

ABSENCE OF ALLOGENEIC RESTRICTION IN HUMAN
T-CELL-MEDIATED CYTOTOXICITY TO
EPSTEIN-BARR VIRUS-INFECTED TARGET CELLS

Demonstration of an HLA-linked Control
at the Effector Level*

BY MARC LIPINSKI, WOLF H. FRIDMAN, THOMAS TURSZ, CLAUDE VINCENT,
DONALD PIOUS, AND MARC FELLOUS

From the laboratoire d'Immunologie Clinique, Institut Gustave-Roussy, Villejuif, France; the laboratoire d'Immunologie Cellulaire, IRSC, Villejuif, France; the laboratoire d'Immunologie, Institut National de la Santé et de la Recherche Medicale U80, Hôpital E. Herriot, Lyon, France; the Department of Pediatrics, University of Washington, Seattle, Washington; and the laboratoire de Génétique de la Transplantation Humaine, Institut National de la Santé et de la Recherche Medicale U93, Hôpital Saint Louis, Paris, France

Genes located in the major histocompatibility complex (MHC)¹ influence T-cell-mediated cytotoxicity against virally infected or hapten-modified target cells at several different levels. First, MHC products act as recognition structures and eventually as restriction elements at the target cell surface. Second, genes in the MHC govern, through various mechanisms, the development of cytotoxic T lymphocyte (CTL) responses. In man, CTL raised against influenza virus-induced antigens are subject to allogeneic restriction (1). Similar data have been reported for human CTL against minor histocompatibility antigens (2) and haptens (3). On the other hand, no evidence for allogeneic restriction has been found in other systems, such as T-cell-mediated cytotoxicity against trinitrophenyl (TNP)-modified human targets (4) or in the mouse against Sendai virus-infected cells (5). However, in these nonrestricted responses, quantitative differences in the levels of cytotoxicity with different individuals and different effector-target cell combinations were obtained (4). Several MHC-linked factors may contribute to this apparent polymorphism. Not only may cross-reactivity occasionally occur, masking the role of the MHC products as restriction elements (6), but the level of CTL responses may be controlled by MHC-located genes such as immune response (Ir) (7) or viral susceptibility genes (8).

In human infectious mononucleosis (IM), peripheral T lymphocytes taken at the acute phase of the disease, are specifically cytotoxic for Epstein-Barr virus (EBV)-positive cell lines (9). We have previously shown in this system that Daudi cells

* Supported by Institut National de la Santé et de la Recherche Medicale (France) (grant 17.42.77.79) and Institut Gustave-Roussy (grant 76 A 22).

¹ Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; CTL, cytotoxic T lymphocyte(s); DMSO, dimethylsulfoxide; EBV, Epstein-Barr virus; IM, infectious mononucleosis; Ir, immune response; MHC, major histocompatibility complex; NK cell, natural killer cell; PBL, peripheral blood lymphocyte(s); RFC, rosette-forming cell(s); TNP, trinitrophenyl.

lacking HLA-A, B, and C antigens, although EBV-positive, were resistant to lysis by anti-EBV CTL (10). On the other hand, preliminary evidence has been reported, arguing against the existence of allogeneic restriction in this system (9–11).

In the present work, we analyze this system further and, first, confirm that HLA is indeed involved in the structure(s) recognized by anti-EBV CTL because, not only are Daudi cells not killed, but also anti- β 2 microglobulin and anti-HLA antibodies inhibit the cytolysis of EBV-positive, HLA-positive target cells. Second, we present further arguments for the absence of allogeneic restriction in this system. Finally, evidence suggesting an HLA-linked gene control of the development of anti-EBV CTL is presented, most individuals exhibiting a strong CTL response being HLA-A1 positive.

Materials and Methods

Effector Lymphocytes. Blood samples were collected at the acute phase of the disease from 25 patients with infectious mononucleosis assessed by a positive Paul-Bunnell-Davidsohn test. Peripheral blood lymphocytes (PBL) were purified by centrifugal sedimentation on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). To avoid any spontaneous nonspecific cytolysis of the lymphoblastoid cells used as targets, further purification of T cells was required. This was achieved by removal of Fc γ -receptor-positive lymphocytes including most natural killer (NK) cells in man (12, 13). EA rosettes were performed according to a previously published method (14). EA rosette-forming cells (RFC) were removed by centrifugation on Ficoll-Hypaque gradients. For complete abrogation of nonspecific killing, it appeared necessary to repeat this procedure twice. In some cases, effector lymphocytes were frozen in 5% dimethylsulfoxide (DMSO) and stored in liquid nitrogen.

Target Cells. Various EBV-positive and -negative lymphoblastoid cell lines were maintained in our laboratory in RPMI (Gibco, Glasgow, U. K.) medium supplemented with 10% heat-inactivated fetal calf serum. In our assays, cells from the following EBV-positive lines were used as targets: Daudi-lacking HLA-A, B, and C products (15) and Raji (HLA A3, A10, B18, and B35), both originating from human Burkitt's lymphomas; T5-1 (HLA A1, A2, B8, B27, DR1, and DR3) and HLA-homozygous cell lines (provided by W. F. Bodmer, Oxford, U. K.) established by the in vitro EBV-transformation of B cells from healthy donors. The latter included PGF (HLA A3-B7), Priess (HLA A2-B15), and Madura (HLA A2-B9). T5-1 MHC variants (16) served as target cells in some experiments. These included variants 4.22.8, 10.9.6, 7.2.8, and 6.1.6. lacking expression of HLA-A2, A2 and B8, A1, DR1, and DR3 antigens, respectively. Finally, EBV-negative HLA antigen lacking K-562 cells served as reference targets for natural killing because of its remarkable sensitivity to NK cell cytotoxicity (17).

HLA Phenotyping. Effector cell HLA phenotypes were determined by the classical microlymphocytotoxicity assay (18).

Cytotoxicity Assay. Target cells (3×10^6 in 0.2 ml medium) were labeled by incubation with 0.1 ml (100 μ Ci) of ^{51}Cr as sodium chromate (New England Nuclear, Boston, Mass.) for 45 min at 37°C, washed twice in 50 ml Hanks' solution (Institut Pasteur, Paris), and resuspended at 10^5 cells/ml. 0.1 ml of this suspension was then mixed with 5×10^5 fresh or thawed purified T lymphocytes in a final vol of 0.2 ml at a 50:1 effector cell to target cell ratio, in flat-bottomed Cook microtiter plates (Greiner, Nürtingen, West Germany). After a 4-h incubation at 37°C in a 5% CO_2 humidified incubator, 0.1 ml of the supernates were harvested for counting of the radioactivity released in a gamma-counter (Intertechnique, Paris, France). A spontaneous release control and a maximum release control were included in all assays and consisted of 0.1 ml medium or HCl 6 N, respectively, added in place of effector lymphocytes. Mean counts in triplicate tests were determined. Percent specific lysis (SL) were calculated as:

$$\% \text{ SL} = \frac{E - S}{M - S} \times 100;$$

where E, S, and M represent mean experimental, spontaneous, and maximum releases,

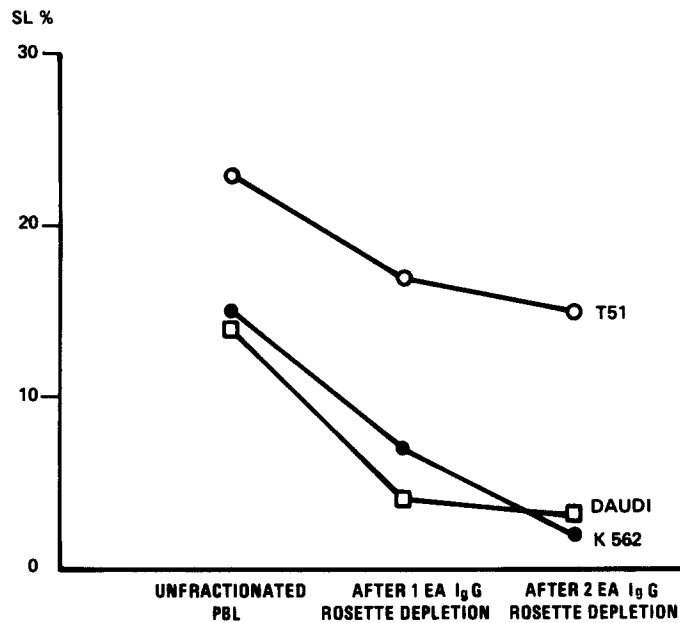


FIG. 1. Effect of one and two EA-IgG rosette-forming cell depletion cycles on cell-mediated cytotoxicity by PBL from IM patients to various target cells: T5-1 (EBV-positive, HLA-positive), Daudi (EBV-positive, HLA-negative), K-562 (EBV-negative, HLA-negative, NK cell sensitive). The cytotoxic activity was measured in a 4-h ^{51}Cr chromium release assay at a 50:1 effector:target cell ratio. Values are the means of three replicate wells. SE are <5% in all cases.

respectively. The spontaneous release usually ranged from 12 to 18% of the maximum ^{51}Cr released in the HCl controls.

Cytotoxicity Inhibition Experiments. Cytotoxicity inhibition experiments were performed by adding various antibodies to the assay medium: rabbit anti-human $\beta 2$ microglobulin antiserum; $\text{F}(\text{ab})'_2$ fragments of polyspecific human IgG anti-HLA-A, B, and C antibodies; monoclonal anti- $\beta 2$ microglobulin (BBM.1) (19); and anti-HLA (W6/32) antibodies, the latter reacting with all HLA-A, B, and C molecules (20), both provided by W. F. Bodmer (Oxford, U. K.); and finally, a polyspecific human anti-HLA-DR serum was used as control.

Results

Involvement of HLA Molecules in Antiviral T-Cell-mediated Lysis. When investigating T-cell-mediated cytotoxicity in man, especially when lymphoblastoid cell lines are used as targets, great care has to be taken to avoid spontaneous lysis by NK cells. Because human NK cells (12-13), as well as B cells, have been shown to carry Fc γ receptors, PBL from IM patients were depleted of EA-IgG RFC. The experiment shown in Fig. 1 illustrates the effect of one or two depletion cycles of EA-IgG RFC on lymphocyte-mediated cytotoxicity towards T5-1, Daudi, and K-562 cells. Unfractionated PBL were able to kill all three targets. One cycle of EA-IgG RFC removal left a lymphocyte population still containing 6% RFC. This effector population exhibited a slightly decreased cytotoxicity on T5-1 target cells and a reduced, but still significant, killing of Daudi and K-562 cells. After retreatment using the same procedure, no EA-IgG RFC could be found in the purified population which no longer killed Daudi or K-562 target cells while still exerting consistent cytotoxicity towards T5-1 cells. This two-step purification is therefore necessary to abrogate fully NK cell activity as

TABLE I
Inhibition of the T-Cell-mediated Lympholysis of T5-1 Target Cells by Various Antibodies

Antibodies*	Exp 1		Exp 2		Exp 3		Exp 4	
	SL‡	Inh§	SL	Inh	SL	Inh	SL	Inh
0	15.7		29.6		35.0		12.0	
Anti- β 2 microglobulin	1.4	91.1			24.6	29.7	6.6	45.0
F(ab)'2 anti-HLA			8.3	72.0	24.7	29.4	7.8	35.0
Anti-HLA-DR	17.0	0	27.8	6.1	36.6	0	18.0	0
0	14.7	6.4						

Antibodies	Exp 5	
	SL	Inh
	22.9	
Anti- β 2 microglobulin	-0.5	100
Monoclonal anti- β 2 microglobulin	7.5	67.2
Monoclonal anti-HLA	14.2	38.0

* Conventional (Exp 1-5) and monoclonal (Exp 5) antibodies described in Materials and Methods were added to the incubation medium during the 4-h ^{61}Cr release assay. Effector to target cell ratio was 50:1. Values are the means of three replicate wells. SE are <5% in all cases.

‡ Percent specific lysis.

§ Percent inhibition of specific lysis.

|| Effector cells preincubated with anti- β 2 microglobulin serum.

assessed by the final absence of killing of the EBV-negative, NK cell-sensitive K-562 target. Moreover, the purified subpopulation was unable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC), as assessed by the absence of lysis of L1210 cells in the presence of rabbit anti-L1210 cell antiserum (data not shown). This rules out the involvement of anti-EBV ADCC in the measured anti-EBV killing mediated by lymphocytes from patients with high titers of anti-EBV antibodies. This purification procedure results in T-effector cells cytotoxic towards the EBV-positive, HLA-positive T5-1 target cells but noncytotoxic to EBV-positive, HLA-negative Daudi cells. We have used this procedure in all these studies.

To further analyze the involvement of HLA molecules in the target structure(s) for antiviral CTL, we have attempted to inhibit T-cell-mediated cytotoxicity of the EBV-genome carrying T5-1 cells with various anti- β 2 microglobulin and anti-HLA antibodies. Table I shows that the addition to the assay medium of rabbit anti-human β 2 microglobulin and of F(ab)'2 fragments of polyspecific human anti-HLA-A, B, and C antibodies consistently inhibited T5-1 cytotoxicity by T cells from patients with IM. In contrast, addition of human polyspecific anti-HLA-DR antibodies was without effect. The use of monoclonal antibodies anti- β 2 microglobulin (BBM-1) and anti-HLA (W6/32) reacting with all the HLA-A, B, and C molecules also inhibited the reaction confirming the previous data (Table I). The observed inhibitory effect was not a result of interaction of the antibodies with the effector cells because preincubation of the latter with anti- β 2 microglobulin serum did not affect their cytotoxic activity (Table I).

TABLE II
Cytotoxicity to T5-1-variant Target Cells. Effect of Differential HLA Antigen Expression

	Target cell	Progenitor type	Variants	
Exp 1	HLA antigen expression	T5-1	4.22.8	10.9.6
		A1 A2	A1 —	A1 —
		B8 B27	B8 B27	— B27
	DR1 DR3	DR1 DR3	DR1 DR3	
	Specific lysis*	44.2 ± 4.9	43.5 ± 2.0	45.1 ± 5.3
Exp 2	HLA antigen expression	T5-1	7.2.8	6.1.6
		A1 A2	— A2	A1 A2
		B8 B27	B8 B27	B8 B27
	DR1 DR3	DR1 DR3	— —	
	Specific lysis	15.1 ± 4.5	14.3 ± 3.1	20.2 ± 4.8

* Values are means ± SE of triplicates.

The presence of accessible HLA molecules on the T5-1 target cell surface having thus been shown to be necessary for anti-EBV T-cell killing, we have studied the respective role of the HLA-A, B, and D determinants, using as targets T5-1 variants which have selectively lost one or two HLA antigens. Table II shows that neither loss of HLA-A2, HLA-A2 and B8, HLA-A1, nor HLA-DR 1 and 3 determinants impaired the antiviral cytolysis, suggesting no preferential involvement of any of these antigens with the target structure(s) recognized by anti-EBV CTL.

HLA-linked Control of the Development of Anti-EBV CTL. In a series of 25 IM patients studied, it appeared that T lymphocytes from only 9 individuals were able to exert significant cytotoxicity towards T5-1 target cells. The data are given in Table III. Strikingly, seven of these nine patients with detectable anti-EBV CTL shared the HLA-A1 antigen, whereas none of the patients lacking detectable CTL were HLA-A1 positive. This observation does not reflect an increase in HLA-A1-positive individuals among IM patients, because the distribution of HLA antigens in this group as well as in groups studied by other authors (21) does not significantly differ from that of the healthy control population. Furthermore, among the patients without detectable anti-EBV CTL, the distribution was similar to that found in a control population for all HLA antigens, except for the total absence of HLA-A1. The mean specific lysis of T5-1 target cells by CTL from HLA-A1-positive patients was 29.7 vs. 0.6% in the HLA-A1-negative group (Fig. 2). This difference is highly significant according to the Student's *t* test ($t = 7.8, P < 10^{-9}$).

Lack of Allogeneic Restriction in Anti-EBV T-Cell-mediated Cytolysis. We have reported previously (10) and in the present work that the expression of HLA molecules at the target cell surface was necessary for lysis by anti-EBV CTL. The question obviously arises as to whether this killing is HLA-restricted. It can be seen from Table III that among patients developing detectable CTL towards T5-1 target cells, the level of cytotoxicity is not correlated with the number of HLA specificities shared between effector and target cells. For example, CTL from patient 1 sharing only one HLA specificity with T5-1, exerted a specific lysis of 44.2%, whereas CTL from patient 2, sharing two HLA specificities with the target cells exerted 42.2% of specific lysis at the same effector:target cell ratio. CTL from patient 8, without any HLA specificity in common with T5-1 cells nevertheless exerted a significant cytotoxicity towards this

TABLE III
Cytolysis of EBV-positive T5-1 Target Cells by Peripheral T Cells from 25 IM Patients

Patients	Effector cell		T5-1 target cell* specific lysis
	HLA phenotype		
	A locus	B locus	
1	<u>1</u> , 11	12, 21	44.2 ± 4.9
2	<u>1</u> , 31	<u>8</u> ,	42.2 ± 2.9
3	<u>1</u> , 25	<u>8</u> , 22	35.0 ± 1.8
4	<u>1</u> , 33	<u>8</u> , 17	29.6 ± 1.9
5	<u>1</u> , 29	<u>7</u> , 12	22.9 ± 0.5
6	<u>1</u> , 32	<u>8</u> , 14	22.4 ± 1.5
7	<u>2</u> , 31	<u>8</u> , 12	16.4 ± 1.0
8	23, 31	12, 15	15.1 ± 4.5
9	<u>1</u> , 26	<u>8</u> , 12	12.0 ± 1.7
10	<u>2</u> , 31	<u>8</u> , 40	3.7 ± 0.9
11	3, 11	<u>8</u> , 40	3.5 ± 1.0
12	28, 32	5, 18	3.2 ± 0.7
13	29, —	12, —	2.2 ± 0.9
14	<u>2</u> , 11	5, 8	1.0 ± 0.8
15	28, 31	<u>27</u> , <u>37</u>	-0.3 ± 0.9
16	<u>2</u> , —	<u>12</u> , 38	-0.6 ± 1.9
17	<u>2</u> , 11	12, —	-1.8 ± 1.7
18	<u>2</u> , 9	12, <u>27</u>	-2.4 ± 1.3
19	23, 25	12, 21	-1.8 ± 0.2
20	3, 28	7, 40	-2.0 ± 1.1
21	3, 11	5, 14	-3.5 ± 0.5
22	<u>2</u> , 9	5, <u>27</u>	-4.3 ± 0.3
23	<u>2</u> , —	7, 12	-4.9 ± 1.0
24	3, 31	21, <u>27</u>	-4.8 ± 0.7
25	<u>2</u> , 3	15, <u>39</u>	-7.1 ± 0.5

The T-cell-mediated cytotoxicity assay is performed as described in Materials and Methods. Specific lysis values are mean percent specific lysis ± SE of triplicates. Effector cell HLA antigens are underlined when present in the T5-1 HLA phenotype.

* T5-1 HLA phenotype: A1, A2, B8, B27.

target (15.1%) as compared, for example, to the cytotoxicity exhibited by CTL from patients 7 (16.4%) and 9 (12%) sharing two HLA specificities with T5-1 cells. On the other hand, among the patients who did not develop detectable CTL towards T5-1 target cells, it is noteworthy that 4 of 16 shared two specificities and 7 shared one specificity with the target cells (Table III).

Tables IV and V report experiments in which T lymphocytes from different IM patients were tested against various EBV-positive target cells. The CTL from five patients which exerted significant cytotoxicity towards T5-1 target cells were also cytolytic towards the other target cells tested (Raji and PGF) regardless of their HLA compatibility (Table IV). In 7 of 12 cytotoxic combinations, there was no HLA specificity in common between effector and target cells, indicating that HLA antigens do not act as restriction elements in this system.

The lack of detectable CTL towards T5-1 target cells in some patients left the possibility that cytotoxicity could be found in fully compatible HLA-A and B effector-

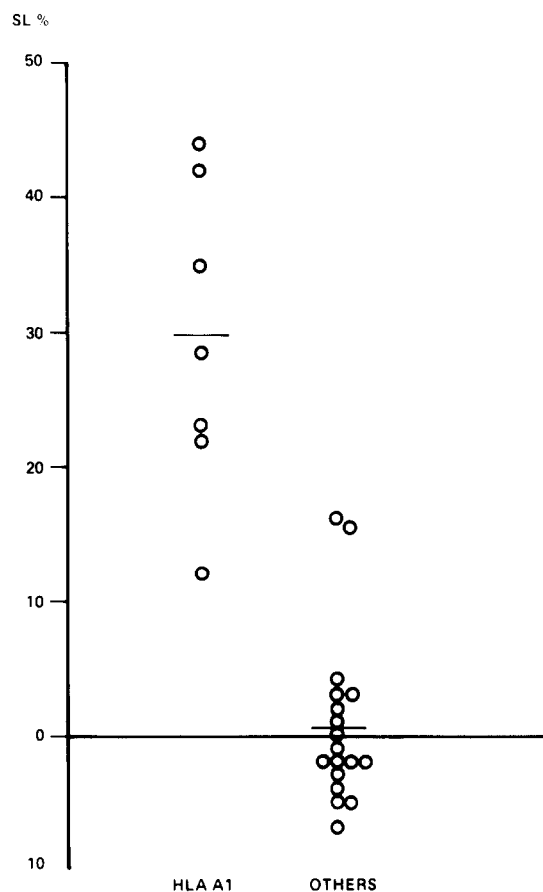


FIG. 2. Cytolysis of T5-1 target cells by peripheral blood T lymphocytes HLA-A1 positive and negative IM patients. Reactions were performed as described in Materials and Methods. Each bar represents the average specific lysis for each group.

TABLE IV
Lack of Allogeneic Restriction in IM Patient T-Cell-mediated Cytotoxicity against EBV-positive Target Cells

Patient	Effector cells HLA phenotype	Target cells*		
		T5-1	Raji	PGF
7	A2 A31 B 8 B12	16.4 ± 1.0	11.6 ± 0.8	17.7 ± 1.2
6	A1 A32 B 8 B14	22.4 ± 1.5	<u>12.7 ± 1.1</u>	<u>16.0 ± 1.8</u>
2	A1 A31 B 8	42.2 ± 2.9	<u>22.1 ± 1.4</u>	<u>12.5 ± 1.0</u>
1	A1 A11 B12 B21	44.2 ± 4.9	<u>62.1 ± 4.2</u>	
5	A1 A29 B 7 B12	22.9 ± 0.5		23.0 ± 0.7

Values represent mean percent specific lysis ± SE of triplicates.

Values are underlined where no HLA-A or B antigen is shared between effector and target cells.

* Target cell HLA phenotypes: T5-1, A1, A2, B8, B27; Raji: A3, A10, B18, B35; PGF A3, B7.

TABLE V
Lack of Killing of HLA-homozygous EBV-positive Lymphoblastoid Target Cells by T Cells from HLA-compatible IM Patients

Effector cells		Target cells			
Patient	HLA phenotype	T5-1	HLA-homozygous lines*		
			Priess	ARNT	Madura
25	A2 A3 B15 B39	-7.1 ± 0.5	-0.4 ± 1.1	-2.5 ± 0.7	
10	A2 A31 B40 B8	3.7 ± 0.9			-11.1 ± 1.7

* HLA-homozygous line phenotypes: Priess, A2, B15; ARNT, A2, B39; Madura A2, B40. Values represent mean percent specific lysis ± SE of triplicates.

target cell combinations. This does not appear to be the case, because T lymphocytes from patient 25 are not cytotoxic towards the EBV-positive, HLA-A and B homozygous Priess and ARNT cells, which are HLA-A and B compatible with the effector cells (Table V). Similar data were obtained using lymphocytes from patient 10 which did not kill the EBV-positive, HLA-A and B homozygous Madura target cells in spite of its HLA-A and B compatibility with the effector cells (Table V).

Discussion

The present study concerns the role of the human MHC in the cytotoxic response of T cells from patients with IM towards EBV genome-containing target cells. First, we investigated the role of HLA products at the target cell surface and second, the possible involvement of MHC genes in the control of the cytotoxic T-cell response. The data herein presented show that, after complete elimination of NK and K cells has been achieved, T cells from patients with IM are cytotoxic towards EBV-positive cells only if HLA-A, B and C molecules are expressed on the target cell membrane. After one cycle of EA-IgG RFC removal, Daudi cells could be occasionally killed. In such cases, the purified lymphocyte population was still able to mediate ADCC against L1210 cells in the presence of anti-L1210 antiserum. We therefore conclude that the observed lysis of Daudi cells could be related to both remaining spontaneous cytotoxicity and ADCC through anti-EBV antibodies. Furthermore, the fact that anti-EBV T cell lysis is consistently inhibited by anti- β 2 microglobulin and anti-HLA-A, B, and C antibodies, argues further for the role of HLA molecules at the target cell surface in this system. This inhibition is not a result of interaction with the effector cells, because preincubation of the latter with anti- β 2 microglobulin antibodies is without effect. The recognition of HLA-D products by T-effector cells is not necessary for anti-EBV killing because anti-HLA-DR antibodies did not affect the cytotoxic reaction. That Daudi cells, though carrying the HLA-DR6 antigen (22) are indeed resistant to anti-EBV T-cell-mediated cytotoxicity, further indicates that HLA-D products cannot act as recognition structures in this system. Moreover, T5-1 6.1.6 variant cells which do not express HLA-D products were as sensitive to anti-EBV CTL as the wild type T5-1 cells.

In this system, despite the fact that the expression of HLA products is necessary for anti-viral T-cell-mediated killing, we did not find any evidence for allogeneic restriction. The lack of restriction was established by the following arguments: (a) CTL

from one given individual could exert a similar magnitude of cytotoxicity towards different EBV-positive cells, regardless of the number of HLA antigens shared by the effector and target cells, (b) in several cytotoxic combinations, CTL from patients with IM were able to kill target cells with which they shared no HLA specificity, (c) finally, variant T5-1 cells, lacking one or two HLA antigens at the A and B loci, were killed to the same extent as the wild-type cell line, indicating the lack of preferential involvement of any antigen in the target structure(s) recognized by the anti-EBV CTL. Cytolysis of EBV-positive allogeneic target cells is indeed EBV-specific and is not a result of alloreactive CTL generated during the response to EBV infection because it is well substantiated that T cells from patients with IM cannot kill allogeneic EBV-negative cells (9, 10). Thus, this situation is clearly different from the murine TNP model in which allo-reactive CTL can cross-react with TNP-modified syngeneic target cells (23).

The lack of allogeneic restriction observed in our data has, however, to be tempered by the fact that EBV-infected cells expressed as much as 36 times more HLA molecules at their surface than normal lymphocytes (24). This could reveal cross-reactivity between allo-antigens that are not detectable using conventional target cells. Furthermore, it remains possible that virus-infected cells, especially when transformed, express extra MHC specificities (25) which may mimic the self antigens of effector T cells. However, it is striking that in no case were CTL from one patient able to show preference for one given EBV-positive cell line. This favors, therefore, the idea that there is a real absence of allogeneic restriction in this system. These results appear to conflict with results from several murine (26–29) and human (1–3) systems. However, unrestricted models such as the CTL response to Sendai-infected murine target cells (5) and to TNP-modified cells (6) have been described. The response of human T cells to TNP-modified targets, though species restricted, is not HLA restricted which argues in favor of the hypothesis that CTL recognize TNP in conjunction with an antigen widely shared among humans but absent from mice (4). Similarly, the *in vivo* generated T cells may recognize EBV-coded antigens in association with a public or constant part determinant born by the HLA-A, B, and C products or with β 2 microglobulin.

The MHC, besides coding for molecules involved in target structures for CTL, contains genes governing the magnitude of immune responses. MHC-linked Ir as well as immune suppression (Is) genes controlling antibody production have been clearly demonstrated (30). It has been shown recently in the mouse that genes located in the H-2I region govern the capacity of CTL to recognize either H-2K or H-2D TNP-modified target cells (31). Similar data have been obtained in studies of the response to H-Y (32) or murine sarcoma virus-coded (33) antigens. In man, in the HLA-restricted T-cell killing of influenza virus-infected target cells (1), it is striking to observe that most individuals carrying the HLA-A2 antigen do not develop anti-viral CTL *in vitro* (34). This result can be interpreted in terms of an HLA-A2-linked gene(s) governing unresponsiveness to influenza-coded antigens. In a similar model, Shaw and Biddison have recently shown that, within a large family, HLA identical siblings exhibit identical patterns of anti-influenza T-cell-mediated cytolysis against different target cells (35).

In the system studied here, using CTL against EBV-coded antigens developed *in vivo* during the course of IM, we have shown that T lymphocytes from only 9 of 25

patients were cytotoxic against EBV-positive target cells. 7 of these 9 patients carried the HLA-A1 antigen, whereas none of the 16 patients lacking detectable CTL were HLA-A1 positive. These data favor the hypothesis of an HLA-A1-linked gene controlling development of anti-EBV CTL. The mean specific lysis of T5-1 target cells by CTL from HLA-A1-positive patients was 29.3 vs. 0.6% for HLA-A1-negative patients ($P < 10^{-9}$). The distribution of HLA-A1-positive individuals within the two groups of patients is strikingly different from the frequency of HLA-A1 in a control population (23.4%). This unusual distribution does not reflect any selection of HLA-A1-positive individuals among IM patients because in our group ($7/25 = 28\%$) as well as in reported studies (21), there is no abnormal frequency of the HLA-A1 antigen nor of any other HLA-A or B antigen tested. However, some features of the course of the disease peculiar to HLA-A1 positive patients cannot be excluded. Larger clinical studies are needed to clarify this point.

That the magnitude of the anti-EBV response in IM patients is controlled by an HLA-linked gene(s) can be tentatively explained in several ways: (a) HLA-A1-linked gene(s) govern some nonimmunological parameters concerning EBV infection such as the level of viremia or the kinetics of virus clearance in the organism (8). Similar H-2-linked genes have been actually described in the control of Moloney viremia (36). (b) Ir gene(s) control the development of CTL. HLA-A1-linked gene(s) may govern either the proliferative response of T cells towards EBV-infected cells, or the differentiation of precursors to mature CTL, or both phenomena. A possible explanation of our observation is that T cell from HLA-A1-negative IM patients do proliferate in response to the virus but do not undergo differentiation to mature CTL to the same extent as T cells from HLA-A1-positive patients. Interestingly, T cells from HLA-A2 individuals which frequently do not generate anti-influenza virus CTL (34), nevertheless exhibit a normal proliferative response in the presence of influenza infected cells (A. J. McMichael. Personal communication.). The existence of Ir genes is well documented (30). A new type of Ir gene(s) controlling the expression of MHC-unrestricted CTL to hapten-modified target cells has been described recently (37). In view of these data, the hypothesis that HLA-A1-positive IM patients develop unrestricted CTL, whereas T cells from HLA-A1-negative patients are subject to strict allogeneic restriction is worth considering. This does not however appear to be the case because T cells from those patients which are not cytotoxic towards EBV-positive allogeneic target cells are equally unable to kill fully HLA-matched EBV-positive cells. (c) An Is gene present in all individuals but the HLA-A1-positive subjects is responsible for the absence of CTL observed in HLA-A1-negative patients.

It is noteworthy that the HLA-A1-linked Ir (or Is) gene postulated in this work would be located close to the HLA-A locus, whereas the HLA-D region has so far been considered to be the human equivalent of the murine H-2I region.

Summary

Peripheral T lymphocytes from patients with infectious mononucleosis (IM) are sensitized *in vivo* against the Epstein-Barr virus (EBV). The expression of HLA-A, B, or C molecules at the target cell surface is necessary for the cytotoxic reaction because (a) EBV-positive Daudi cells lacking HLA-A, B, and C determinants are resistant to anti-EBV T-cell lysis, (b) cytolysis of EBV-positive target cells can be consistently inhibited by anti-HLA-A, B, and C and anti- $\beta 2$ microglobulin antibodies. However,

no evidence for allogeneic restriction in this system was apparent as (a) cytotoxic T lymphocytes (CTL) from one given individual could exert a cytotoxicity of a similar magnitude on different EBV-positive target cells, regardless of the number of HLA-A or B specificities shared by the effectors and targets; (b) CTL from IM patients were able to kill target cells without any HLA-A or B antigen in common; and (c) T5-1 variants lacking one or two HLA antigens at the A, B, or D locus are killed to the same extent as the parental cells. 7 of the 9 IM patients with detectable circulating anti-EBV CTL carried the HLA-A1 antigen, whereas none of the 16 IM patients lacking detectable peripheral CTL were HLA-A1 positive (mean specific lysis of T5-1 target cells by T cells from HLA-A1-positive patients: 29.3 vs. 0.6% in HLA-A1-negative patients) ($P < 10^{-9}$). These data suggest an HLA-A1-linked gene control of the magnitude of the anti-EBV CTL response. Thus, the HLA region appears to act at two different levels in the T-cell-mediated lysis of EBV-infected cells by controlling first, the development of anti-EBV and second, the expression of HLA-A, B, and C molecules involved as recognition structures at the target cell surface.

We wish to thank Professeur J. Dausset and Dr. A. Senik for fruitful help. We are grateful to Ms. Y. Finale, M. Y. Guillard, and M. Duteil for expert technical assistance, to Professor W. F. Bodmer who donated monoclonal anti-HLA and anti- β 2 microglobulin antibodies and HLA-homozygous cell lines, to Doctors Y. Coquin and J. P. Ghanassia and Ms. R. Kerdilès for providing blood samples from IM patients, and to Dr. P. Avner for critical reading of the manuscript.

Received for publication 9 August 1979.

References

1. Mc Michael, A. J., A. Ting, H. J. Zweerink, and B. A. Askonas. 1977. HLA-restriction of cell-mediated lysis of influenza virus-infected human cells. *Nature (Lond.)* **270**:524.
2. Goulmy, E., A. Termijtelen, B. A. Bradley, and J. J. Van Rood. 1977. Y-antigen killing by T cells of women is restricted by HLA. *Nature (Lond.)* **266**:544.
3. Dickmeiss, E., B. Soeberg, and A. Svejgaard. 1977. Human cell-mediated cytotoxicity against modified target cells is restricted by HLA. *Nature (Lond.)* **270**:526.
4. Shaw, S., D. L. Nelson, and G. M. Shearer. 1978. Human cytotoxic response in vitro to trinitrophenyl-modified autologous cells. I.T cell recognition of TNP in association with widely shared antigens. *J. Immunol.* **121**:281.
5. Finberg, R., S. J. Burakoff, H. Cantor, and B. Benacerraf. 1978. Biological significance of alloreactivity: T cells stimulated by Sendai virus-coated syngeneic cells specifically lyse allogeneic target cells. *Proc. Natl. Acad. Sci. U. S. A.* **75**:5145.
6. Burakoff, S. J., R. N. Germain, and B. Benacerraf. 1976. Cross-reactive lysis of trinitrophenyl (TNP)-derivatized H-2 incompatible target cells by cytolytic T lymphocytes generated against syngeneic TNP spleen cells. *J. Exp. Med.* **144**:1609.
7. Schmitt-Verhulst, A. M., and G. M. Shearer. 1975. Bifunctional major histocompatibility-linked genetic regulation of cell-mediated lympholysis to trinitrophenyl-modified autologous lymphocytes. *J. Exp. Med.* **142**:914.
8. Lilly, F., and H. A. Freedman. 1976. Mechanisms of the H-2 effect on viral leukemogenesis. In *Animal Virology*. D. Baltimore, A. S. Huang, and C. Fred Fox, editors. Academic Press, Inc., New York. 359.
9. Svedmyr, E., and M. Jondal. 1975. Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr Virus are present in patients with infectious mononucleosis. *Proc. Natl. Acad. Sci. U. S. A.* **72**:1622.
10. Tursz, T., W. H. Fridman, A. Senik, A. Tsapis, and M. Fellous. 1977. Human virus-

infected target cells lacking HLA antigens resist specific T-lymphocyte cytolysis. *Nature (Lond.)*. **269**:806.

11. Bakacs, T., E. Svedmyr, and E. Klein. 1978. EBV-related cytotoxicity of Fc receptor negative T lymphocytes separated from the blood of infectious mononucleosis patients. *Cancer Lett.* **4**:185.
12. West, W. H., G. B. Cannon, H. D. Kay, G. D. Bonnard, and R. B. Herberman. 1977. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. *J. Immunol.* **118**:355.
13. Nelson, D. L., B. M. Bundy, and W. Strober. 1977. Spontaneous cell-mediated cytotoxicity by human peripheral blood lymphocytes in vitro. *J. Immunol.* **119**:1401.
14. Peter, H. H., J. Pavie-Fischer, W. H. Fridman, C. Aubert, J. P. Cesarini, R. Roubin, and F. Kourilsky. 1975. Cell-mediated cytotoxicity in vitro of human lymphocytes against a tissue culture melanoma cell line (IGR 3). *J. Immunol.* **115**:539.
15. Fellous, M., F. Mortchelewicz, M. Kamoun, and J. Dausset. 1975. The use of a lymphoid cell line to define new B lymphocyte specificities probably controlled by the MHC region. In *Histocompatibility Testings*, F. Kissmeyer-Nielsen, editor, Munksgaard, Copenhagen. 708.
16. Gladstone, P., and D. Pious. 1978. Stable variants affecting B cell alloantigens in human lymphoid cells. *Nature (Lond.)*. **271**:459.
17. Klein, E., H. Ben-Bassat, H. Neumann, P. Ralph, J. Zeuthen, A. Polliack, and F. Vanky. 1976. Properties of the K562 cell line, derived from a patient with chronic myeloid leukemia. *Int. J. Cancer.* **18**:421.
18. Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation (Baltimore)*. **6**:913.
19. Brodsky, F. M., W. F. Bodmer, and P. Parham. 1979. Characterization of a monoclonal anti- $\beta 2$ microglobulin antibody and its use in the genetic and biochemical analysis of major histocompatibility antigens. *Eur. J. Immunol.* **9**:536.
20. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens. New tools for genetic analysis. *Cell*. **14**:9.
21. Rosdahl, N., and A. Svejgaard. 1979. HLA types and ABO blood groups in patients with infectious mononucleosis. *Tissue Antigens*. **13**:223.
22. Jones, E. A., P. N. Goodfellow, J. G. Bodmer, and W. F. Bodmer. 1975. Serological identification of HLA-linked human "Ia-type" antigens. *Nature (Lond.)*. **256**:650.
23. Lemonnier, F., S. J. Burakoff, R. N. Germain, and B. Benacèraf. 1977. Cytolytic thymus-derived lymphocytes specific for allogeneic stimulator cells crossreact with chemically modified syngeneic cells. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1229.
24. McCune, J. M., R. E. Humphreys, R. R. Yocum, and J. L. Strominger. 1975. Enhanced representation of HLA antigens on human lymphocytes after mitogenesis induced by PHA or EBV. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3206.
25. Garrido, F., V. Schirmacher, and H. Festenstein. 1976. H-2 like specificities of foreign haplotypes appearing on a mouse sarcoma after vaccinia virus infection. *Nature (Lond.)*. **259**:228.
26. Doherty, P. C., R. V. Blanden, and R. N. Zinkernagel. 1976. Specificity of virus immune effector T cells for H-2K and H-2D compatible interactions: implications for H antigen diversity. *Transplant. Rev.* **29**:89.
27. Bevan, M. J. 1975. Interaction antigens detected by cytotoxic T cells with the major histocompatibility complex as modifier. *Nature (Lond.)*. **256**:419.
28. Shearer, G. N., T. G. Rehn, and A. M. Schmitt-Verhulst. 1976. Role of the murine major histocompatibility complex in the specificity of in vitro T cell-mediated lympholysis against syngeneic trinitrophenyl-modified targets. *Transplant. Rev.* **29**:222.

29. Germain, R. N., M. E. Dorf, and B. Benacerraf. 1975. Inhibition of T lymphocyte-mediated tumor specific lysis by alloantisera directed against the H-2 serological specificities of the tumors. *J. Exp. Med.* **142**:1023.
30. Benacerraf, B., and M. Dorf. 1976. The nature and function of specific H-linked immune response genes and immune suppression genes. The activities of complementing histocompatibility-linked Ir genes in the control of immune responses to synthetic antigens. In *The Role of Products of the Histocompatibility Gene Complex in Immune Responses*. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 228.
31. Schmitt-Verhulst, A. M., and G. M. Shearer. 1976. Multiple H-2-linked immune response gene control of H-2D-associated T-cell-mediated lympholysis to trinitrophenyl-modified autologous cells: Ir-like genes mapping to the left of I-A and within the I region. *J. Exp. Med.* **144**:1701.
32. Simpson, E., and R. D. Gordon. 1977. Responsiveness to H-Y antigen, Ir gene complementation and target cell specificity. *Immunol. Rev.* **35**:59.
33. Gomard, E., V. Duprez, T. Reme, M. J. Colombani, and J. P. Levy. 1977. Exclusive involvement of H-2D^b or H-2K^d product in the interaction between T-killer lymphocytes and syngeneic H-2^b or H-2^d viral lymphomas. *J. Exp. Med.* **146**:909.
34. McMichael, A. 1978. HLA-restriction of human cytotoxic T lymphocytes specific for influenza virus. Poor recognition of virus associated with HLA A2. *J. Exp. Med.* **148**:1458.
35. Shaw, S., and W. E. Biddison. 1979. HLA-linked genetic control of the specificity of human cytotoxic T cell responses to influenza virus. *J. Exp. Med.* **149**:565.
36. Debre, P., S. Gisselbrecht, and J. P. Levy. 1979. H-2 control of Moloney induced leukemia in mice. *J. Immunol.* In press.
37. Billing, P., S. J. Burakoff, M. E. Dorf, and B. Benacerraf. 1978. Genetic control of cytolytic T lymphocyte responses. I. Ir gene control of the specificity of cytolytic T lymphocyte responses to trinitrophenyl-modified syngeneic cells. *J. Exp. Med.* **148**:341.