-M and Ba-L were expanded by infecting monocyte-derived macrophages (MDM). Prior to infection, the MDMs used for Ba-L propagation were grown in complete medium in gelatin-coated flasks (10); whereas those used for propagation of Ada-M were grown in complete medium supplemented with 1,000 U of recombinant macrophage colony-stimulating factor (Cellular Products, Buffalo, N.Y.) per ml and in flasks coated with both gelatin and fibronectin (3). Supernatants were collected at 10 to 20 days postinfection.

A primary HIV isolate was passaged for 4 days in peripheral blood mononuclear cells (PBMCs) that had been previously stimulated with 3 μg of phytohemagglutinin P (PHA-P; Sigma, St. Louis, Mo.) per ml plus 25 U of interleukin-2 (Boehringer Mannheim, Indianapolis, Ind.) per ml in complete media for 3 days.

Plasma virus was isolated as described elsewhere (21). Briefly, plasma from HIV-infected individuals was diluted 1:3 with saline, layered over 20% (wt/vol) sucrose, and ultracentrifuged for 1 h at 120,000 × *g*. Virus was resuspended in RPMI 1640 medium. Samples were tested for p24 positivity by enzyme immunoassay (EIA; Immunotech Inc., Westbrook, Maine).

Immunocapture procedures. Four immunocapture procedures were compared for the ability to capture HIV with a monoclonal antibody to HLA-DR. Several antibody preparations were used for these experiments: Fc-specific rabbit anti-mouse (RAM) immunoglobulin G (IgG) (Jackson Immunoresearch Laboratories Inc., West Grove, Pa.), an HLA-DR mouse IgG2a monoclonal antibody (clone L243; American Type Culture Collection, Rockville, Md.), a mouse IgG2a isotype control (Becton Dickinson, San Jose, Calif.), and a mouse IgG control containing all isotypes (Sigma). The total incubation volume for all of the procedures was 225 μl. The first procedure was performed as described by Orentas and Hildreth, with some modifications (14). Briefly, wells from a Falcon Microtest assay plate (Becton Dickinson) were coated first with RAM in phosphate-buffered saline (PBS; pH 7.4) and then with the HLA-DR antibody or an IgG control. Intact HIV_{MN} was added to the plate, which was then incubated overnight at 4°C. Bound virus was lysed with 0.5% Triton X-100 detergent in PBS.

For the other procedures, fixed *Staphylococcus aureus* cells (Pansorbin; Calbiochem, San Diego, Calif.), protein A coupled

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TABLE 1. Recovery of HIV_{MN} after incubation with HLA-DR antibody immobilized by various procedures[a]

Antibody used	Avg % recovery ± SD[b]			
	96-well plate	GAM-agarose	Protein A-agarose	*S. aureus* cells
None	0.10	ND[c]	0	9.6
Control mouse IgG	0.13	5.2 ± 0.8	0	9.8
Anti-HLA-DR	0.65	26.0 ± 1.5	1.6	87.5 ± 3.6
% Specific binding	0.52	20.8	1.6	77.7

[a] HIV_{MN} from AA-2 B-lymphoblastoid cells was used. The 96-well plate, *S. aureus* cells, and protein A-agarose were loaded with RAM IgG prior to addition of mouse antibodies (or no antibody). Only the anti-HLA-DR monoclonal antibody or control mouse IgG was added to the GAM-agarose. The virus was then added to the immobilized antibody and incubated overnight at 4°C. Bound virus was detected by measuring p24 after treatment with 0.5% Triton X-100 detergent.

[b] Values are percentages of p24 detected, where the total amount of virus loaded per sample was 932.85 pg of p24 per ml, except for the protein A-agarose method, where 1,033 pg/ml was loaded. Results are representative of two to six experiments for each procedure.

[c] ND, not done.

TABLE 2. Recovery of monocytotropic viruses after incubation with immobilized anti-HLA-DR antibody

Virus type	% of p24 detected[a]		Total p24 (pg/ml)	% Specific p24 binding
	IgG control	Anti-HLA-DR		
HIV_{MN}[b]	0.6	86.8	1,248	86.2
HIV_{Ada-M}	3.5	53	6,000	49.5
HIV_{Ba-L}	1.8	54.3	4,170	52.5

[a] Values are percentages of p24 detected after treatment of pelleted virus with 0.5% Triton X-100 detergent.

[b] Control virus grown in B-lymphoblastoid AA-2 cells (nonmonocytotropic).

to agarose (Pierce Chemical Co., Rockford, Ill.), and goat anti-mouse (GAM) IgG covalently coupled to agarose (Sigma) were washed with PBS containing 1% bovine serum albumin (BSA). The *S. aureus* cells (50 μl) and protein A-agarose (25 μl) were incubated with 250 μg of RAM antibody for 2 h at room temperature with rocking. Cells and agarose were washed three times in 1 ml of PBS plus 1% BSA and then incubated for an additional 2 h at room temperature with 2 μg of HLA-DR antibody or 2 to 10 μg of control antibody with rocking. GAM-agarose (25 μl) was similarly incubated with anti-HLA-DR antibody or an IgG control. Treated immunosorbents were washed three times in PBS plus 1% BSA and incubated overnight with virus at 4°C on a rocking platform. Virus-bound immunosorbents were washed four more times, lysed with detergent, and then added directly to p24 EIA wells to measure virus recovery.

The above methods were compared for overall efficiency in immobilizing HIV_{MN} from AA-2 cells. Table 1 shows that greater than 77% of virus was specifically recovered by immunocapture with *S. aureus* cells compared with the IgG control in this representative experiment. In six experiments, an average of 6.5% ± 4.85% of HIV_{MN} bound to control *S. aureus* cells, while 78.8% ± 7.6% bound to immobilized anti-HLA-DR, for an overall specific recovery of 72.2%. A lower but significant level (20.8%) of HIV_{MN} was recovered by immunoprecipitation with GAM-agarose, while less than 2% was captured by the 96-well plate and protein A-agarose methods (Table 1). Quantitation of the amount of RAM antibody bound to both sources of protein A was done by Western blotting and densitometry analysis. Results revealed that similar amounts of antibody bound to both of the immunosorbents (data not shown), suggesting that some other factor(s) was responsible for the difference in virus recovery. Since the greatest degree of virus recovery was observed with *S. aureus* cells, all further experiments employed this immunosorbent.

HLA-DR detection on primary and plasma-derived HIV isolates. Anti-HLA-DR antibody captured approximately 50% of HIV_{Ada-M} or HIV_{Ba-L} monocytotropic virus (Table 2) produced from MDMs cultured with or without fibronectin and macrophage colony-stimulating factor, respectively. Approximately 18 to 20% of the HIV derived from mitogen-stimulated PBMCs was also immunocaptured by immobilized anti-HLA-DR monoclonal antibody, compared with an IgG control (Table 3).

The degree of specific binding of anti-HLA-DR antibody to plasma virus was significant in three of eight plasma virus samples tested, with binding ranging from 16.5% for patient 4 to 36.8% for patient 5, compared with 0% for IgG controls ($P \leq 0.05$ for all three samples as tested by Student's t test) (Table 3). In addition, 10% of the plasma virus from patient 6 also bound to the anti-HLA-DR antibody, with the degree of binding approaching significance ($P = 0.12$) (Table 3). The remaining four of the eight samples (from patients 1 to 3 and 7) did not detectably bind to the immobilized anti-HLA-DR antibody ($P > 0.05$ for immobilized anti-HLA-DR versus IgG controls of replicate samples, t test). Interestingly, in two of three HLA-DR-negative samples (virus from patients 2 and 3), 36 to 37% of the total HIV was captured by the nonspecific IgG control alone (Table 2). Previous studies have suggested that the high background may be due to bound antiviral antibody interacting with protein A (21).

In this study, we showed that HLA-DR was associated with primary isolate virus produced by mitogen-stimulated PBMCs and monocyte-derived macrophages. HLA-DR was also detected on three of the eight plasma virus samples tested. Since the anti-HLA-DR monoclonal antibody used in these experiments recognizes only the αβ heterodimer (11), virus that was immunocaptured possessed the heterodimers. Ordinarily, on HLA-DR-expressing cells, the initial expression of HLA-DR on a cell surface is preceded by binding of the specific peptide to the heterodimer before it is transported to the cell membrane. Once bound, the peptide dissociates with a very slow dissociation constant (17). Thus, it is quite likely that the HLA-DR on the viral surface contains bound peptide and that the peptide–HLA-DR complex is functional in binding to the T-cell receptor on $CD4^+$ lymphocytes.

The presence of cell surface antigens, including class I and II antigens, on HIV derived from cell lines and primary isolates has been reported by others (1, 4, 5, 12, 16). Arthur et al. have shown that a large fraction of virus produced by the H9 cell line could be immunocaptured by antisera to MHC class I or II (1). By using a slightly different immunoprecipitation method, Capobianchi et al. showed that both laboratory and primary clinical virus isolates incorporated HLA-DR (5). Since the captured virus was detected by measuring infectious virus produced after addition of indicator cells, a quantitative value for the degree of cell membrane proteins present on the primary and laboratory isolates was not obtained. More recently, Cantin et al. also reported the detection of HLA-DR on PHA-stimulated primary isolates. Analysis was semiquantitative and employed immunomagnetic beads and subsequent p24 analysis (4). Approximately 12 to 44% virus recovery with anti-HLA-DR-coated beads was observed (4). Our data support these

TABLE 3. Recovery of plasma virus, primary isolates, and cell line-derived HIV by immobilized anti-HLA-DR

Expt no. and virus type (individual no.)[a]	Initial p24 level (pg/ml of plasma)[b]	% (avg amt [pg/ml] ± SD) of p24 detected after treatment[c] with:			Amt of p24 loaded (pg/ml)	% Specific p24 recovered
		No antibody	IgG antibody	Anti-HLA-DR antibody		
1						
Plasma virus (1)	271.6	ND	5.3 (17.39 ± 9.4)	3.2 (11.57 ± 1.8)	328.4	−2.1
HIV_{MN}	NA	2.8 (31.3)	1.5 (16.65 ± 0.97)	72.4 (796.7 ± 5.7)	1,100	70.9
2						
Plasma virus (2)	119.3	ND	36.4 (52.1)	40.7 (58.2)	143.2	4.3
HIV_{MN}	NA	ND	8.9 (85.14)	73.7 (701.8)	952.5	64.8
Primary isolate	NA	ND	5.6 (59.06)	25.1 (265.4)	1,058	19.5
3						
Plasma virus (3)	75.38	ND	37.3 (18.28 ± 4.2)	36.7 (16.87 ± 5.7)	49.0	−0.6
Primary isolate	NA	ND	2.8 (38.15)	21.47 (289.9)	1,350	18.67
4						
Plasma virus (4)	206	ND	16.0 (6.6 ± 2.5)	32.5 (13.4 ± 0.3)	41.2	16.5
HIV_{MN}	NA	ND	12.7 (46.4)	70.4 (258)	366.6	57.7
5						
Plasma virus (5)	1.3×10^6	ND	33.6 (134.8 ± 0.4)	70.4 (282.3 ± 36.7)	401.1	36.8
HIV_{MN}	NA	ND	0.4 (25.23 ± 4.2)	81.8 (4,800 ± 102.8)	5,870.5	81.4
6						
Plasma virus (6)	110.3	41.9 (144.5 ± 6.9)	35.6 (122.65 ± 9.4)	45.8 (157.95 ± 16.3)	344.8	10.2
Plasma virus (7)	78.1	13.7 (16 ± 0.9)	6.7 (7.84 ± 0.03)	2.6 (3.06 ± 1.1)	116.2	−4.1
Plasma virus (8)	3,151.2	4.0 (15.6 ± 2.6)	4.1 (16.3 ± 0.2)	33.6 (132.4 ± 31.4)	393.9	29.5
HIV_{MN}	NA	1.9 (25.6 ± 4.7)	2 (29.0 ± 8.0)	52.7 (730.4 ± 42.8)	1,386	50.7

[a] Plasma virus isolated from eight individuals was tested in six separate experiments alongside HIV_{MN} from the AA-2 cell line and/or an HIV primary isolate produced in PHA-stimulated PBMCs.

[b] These values were derived by determining p24 recovery from ultracentrifuged plasma and adjusting for the overall volume of plasma used. NA, not applicable.

[c] Values are percentages and averages of duplicate amounts of p24 detected, unless indicated otherwise. Virus recovery was quantitated by measuring the amount of p24 bound to protein A-*S. aureus* after incubation with 0.5% Triton X-100 and is expressed as a percentage of the total p24. ND, not done.

studies. In addition, our study shows the presence of HLA-DR on monocytotropic virus produced in primary cultured cells. The results showed that higher levels of the monocytotropic virus than the primary isolate were captured, suggesting that HLA-DR levels were higher on the monocytotropic virus.

This is also the first study to show that HLA-DR is associated with plasma virus. Although the cell origin of plasma virus has not been identified, it was anticipated that all plasma virus samples would possess HLA-DR since expression of HIV in vivo has been shown to occur predominantly in stimulated $CD4^+$ T lymphocytes and macrophages (9, 15, 19, 20), which both express HLA-DR. It was somewhat unexpected, then, to find that HLA-DR was not detected on all of the plasma virus samples tested. While the high background binding may have obscured HLA-DR-specific binding in the HLA-DR-negative samples, it is also possible that anti-p24 antibodies were present in several of the plasma virus samples tested, thereby blocking p24 from being detected. In a control experiment, two of three ultracentrifuged plasma samples from additional seropositive (but p24 negative) individuals contained anti-p24 antibodies capable of blocking p24 in a p24 EIA to some degree (data not shown). Alternatively, it is possible that plasma virus from samples testing negative for HLA-DR was derived from cells that expressed lower levels of HLA-DR in vivo. In fact, Clerici et al. have observed that HLA-DR levels on monocytes can substantially decrease during HIV infection (7). Additionally, we have observed that PHA stimulation increases HLA-DR levels on $CD4^+$ $HLA\text{-}DR^+$ T cells two- to threefold (unpublished observation). Thus, the variation in levels of HLA-DR expression on plasma virus may be due to the plasma virus budding from more than one cell type or, alternatively, from several subpopulations of one cell type.

In summary, these results show that HLA-DR was associated with primary isolate virions, virions produced by macrophages, and plasma virus. These results indicate that it is possible that engagement of the T-cell receptor on $CD4^+$ T cells by HLA-DR on plasma virus could occur in vivo.

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