HUMAN CYTOTOXIC T-CELL RESPONSES TO TYPE A AND TYPE B INFLUENZA VIRUSES CAN BE RESTRICTED BY DIFFERENT HLA ANTIGENS

Implications for HLA Polymorphism and Genetic Regulation

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Since the discovery of the HLA-A and -B antigens, two questions which have been of particular interest are, (*a*) What is the biological function of these molecules? and (*b*) Why are they the most polymorphic genetic system known in man? The answer to these questions may be related because an understanding of the function of these molecules might provide insight into the origins and maintenance of such remarkable polymorphism. Until recently, the only known function of these antigens was as a barrier to allogeneic tissue transplantation, which was of no obvious relevance to individual survival during evolution. However, studies in rodents (1, 2) and more recently in humans (3–8) have provided convincing evidence that antigens coded by the major histocompatibility complex (MHC)[1] are cell surface structures that play a crucial role in immunologic interactions between thymus-derived (T) lymphocytes and other cells. In vitro studies of human cytotoxic T-cell responses have demonstrated that T cells must recognize foreign antigen (such as the male H-Y antigen [3], dinitrophenol [4], and influenza virus [5–8]) in conjunction with cell surface HLA-A or -B antigens. This dual requirement for T-cell activation raises the possibility that any individual's response to a particular foreign antigen may be determined not only by the molecular properties of the foreign antigen, but also by the repertoire of cell surface histocompatibility antigens. That this might account for the observed polymorphism of MHC genes was proposed by Doherty and Zinkernagel (9).

The potential evolutionary advantage of HLA-A and -B polymorphism has become evident in our studies of HLA-linked genetic control of in vitro cytotoxic responses to two closely related human pathogens: type A and type B influenza viruses. Previous studies have shown that in the cytotoxic T-cell response to type A influenza virus (A/HK), the viral antigens are recognized in conjunction with self antigens; these self antigens are coded by HLA-linked genes (7) and are highly associated with the HLA-A and -B antigens (5, 6, 8). In addition to coding for these HLA antigens, HLA-linked genes can control the magnitude of cytotoxic responses to virus in conjunction with these self antigens (7). Our study extends these observations in parallel studies of the cytotoxic response to type B influenza virus (B/HK) and demonstrates that the

[1] *Abbreviations used in this paper*: A/HK, HK-X-31 strain of type A influenza virus; B/HK, HK strain of type B influenza virus; E:T, effector:target; MHC, major histocompatibility complex; PBL, peripheral blood mononuclear leukocytes.

HLA-linked regulation is virus-specific and differs even between these two closely related viruses. Furthermore, the data indicate that each HLA-A and -B specificity of a given donor may function in the recognition of some but not all foreign antigens. Because MHC-restricted influenza-specific cytotoxic effectors have been shown to enhance host survival in mice (10, 11), the findings presented here for the human T cell response may provide a biologically relevant model by which to account for the maintenance of HLA-A and -B polymorphism.

Materials and Methods

Human Blood Reagents. Peripheral blood mononuclear leukocytes (PBL) and plasma were collected by batch leukapheresis from normal adult volunteers, and the PBL were separated by flotation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) as previously described (12). Plasma from five such male donors was pooled, frozen in portions at −20°C, and used after thawing as the normal plasma pool. Fresh PBL were cryopreserved and thawed before use as described by Holden et al. (13). HLA-A and -B serotyping was kindly performed by Dr. A. H. Johnson, Duke University, Durham, N. C. Family HLA haplotype assignment was made on the basis of the HLA serotyping data for all family members and the results of intrafamilial mixed lymphocyte culture, as previously described (7).

Viruses. A/HK (A/Hong Kong/8/68-X-31[H3N2]), and B/HK (B/Hong Kong/8/73), were grown in embryonated eggs (14), aliquotted, and stored at −70°C. Hemagglutination titers were measured as described (14).

Generation of Effectors. PBL were thawed and resuspended at 3×10^6/ml in RPMI-1640 with glutamine (Grand Island Biological Co., Grand Island, N. Y.) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). Virus stock was thawed and diluted to a concentration of 0.2–2.0 hemagglutination units per milliliter in the same media. 4 ml each of virus suspension and cell suspension were mixed in tissue culture flasks (Falcon 3013, Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) that were incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air. After 1 h, 0.5 ml of the normal human plasma pool was added, and the flasks were incubated for 7 d.

Target Cells and Cytotoxicity Assay. Cytotoxicity was assessed in a standard 6 h ^{51}Cr release assay as previously described (15). The targets cells were phytohemagglutinin (M form, Grand Island Biological Co.)-stimulated lymphoblasts that were infected with virus on the day of the assay (15).

Data Analysis. Data from cytotoxicity assays are expressed as the mean percent specific lysis of triplicate determinations (12). Spontaneous release of ^{51}Cr from all target cell preparations was <25% of the detergent-releasable counts. To analyze the role of HLA-linked determinants in family studies of virus recognition, the effector-target combinations were subdivided into four groups with respect to the genetic relationship between the effector and the target: (*a*) autologous; (*b*)HLA-identical; (*c*)HLA-haploidentical; and (*d*)HLA-different. The mean lysis was calculated at each effector:target (E:T) ratio for each group, and the statistical significance of differences was tested by the two-tailed Student's *t* test.

Conventional lytic unit calculations were made as follows. Percent specific lysis was plotted as a function of the $\log_{10}$ of the effector cell concentration for three different dilutions, and the best linear approximation was determined by a least squares fit. The number of cells required to generate 30% lysis was determined from the line, and the lytic activity was expressed as its reciprocal.

Results

HLA-Linkage of Target Antigens. We have previously demonstrated in studies of two families that human cytotoxic T cells sensitized against A/HK recognize viral antigens in conjunction with self-determinants that are coded by genes linked to HLA (7). Although A/HK and B/HK are closely related viruses, human cytotoxic T-cell

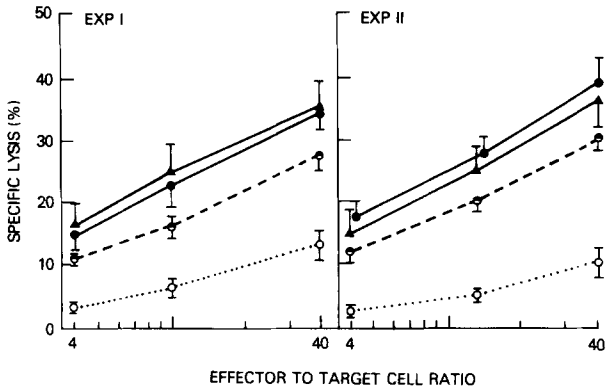

FIG. 1. HLA restriction of cytotoxicity generated against B/HK. In exp. I, PBL from all nine siblings of family B were sensitized in vitro against B/HK, and effectors from each sibling were assayed on B/HK-infected target cells from seven siblings. Mean lysis was calculated at each of three E:T ratios for the seven combinations of effectors on infected autologous targets (▲), the 13 combinations of effectors on HLA-identical targets (●), the 31 combinations of effectors on HLA-haploidentical targets (◓), and the 12 combinations of effectors on HLA-different targets (○). Exp. II was similar except that effectors were assayed on infected targets from all nine siblings. Vertical bars designate the standard error of the mean.

responses to these viruses show little or no cross-reactivity (Table II) (5, 15). Members of one of these families (family B) have been restudied to analyze the self specificity of their cytotoxic responses to B/HK; the results indicate that the response to this closely related virus is also restricted by HLA-linked genes (Fig. 1). Mean lysis of HLA-identical target cells was comparable to that observed with autologous targets. In contrast, mean lysis of HLA-different siblings' targets was much lower than of either autologous or HLA-identical targets ($P < 0.001$ at each E:T ratio in both experiments). In haploidentical effector-target combinations lysis was less than in autologous or HLA-identical combinations but greater than in HLA-different combinations ($P < 0.005$ in each experiment). Lytic unit calculations indicate that on the average 25–50% of the activity on autologous cells is detected on HLA-haploidentical targets, and that <5% of the activity is detected on HLA-different siblings' targets (these conclusions are independent of whether the lytic unit calculations are made from the average data in Fig. 1 or made from the titrations of individual effector populations and subsequently averaged).

Antigen-specific HLA-linked Genetic Control of Self Specificity. Previous family studies have demonstrated that individuals may generate a stronger cytotoxic response to A/HK in conjunction with products of one HLA haplotype than in conjunction with the other haplotype, and that this preferential responsiveness is under HLA-linked genetic control (7). These studies investigated whether the cytotoxic response to B/HK was under similar HLA-linked control, and whether this control resulted in the recognition of different self specificities in conjunction with the two closely related viruses, A/HK and B/HK. The findings are illustrated by the data from two representative experiments with cells from four HLA-identical siblings in family B (Fig. 2). In each experiment, cells from each sibling were stimulated in vitro with A/HK and B/HK, and the effectors were assayed to determine how much of the cytotoxic activity for HLA antigens coded by the maternal haplotype (A31-B40) and how much was specific for HLA antigens coded by the paternal haplotype (A25-B15).

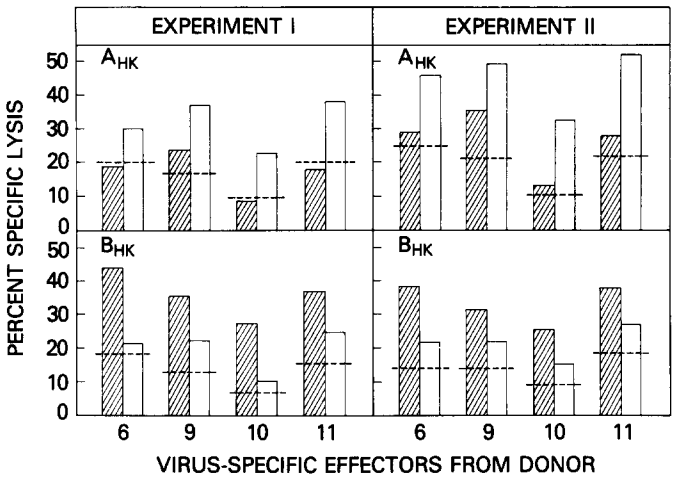

FIG. 2. Virus-specific HLA-linked genetic control of cytotoxic responses to A/HK and B/HK. PBL from four HLA-identical siblings of family B were sensitized against either A/HK (upper panels) or B/HK (lower panels) and assayed at three E:T ratios on panels of siblings' targets infected with virus of the immunizing strain. For each effector, the bars compare the mean lysis (at 40:1 E:T ratio) on targets from family members who share only HLA genes of the maternal HLA haplotype (HLA-A31-B40) (hatched bars) and the mean lysis on targets who share only HLA genes of the paternal HLA haplotype (HLA-A25-B15) (open bars). Also shown, as a base line, is the mean lysis on siblings' targets who are mismatched for both HLA haplotypes (dashed line).

The three notable features of the results are: (*a*) each of these donor's cells recognizes virus preferentially in conjunction with products of one HLA haplotype; (*b*) for responses to a given virus, all four HLA-identical siblings show the same haplotype preference; and (*c*) for each of these siblings, the haplotype preference in the response to A/HK is the opposite of that observed in the response to B/HK. Lytic unit calculations assist in more accurately estimating the magnitude of the haplotype preferences observed, and indicate that the differences are quite marked. For example, in the cytotoxic response to A/HK in experiment I, recognition of products of the paternal haplotype is ~4.7 times greater on the average than recognition of products of the maternal haplotype; conversely, in the cytotoxic response to B/HK, there is an ~2.3-fold difference in the opposite direction (mean haplotype preferences for the four siblings were significantly different in responses to the two viruses, $P < 0.002$).

The finding that HLA-identical siblings are similar to one another with respect to the haplotype preference in their cytotoxic responses to B/HK suggests that this preference is controlled by HLA-linked genes. This extends the previous observations that there is HLA-linked genetic control of the cytotoxic response to A/HK among members of this family (7). Moreover, the striking contrast between the haplotype preferences observed in the A/HK and B/HK responses demonstrates that there is antigen specificity in this HLA-linked genetic control, which can result in differences in the self specificity of cytotoxic responses to two closely related viruses.

Virus-Specific Differences in HLA-A2 Recognition among Unrelated Donors. Population studies also indicate that the repertoire of self determinants to which cytotoxic T cells respond can be influenced by the immunizing virus. Cells from unrelated HLA-A2-positive donors have been analyzed with respect to cytotoxic T-cell recognition of self HLA-A2 in conjunction with either A/HK or B/HK. As seen in a representative

TABLE I

Some Donor's Cytotoxic T Cells Recognize HLA-A2 in Conjunction with Influenza A/HK but not B/HK

Effector cell donor	Immunizing virus	Percent lysis of target cells infected with homologous virus*									Summary of mean percent lysis on targets that are:		
		UB17	S11	H7	UB16	G6	P5	S2	S8	M	HLA-A and -B matched	HLA-A2 matched	HLA-mismatched
UB17‡	B/HK	*21*	25	26	5	7	4	8	6	5	25	6	5
	A/HK	*24*	32	26	33	29	20	31	42	8	29	31	8
HLA antigens shared		Auto	A1,2 B7,8	A1,2 B7,8	A2	A2	A2	A2	A2	—	A1,2 B7,8	A2	—
S11	B/HK	27	*32*	33	4	3	1	3	5	3	30	3	3
	A/HK	23	*28*	29	28	21	16	26	33	9	26	25	9
HLA antigens shared		A1,2 B7,8	Auto	A1,2 B7,8	A2	A2	A2	A2	A2	—	A1,2 B7,8	A2	—

Figures denoting autologous lysis are italicized, and designated "auto."

* Indicates targets infected with virus strain identical to the immunizing strain.

‡ Results of HLA-serotyping on donors were as follows: UB16:-A2,3,-Bw35; UB17:-A1,2,-B7,8; G6:-A2,3,-B15; H7:-A1,2,-B7,8; M:-A3,-Bw35; P5:-A2,28,-Bw44; S2:-A2,-B5; S8:-A2,18,-Bw35; S11:-A1,2,-B7,8.

experiment (Table I), cells from two such donors failed to recognize HLA-A2 in conjunction with B/HK despite recognition of HLA-A2 in conjunction with A/HK. Thus, the results of population studies are consistent with those in the family studies that: (*a*) for each donor, some but not all of his HLA specificities may be recognized in conjunction with a particular immunizing antigen; and (*b*) each of a donor's HLA specificities may function in cytotoxic responses to some but not all foreign antigens.

Is the failure to respond to B/HK in conjunction with HLA-A2 a consistent finding with HLA-A2-positive donors? No, additional experiments (Table II) confirm that donors S11 and UB17 are low responders to B/HK plus HLA-A2, and clearly demonstrate that other HLA-A2-positive donors such as S2 and UB14 are relatively high responders to B/HK plus HLA-A2. Although cells of donors UB17 and S11 generate strong virus-specific cytotoxicity against autologous cells infected with either A/HK or B/HK, the A/HK response is characterized by considerable HLA-A2-restricted cytotoxic activity, whereas there is minimal HLA-A2-restricted cytotoxic activity generated in the response to B/HK. (Although in this experiment the overall response of these two donors to B/HK is somewhat less than that to A/HK, lytic unit calculations indicate that <1% of the overall B/HK response is HLA-A2 restricted compared with ~15% of the A/HK response.) It should be noted that although UB17 and S11 respond poorly to B/HK in conjunction with HLA-A2 they respond to B/HK in conjunction with HLA-B7 and -B8 as evidenced by lysis of targets sharing those antigens (in addition to HLA-A2). In contrast, cells from UB14 and S2 generate a strong HLA-A2-restricted response to both A/HK and B/HK. The differences between the high responders and the low responders to B/HK plus HLA-A2 cannot be a result of heterogeneity of the HLA-A2-associated target antigen or inadequate viral presentation on the targets cells of the low responders because effectors from the high responders kill the virus-infected targets from the low responder donors. Thus, the ability to evoke a cytotoxic T-cell response to B/HK in conjunction with HLA-A2 is not determined solely by the serologically defined antigens present on the HLA-A2 molecule.

TABLE II

HLA-A2-Positive Donors Differ in Their Cytotoxic Responses to B/HK in Conjunction with HLA-A2

Effector cell donor	Immunizing virus	E:T Ratio	Percent lysis of target cells from indicated donor infected with:									
			Homologous* virus								Heterologous‡ virus	
			UB14	S2	UB17	S11	H7	FC4	W5	UB18	FC4	S11
UB14§	B/HK	40	*30*	27	26	27	25	27	31	6		
		10	*21*	19	18	19	16	19	23	2	10	8
	A/HK	40	*45*	62	40	47	51	29	59	5		
		10	*38*	49	33	35	38	21	40	3	0	1
HLA antigens shared			Auto	A2	A2	A2	A2	A2	A2	—	A2	A2
S2	B/HK	40	24	*38*	28	31	23	27	29	6		
		10	15	*27*	17	19	12	17	19	0	4	2
	A/HK	40	32	*56*	40	44	43	26	53	7		
		10	16	*40*	22	29	29	16	34	1	0	0
HLA antigens shared			A2	Auto	A2	A2	A2	A2	A2	—	A2	A2
UB17	B/HK	40	5	8	*22*	24	21	18	13	20		
		10	2	4	*15*	14	12	11	7	11	4	0
	A/HK	40	24	38	*40*	50	51	20	49	26		
		10	9	16	*30*	36	36	10	30	14	−2	−1
						A1,2	A1,2	A2	A2	A1	A2	A1,2
HLA antigens shared			A2	A2	Auto	B7,8	B7,8	B7	B8	B7	B7	B7,8
S11	B/HK	40	6	7	30	*33*	31	17	22	30		
		10	3	5	19	*21*	19	8	13	16	2	*0*
	A/HK	40	20	33	38	*48*	46	18	44	26		
		10	10	14	29	*33*	37	12	28	14	1	*2*
					A1,2		A1,2	A2	A2	A1	A2	
HLA antigens shared			A2	A2	B7,8	Auto	B7,8	B7	B8	B7	B7	Auto
UB18	B/HK	10	3	4	29	31	30	28	3	*35*	4	1
	A/HK	10	2	6	51	56	61	23	0	*48*	−1	0
					A1	A1	A1					A1
HLA antigens shared			—	—	B7	B7	B7	B7	—	Auto	B7	B7

Figures denoting autologous lysis are italicized, and designated "auto."

* Indicates targets infected with virus strain identical to immunizing strain.

‡ Indicates B/HK-infected targets for A/HK-immune effectors and vice versa.

§ Results of HLA-serotyping of donors was as in Table I and also: UB14:-A2,-B15,w44; UB18:-A1,-B7; FC4:-A2,-B7; W5:A2,-B8.

Discussion

The foregoing studies were designed to investigate genetically controlled similarities and differences in the cytotoxic responses to two closely related viruses: A/HK and B/HK. The results indicate that the in vitro human cytotoxic response to B/HK, like the response to A/HK (5–8), is HLA restricted. Investigation of the genetic regulation of these HLA-restricted responses indicates that: (*a*) HLA-linked genes can determine which of the repertoire of HLA-coded self antigens will be recognized in conjunction with particular viral determinants; (*b*) this HLA-linked regulation is specific for the immunizing virus; and (*c*) this genetic control in some situations may be a result of a regulatory gene distinct from the HLA-linked gene that codes for the target antigen.

These findings that HLA-linked regulation of the cytotoxic response differs even between responses to two closely related viruses (i.e., is antigen specific) has important implications not only related to possible mechanisms of regulation of T-cell responses but also related to the evolution of HLA polymorphism. That cytotoxic T-cell responses can be beneficial in recovery from viral infections has been suggested by murine studies of recovery from infections with influenza virus (10, 11). Consequently,

individuals with stronger cytotoxic respones to a particular viral pathogen may have a higher probability of surviving an infection by that pathogen. Thus, any viral pathogen that has produced significant mortality during evolution may have generated a selective pressure for preservation of host genes that confer higher cytotoxic responses against that pathogen. Independent of whether influenza virus itself has exerted a strong selective pressure in human evolution, the genetic mechanisms involved in cytotoxic responses to influenza viruses provide a model that may be applicable to human cytotoxic responses to a variety of pathogens.

A model to account for the selective advantage of HLA-A and -B polymorphism can be developed from the following three considerations. First, available data strongly suggest that products of the HLA-A and -B loci are major self components involved in human cytotoxic T-cell recognition of foreign antigens. Previous studies (5–8) have demonstrated that the self antigens recognized in conjunction with influenza virus are not only coded by HLA-linked genes, but are very highly associated with HLA-A and -B specificities. The results in studies of other human cytotoxic responses (3, 4) are consistent with recognition of foreign antigens in conjunction with HLA-A and -B. Second, human cytotoxic responses are often characterized by preferential recognition of the immunogen in conjunction with some, but not others, of the individual's HLA-A and -B antigens (4, 6–8). For example, this study demonstrates that the HLA-identical siblings tested responded to B/HK in conjunction with products of the HLA-A31-B40 haplotype better than in conjunction with products of the HLA-A25-B15 haplotype; similarly other donors (UB17 and S11) (Table II) reacted to B/HK in conjunction with some of their HLA antigens but not in conjunction with HLA-A2. Third, the preferential recognition of HLA target antigens is specific for the immunizing virus, as shown in this study. The preferred HLA target antigens in a donor's cytotoxic response to B/HK (e.g., products of the HLA-A31-B40 haplotype for siblings 6, 9, 10, 11) may be different from the preferred HLA target antigens in that donor's response to A/HK (e.g., products of the HLA-A25-B15 haplotype for the siblings).

These three observations suggest there would be a selective advantage for any individual who had more than one specificity of HLA target molecule, because the virus-specific strong cytotoxic responses facilitated by each of his HLA antigens would compensate for some of the virus-specific deficiencies characteristic of his other HLA antigens. The finding described in this study that there may be regulatory genes distinct from HLA-A and -B (e.g., regulatory genes accounting for differences between donors in recognition of HLA-A2 plus B/HK) does not invalidate this proposed explanation of HLA polymorphism; many possible mechanisms of regulatory gene function would confer selective advantage of HLA-A and -B heterozygous individuals. The simplest example would be a regulatory mechanism that operates by determining the T-cell repertoire; if so, there would be genetically determined holes in the T-cell repertoire that would result in the failure of T cells to recognize a particular foreign antigen in conjunction with a given self specificity. Because donors have different regulatory genes, there will be differences between donors in the specific defects in their T-cell repertoires (e.g., presence or absence of T cells that recognize HLA-A2 plus B/HK). Regardless of which specific defects there are in an individual's T-cell repertoire, HLA heterozygosity would increase the number of possible contexts in which any particular virus would be presented, and thereby decrease the likelihood

that there would be a total absence of cytotoxic T cells capable of responding to that virus.

Family studies have demonstrated that the magnitude of HLA-restricted cytotoxic responses to influenza viruses are genetically controlled, principally by genes linked to HLA (7) (although preliminary results suggest that in some instances non-HLA genes may contribute to regulation [W. E. Biddison and S. Shaw. Unpublished observations.]). Where within the HLA complex do these regulatory genes map and by what mechanisms do they operate? To simplify subsequent discussion of these questions, the hypotheses will be formulated in terms of the recognition of a particular HLA antigen (i.e., HLA-A2) in conjunction with a specific immunizing virus (i.e., B/HK). Three general hypotheses will be considered for the location of the regulatory gene(s) within the HLA complex, and possible mechanisms of regulation will be proposed for each.

First, recognition of HLA-A2 plus B/HK could be regulated by the structural gene that codes for HLA-A2. For example, if T-cell responses require a cell surface molecular complex of HLA-A2 and B/HK (altered self) (1, 2, 16), then the structural gene coding for HLA-A2 will determine how well it will form such an immunogenic complex. Alternatively, if the generation of a donor's HLA-A2-restricted cytotoxic T-cell repertoire is governed by recognition of self HLA-A2 during differentiation in the thymus (17, 18), then the HLA-A2 gene acts to regulate the responses by shaping the T-cell repertoire. Although there are no data to refute this hypothesis that the structural gene is also regulatory, there are data that necessitate postulating either an alternative or an additional location for a regulatory gene. Specifically, this hypothesis (that the HLA-A2 structural gene regulates recognition of HLA-A2 plus B/HK) cannot readily explain why donors who share HLA-A2 can differ in their B/HK-immune T-cell responses to HLA-A2 (unless there are differences between donors' HLA-A2 genes that cannot yet be detected serologically or by target cell function in cytotoxic T-cell recognition).

The second possible location for HLA-linked regulatory genes governing recognition of HLA-A2 plus B/HK would be the other HLA-A and -B genes expressed by the donor, in analogy to the *H-2K*-region control of *H-2D*-restricted cytotoxic T-cell responses in the mouse (19–21). For example, if the generation of a donor's HLA-A2-restricted T-cell repertoire is affected by the presence of other HLA-A and -B gene products during thymic differentiation, then those other HLA-A and -B gene products would contribute to regulation of T-cell recognition of HLA-A2 plus B/HK. Alternatively, the response to HLA-A2 plus B/HK might be modulated by a population of regulatory cells specific for B/HK in conjunction another HLA-A or -B antigen; for example, the response to HLA-A2 plus B/HK might be suppressed in a HLA-B7-positive donor if he had a high frequency of cytotoxic precursors specific for HLA-B7 plus B/HK that rapidly eliminated the virus-infected stimulating cells. This second hypothesis (that regulation can be a result of other HLA-A and -B gene products) is consistent with the population studies reported in this and a previous study (8). However, preliminary studies indicate there are differences in selective recognition among unrelated donors matched for all four HLA-A and -B antigens (W. E. Biddison and S. Shaw. Unpublished observations.); this finding does not rule out either of the first two locations considered for regulatory genes, but necessitates postulating either an alternative or additional location for a regulatory gene.

The third hypothesis is that the HLA-linked regulatory genes are distinct from

HLA-A and -B and code for products that are not recognized as target antigens by virus-immune cytotoxic T cells. In analogy to murine studies of *I*-region control of cytotoxic responses to the male H-Y antigen (22, 23), these regulatory genes may map to the HLA-D region and may act by controlling helper and/or suppressor cell functions. None of the available data excludes this possibility.

Proposed mechanisms for the HLA-linked genetic regulation must account for the fact that regulation is specific both for the self HLA determinant and for the immunizing virus. Because donors who fail to recognize B/HK in conjunction with HLA-A2 do recognize A/HK in conjunction with HLA-A2, it is difficult to argue that the defect in B/HK recognition results from inadequate expression of the HLA-A2 on the stimulator cell. Similarly, the defect in recognition of HLA-A2 plus B/HK cannot reflect a total absence of cytotoxic cells specific for HLA-A2; instead discrete subpopulations of cytotoxic lymphocyte precursors specific for both HLA-A2 and B/HK might be absent (or greatly reduced in frequency) in the T-cell repertoire. In any case, our observations do not distinguish between a one-receptor and a two-receptor model for the recognition of influenza virus in association with HLA-coded self products.

HLA-linked regulation of self-restricted cytotoxic T-cell recognition may be rather complex, and may reflect concurrent operation of several of the proposed regulatory mechanisms. However, the clear evidence that the regulation is specific for both the immunizing virus and for the HLA determinants may account for the selective advantage of HLA heterozygosity.

Summary

The present study compares human cytotoxic T-cell responses to two closely related viruses (type A and type B influenza) to understand the antigen-specific elements involved in HLA-linked genetic control of cytotoxic T-cell responses. The HLA antigens function as self antigens that are recognized by cytotoxic T cells sensitized against either virus. However, studies in an informative family indicate that in this family, the HLA antigens preferentially recognized in conjunction with type A influenza (A/HK) differ from the HLA antigens preferentially recognized in conjunction with type B influenza (B/HK). Similarly, population studies demonstrate that some (but not all) donors whose T cells recognized A/HK in conjunction with HLA-A2 failed to recognize B/HK in conjunction with HLA-A2. Thus, HLA-linked regulation must operate by a mechanism(s) that is specific both for the self HLA antigen and the viral antigen. Furthermore, these findings indicate that different HLA antigens may facilitate T-cell responses to different pathogens, which would result in an evolutionary advantage for HLA heterozygosity.

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