HLA-LINKED GENETIC CONTROL OF THE SPECIFICITY OF HUMAN CYTOTOXIC T-CELL RESPONSES TO INFLUENZA VIRUS

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The major histocompatibility complex (MHC)[1] has been shown to exercise two kinds of control over the cytotoxic response of murine T cells to foreign antigens. First, cytotoxic T cells recognize foreign antigens such as viruses, minor histocompatibility antigens, and trinitrophenol (TNP) in association with *H-2K* and *H-2D* region gene products (1, 2). Second, MHC genes can also affect the magnitude of the cytotoxic response to specific foreign antigens in association with selected H-2K or H-2D antigens (3–6), an effect which may be analogous to the Ir gene control of antibody responses (7). Recent studies of the human cytotoxic response to the male Y antigen (8), dinitrophenol (9), and influenza virus (10) demonstrated that human T cells also recognize these antigens in conjunction with determinants that are associated with HLA-A and -B. Furthermore, in each of these human studies there was a suggestion of selective recognition of MHC gene products, because T cells did not appear to recognize these antigens in association with all the HLA-A and -B specificities that were analyzed.

To better define the genetic controls of human T-cell cytotoxic responses to foreign antigens, we have investigated responses to influenza virus among members of a large family. Analysis of their cytotoxic effectors induced in vitro by exposure to influenza virus demonstrates that: (*a*) effector cells recognize HLA-A and -B-linked gene products in association with influenza virus; (*b*) many of these family members' T cells respond preferentially to influenza in association with gene products of only one of their HLA haplotypes; (*c*) HLA-identical siblings have the same pattern of preferential response, indicating control by HLA-linked genes.

Materials and Methods

Human Blood Reagents. Peripheral blood mononuclear leukocytes (PBL) and plasma were collected by batch leukapheresis from healthy adult volunteers on no medications and separated by flotation on Ficoll-Hypaque as previously described (11). Plasma from five such male donors was pooled, frozen in portions at −20°C, and used as the normal human plasma pool. Fresh PBL were cryopreserved and thawed when desired by using protocols previously described by Holden et al. (12). HLA-A, -B, and -C locus serotyping of members of a large family was kindly performed by Dr. A. H. Johnson, Duke University, Durham, N. C. (Fig. 1).

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[1] Abbreviations used in this paper: E:T, effector to target; MHC, major histocompatibility complex; PBL, peripheral blood leukocytes; PHA, phytohemagglutinin; TNP, trinitrophenol.

Viruses. An influenza type A virus, A/HK [A/Hong Kong/8/68-X-31(H3N2)], and an influenza type B virus B/HK (B/Hong Kong/8/73) were grown in embryonated eggs (13), aliquotted, and stored at −70°C. Hemagglutination titers were measured as described (13).

Immunizations. Fresh PBL or thawed cryopreserved PBL (at a concentration of 4×10^6/ml) were exposed to influenza virus (2-20 hemagglutination units/ml) in Hank's balanced salt solution for 1 h at 37°C. The cells were then pelleted, resuspended to 1.5×10^6/ml in RPMI-1640 with glutamine (Grand Island Biological Co., Grand Island, N.Y.) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 5% vol/vol pooled normal human plasma (culture media) and incubated in upright plastic flasks (12×10^6 cells/flask, Falcon 3013, Oxnard, Calif.) for 7 d at 37°C in a humidified atmosphere of 5% CO_2-95% air. Effectors were harvested and assayed immediately or cryopreserved (12). Previous experiments have demonstrated that the specificity of the cytotoxic response is unaffected by cryopreserving the PBL before induction of the in vitro cytotoxic response or by cryopreserving the effectors and assaying immediately after thawing.

Target Cells and Cytotoxicity Assay. Cryopreserved PBL were thawed, resuspended in culture media, and incubated for 3 d in 3013 flasks (8×10^6 cells/flask) in the presence of phytohemagglutinin (PHA M form, Grand Island Biological Co., final concentration 1:100 of stock). Virus-infected ^{51}Cr-labeled target cells were prepared by exposure of PHA-stimulated cells to 100–200 hemagglutination units of influenza virus and 200 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, Mass.) at 37°C for 90 min in 1 ml of assay medium (RPMI-1640 with glutamine supplemented with penicillin, streptomycin, glutamine, [300 μg/ml], nonessential amino acids 1% vol/vol [Microbiological Associates, Walkersville, Md.], and 10% vol/vol heat-inactivated fetal bovine serum). The cells were then washed, resuspended in assay medium at 1×10^6/ml, cultured for 4 h at 37°C, washed, and used as target cells.

Fresh or cryopreserved effector cells and ^{51}Cr-labeled target cells (10^4/well) were incubated in round bottom microtiter plates (Linbro Scientific, Inc., Hamden, Conn.) at 37°C for 6 h in a standard ^{51}Cr-release assay (11).

Data Analysis. Data from cytotoxicity assays are expressed as mean percent-specific lysis of triplicate determinations (11). Spontaneous release of ^{51}Cr from all target cells during the 6-h assay was ≤25% of detergent-releasable counts. The activity of each effector was measured on each target at three effector to target cell(E:T) ratios. To analyze the role of HLA-linked determinants in virus recognition, the effector-target combinations were subdivided into four groups with respect to the genetic relationship between the effectors and the siblings' targets: (*a*) autologous; (*b*) HLA-identical; (*c*) HLA-haploidentical, and (*d*) HLA-different. The mean lysis (and standard error of the mean) was calculated at each E:T ratio for each group and the statistical significance of differences was tested by the two-tailed Student's *t* test.

Based on the matrix of lytic data for all combinations of siblings' effectors and targets, it is possible to deduce the pattern of inheritance of determinants which are recognized in association with influenza. If the cytotoxic effectors recognize virus in association with determinants coded by genes of a single region, then each sibling could have one of four possible genotypes, and there would be 4^8 possible combinations for inheritance of these four genotypes by eight siblings. With the aid of a computer, each of these possible combinations was tested to determine which best explained the matrix of lytic data. Specifically, for each possible combination, mean lysis was calculated for the groups of all effector-target combinations which were (*a*) identical with respect to this region or (*b*) different with respect to this region. The combinations selected as correct were the ones which were associated with the maximum cross-reactivity between siblings who shared this region and the minimum cross-reactivity between siblings who were different with respect to this region. The 8/65,536 combinations which were equally correct corresponded to rotations and mirror images of one unique configuration (when the genotypes are represented as quadrants, as for HLA genotypes in Fig. 1).

Results

Virus Specificity. As previously shown in detail,[2] the human in vitro cytotoxic T-cell response to influenza-infected autologous cells is dependent upon exposure to

[2] Biddison, W. E., and S. Shaw. 1979. Virus specificity of human influenza virus-immune cytotoxic T cells. Manuscript submitted for publication.

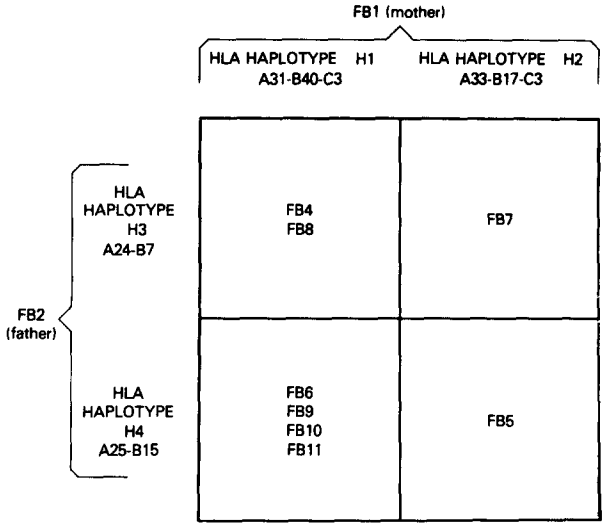

FIG. 1. HLA-haplotype assignments in the family B. By HLA-A, -B, and -C serotyping, the eight siblings were assigned to one of four quadrants which represent the four possible inheritance patterns of parental HLA-haplotypes.

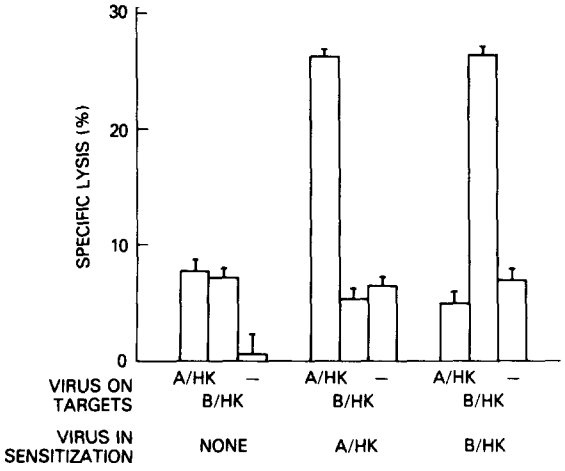

FIG. 2. Virus specificity of human influenza virus-immune cytotoxic T cells. PBL from a donor were sensitized to A/HK, or B/HK or cultured in the absence of virus, and each population of effectors was assayed at an E:T ratio of 3:1 on ^{51}Cr-labeled autologous target cells which were uninfected, or infected with A/HK or B/HK.

virus in the sensitization culture and upon infection of the autologous target leukocytes (Fig. 2). Furthermore, there is reciprocal exclusion of cytotoxic activity generated against type A (A/HK) and against type B (B/HK) influenza viruses, indicating at least type specificity for the immunizing virus.

Recognition of HLA-Linked Determinants in Association with Virus. To identify the self determinants which are recognized in association with influenza, the cross-reactivity of influenza-immune cytotoxic activity was evaluated among members of a large family. The cytotoxic activity of virus-immune effectors on siblings' virus-infected targets correlated with the number of HLA haplotypes shared between siblings'

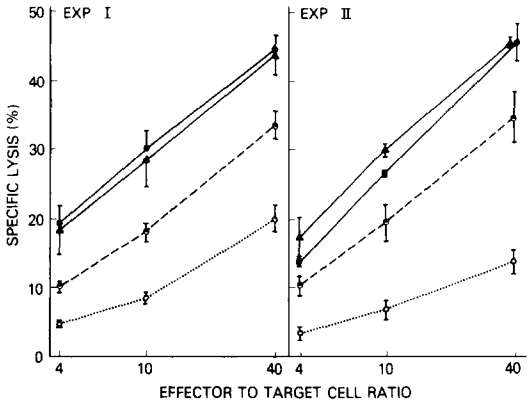

FIG. 3. HLA-restriction of influenza virus-immune cytotoxicity. In exp. I, PBL from the eight siblings of family B were sensitized in vitro against A/HK and effectors from each were assayed on A/HK-infected target cells from the eight siblings at three E:T ratios. Mean lysis was calculated at each E:T ratio for the eight combinations of effectors on infected autologous cells (▲), the 14 combinations of effectors on HLA-identical targets (●), the 30 combinations of effectors on HLA-haploidentical targets (◓), and the 12 combinations of effectors on HLA-different targets (○). Vertical bars represent the standard error or the mean. In exp. II, effectors from siblings FB5, FB7, and FB11 were assayed on targets from siblings FB5, FB6, FB7, FB8, FB9, and FB11 and similar calculations were made of mean lysis on autologous, HLA-identical, HLA-haploidentical, and HLA-different siblings' targets.

effector cells and target cells, as shown in the summaries of two representative experiments (Fig. 3). Mean lysis of HLA-identical target cells was indistinguishable from that of autologous cells. Mean lysis of HLA-different siblings' target cells was much less than that on either autologous or HLA-identical targets ($P < 0.003$ for each comparison at each E:T ratio in both experiments). Semiquantitative analysis suggests that less than 10% of the activity of these effectors was directed at HLA-independent determinants, since the activity of effectors on autologous or HLA-identical targets at a 4:1 ratio was equivalent to or greater than the activity on HLA-different siblings' cells at a 40:1 E:T ratio. There was a small amount of cytotoxic activity of virus-immune effectors on targets from genetically HLA-different siblings, which could have resulted from recognition of virus in association with the HLA-C3 antigen that they all share. Mean cytotoxicity on HLA-haploidentical targets (i.e. target cells which share only one HLA haplotype with the effector cells) was less than that on autologous or HLA-identical targets ($P < 0.03$ and $P < 0.003$, respectively, in the larger experiment, exp. I), but greater than that on HLA-different targets ($P < 0.007$ in each experiment).

Although the results of the foregoing analysis are consistent with HLA-linkage of the determinants recognized in association with virus, formal proof requires identification of the inheritance of these determinants within the family. This can be deduced by inspection of, or more rigorously by computerized analysis of, the matrix of lytic data of all siblings' effectors on all siblings' targets. Analysis of the data in exp. I demonstrates that the cross-reactivity among siblings is best explained by a pattern of inheritance which is precisely concordant with the known inheritance of HLA-A and -B. This establishes with a high degree of certainty ($P < 0.0002$) that the determinants in this family which are recognized in association with virus are coded by HLA-linked genes.

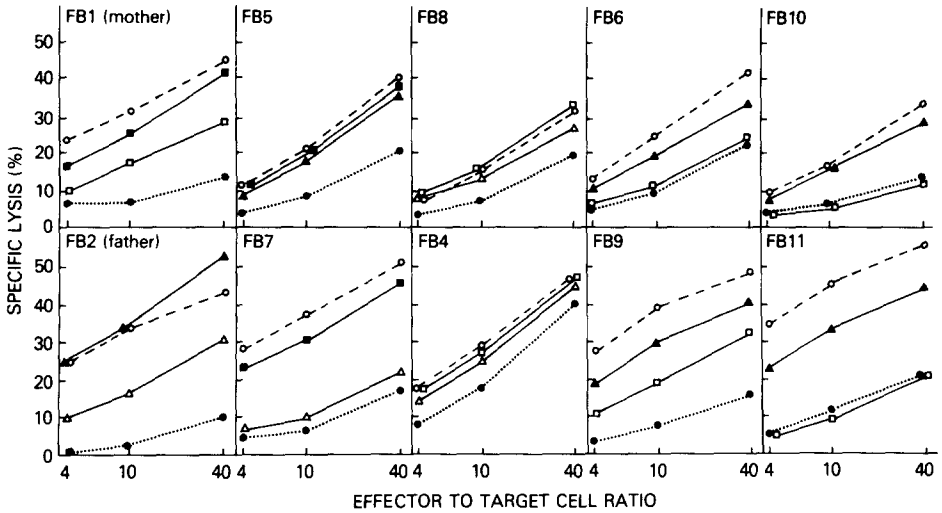

FIG. 4. Differential responsiveness to HLA-linked determinants coded by different haplotypes. PBL from each of the members of family B were sensitized against A/HK and assayed on infected target cells from each member of the family at three E:T ratios. Data shown in each panel is the mean lysis of effectors from one donor on several categories of targets. To determine the relative activity directed against gene products of each HLA haplotype, mean lysis is shown at each E:T ratio for the group of targets which share one HLA haplotype and for the group which share the other HLA haplotype. The HLA haplotype shared in these haploidentical combinations is indicated by the following symbols: H1 (□), H2 (■), H3 (△), or H4 (▲). Each panel also shows the lysis mediated by the same effectors on autologous targets (○), and mean lysis on HLA-different targets (●). For clarity, error bars have been omitted, but the statistical significance of differences are as follows. Effectors from donor FB2 preferentially lysed HLA-haploidentical targets with which they shared H4, relative to those with which they shared H3 ($P < 0.002$ at each E:T ratio). Similarly, effectors from donor FB7 lysed H2-matched targets better than H3-matched targets ($P < 0.004$ at each E:T ratio). Effectors from donors FB6, FB9, FB10, and FB11 all preferentially lysed targets which shared the H4 haplotype relative to those which shared the H1 haplotype ($P < 0.02$ in at least two of three E:T ratios). Effectors from donors FB1, FB5, FB4, and FB8 did not show statistically significant preferential recognition at two or more E:T ratios.

Inherited Patterns of Recognition of HLA-Linked Determinants. Cytotoxic activity of effectors from each family member was analyzed to determine the relative contribution of HLA-linked determinants coded by each haplotype for virus-immune T-cell recognition (Fig. 4). As observed in the foregoing analysis of average cytotoxicity, the greatest cytotoxicity was generally evident on autologous and HLA-identical targets, and the least cytotoxicity on HLA-different siblings' targets. Cytotoxicity on HLA-different siblings' cells was evident with all effectors, but was consistently most pronounced with effectors from donors FB4 and FB8. This cross-reactivity might be due to subpopulations of their effectors which recognize determinants coded by genes not linked to HLA or determinants that are shared by all siblings (but with differences between siblings in their responses to such shared determinants).

The lysis of HLA-haploidentical targets generally ranged between lysis of autologous targets and of HLA-different targets. If all HLA haplotypes coded for determinants which could be equally well recognized in association with influenza, then one might expect that virus-immune effector T cells would show equivalent lysis of targets which were haploidentical with respect to either HLA haplotype. This was observed with effectors from some donors, such as FB5, whose effectors lysed equivalently

TABLE I

Patterns of Recognition of HLA-Linked Determinants by Influenza-Immune Cytotoxic T Cells

Effector cell donor	Virus immune*	E:T	Percent specific lysis of HK-infected target cells from donors:							
			FB1	FB2	FB5	FB7	FB11	FB6	FB9	FB8
Exp. I										
FB5	−	40:1	−6.3‡	−2.5	−2.6	−1.9	−1.9	−2.6	0.0	−1.2
	+	40:1	43.2	29.3	44.0	50.1	47.4	44.8	41.5	16.4
	+	10:1	30.8	16.8	29.0	32.9	25.5	26.6	26.3	10.9
HLA haplotype shared:			H2	H4	H2, 4	H2	H4	H4	H4	—
FB7	+	40:1	44.7	11.2	37.7	48.3	7.0	4.9	6.8	18.0
	+	10:1	23.1	2.3	18.1	25.8	2.6	−1.2	1.2	4.5
HLA haplotype shared:			H2	H3	H2	H2,3	—	—	—	H3
FB11	−	40:1	−4.3	0.8	3.8	3.4	0.9	1.1	3.6	0.9
	+	40:1	6.1	23.7	33.8	10.5	46.7	43.0	48.6	15.0
	+	10:1	1.0	15.3	18.9	4.9	25.9	26.2	26.8	7.3
HLA haplotype shared:			H1	H4	H4	—	H1,4	H1,4	H1,4	H1
Exp. II										
FB5	−	40:1	−3.0	−6.7	−4.0	−2.6	−1.9	−0.2	−0.5	−1.0
	+	40:1	33.7	21.7	43.3	45.0	40.5	36.2	33.0	8.0
	+	10:1	21.8	11.9	24.3	28.5	20.8	19.1	19.4	7.0
FB7	−	40:1	−1.3	−3.4	−0.2	0.2	2.1	2.2	3.4	2.4
	+	40:1	36.2	−4.2	37.7	46.9	4.3	4.4	3.1	11.1
	+	10:1	19.3	−4.8	22.0	27.8	−1.8	0.5	−0.9	2.2
FB11	−	40:1	1.0	−4.4	−0.3	1.5	−1.0	0.7	0.5	1.4
	+	40:1	6.3	16.7	31.5	11.0	32.9	30.2	25.0	8.1
	+	10:1	1.9	6.2	14.4	1.8	15.0	12.2	12.7	4.2

* Donor PBL were either exposed (+) or not exposed (−) to influenza virus before culturing.

‡ Numbers are mean percent specific lysis of triplicate values. Mean standard error of the mean was 0.9% (range 0.4–4.5%) in exp. I and 1.1% (0.5–4.4%) in exp. II. Underlined values represent cytolysis of autologous cells.

targets that shared either the maternal H2 or the paternal H4 haplotypes. However, effectors from many family members preferentially lysed target cells with which they shared one or the other HLA haplotype. For example, effectors from donor FB7 preferentially lysed targets which shared the maternal H2 haplotype, and effectors from donor FB11 preferentially lysed targets which shared the paternal H4 haplotype. The pattern of cytotoxicity of effectors from individual donors was reproducible in independent experiments, as illustrated by two other comparable studies of donors FB5, FB7, and FB11 (compare Fig. 4 and Table I).

Although the magnitude of the cytotoxic responses varied among HLA-identical siblings, their patterns of virus-immune cytotoxicity were reproducibly similar with respect to haplotype preferences (Fig. 4). Specifically, HLA-identical donors FB4 and FB8 both lysed equally targets with which they shared either the H1 or the H4 haplotype. Similarly, HLA-identical donors FB6, FB9, FB10, and FB11 all preferen-

tially lysed HLA-haploidentical targets with which they shared the H4 haplotype to those with which they shared H1. These data are consistent with control of preferential responsiveness by genes linked to HLA.

Discussion

The present study investigates genetic controls of the in vitro response of human T cells to influenza virus-infected autologous cells. Analysis of virus-immune cytotoxic effectors from siblings in a large family has provided the first formal demonstration of the genetic linkage between genes coding for HLA-A and -B antigens and genes coding for the major determinants recognized in association with virus. Similar studies of a second family of seven siblings have confirmed these conclusions (data not shown). Thus, the HLA region controls the T-cell response to influenza in at least one way, because HLA-linked structural genes code for the determinants which are recognized in association with influenza. These findings are analogous to those in many murine systems in which cytotoxic T cells recognize foreign antigens in association with *H-2K* and/or *H-2D* region products (1, 2), and are consistent with the results of human population studies in which cytotoxic responses to the male Y antigen (8), dinitrophenol (9), and influenza (10) have been shown to be restricted by determinants which are associated with HLA-A or -B. The data available at present do not prove that the structural genes that code for the determinants that are recognized in conjunction with virus are identical to the HLA-A and -B loci genes, but only that they are linked in family studies and associated in population studies.

The second feature of genetic control demonstrated in the present study is that many donors generate a cytotoxic response in which there is a preferential response to virus in association with determinants coded by only one HLA haplotype. Furthermore, effectors from HLA-identical siblings have the same patterns of preferential responsiveness, which suggests that the patterns are controlled by genetic rather than by environmental factors. This remarkable similarity in specificity of the responses of HLA-identical siblings, despite their genetic differences at regions not linked to HLA, suggests that the primary genetic control of this preferential responsiveness is mediated by HLA-linked genes.

HLA-linked control of the magnitude of T-cell responses to influenza in association with a given self determinant could be explained in either of two ways: either the structural gene which codes for the determinant also influences the magnitude of the cytotoxic response to that determinant, or there are other HLA-linked genes which regulate the magnitude of that response. With respect to the first alternative, there are a variety of mechanisms by which a structural gene could influence the strength of the T-cell response to influenza in association with its gene product. First, it is possible that T-cell recognition requires a physical association between virus and HLA antigens on the cell surface, so that differences in the ability of the HLA antigens to form altered self complexes with viral antigen (14) on the stimulator cells or target cells would alter the amount of cytotoxicity observed. Alternatively, there may be differences in the size of the cytotoxic T-cell repertoires which recognize different HLA determinants in association with influenza. Thus, preferential responsiveness could be controlled by the structural genes which code for the determinants that are recognized in association with influenza, and that control could be mediated at the level of the responder cell, stimulator cell or target cell.

The second possibility as to the site of controlling genes is that preferential responsiveness is controlled by HLA-linked genes which are distinct from the structural genes that code for the HLA determinants which are recognized in association with virus. If this were the case, it might be possible to demonstrate differences in the specificity of the responses of donors who share HLA-A and -B-linked recognition structures but who differ with respect to other HLA-linked genes. However, two different approaches used in the present study have failed to demonstrate that there are HLA-linked regulatory genes which are distinct from the structural genes whose gene products are recognized in association with virus. One general approach was to test for regulation of the response to genes on one HLA haplotype by genes carried on the other haplotype (i.e. *trans*-HLA control), by comparing responses of family members who shared a given HLA haplotype but who differed with respect to their second HLA haplotype. Valid comparisons must take into account the fact that, although these donors have been exposed to influenza frequently, their exposure histories can not be known precisely. Consequently, we have not compared donors with respect to the absolute magnitude of their responses to influenza in association with antigens of a given haplotype, since such differences may not reflect genetically controlled differences in recognition of these antigens, but rather differences in exposure history (e.g. how recently the donor has been challenged with influenza). Instead, for each donor, the relative responses to antigens of each haplotype (i.e. haplotype preferences) were evaluated. Analysis of the haplotype preferences of all family members suggests that they can all be explained by a consistent hierarchy of their HLA haplotypes with respect to the strength of the T-cell responses which are generated against influenza in association with the gene products of these haplotypes; the hierarchy in this family is H2 $\cong$ H4 > H3 $\cong$ H1. Because there are no family members whose T-cell responses deviate dramatically from this hierarchy, this approach provides no evidence for *trans*-HLA control in the present family or in another family which has been studied.

A second approach to identify HLA-linked regulatory genes distinct from the HLA-linked structural genes would be to compare the specificity of effectors from siblings who are HLA-A and -B region-identical, but who differ with respect to other MHC-linked determinants as a result of recombination. In this context, donor FB11 could have been informative, since the results of intrafamilial mixed lymphocyte culture and primed lymphocyte typing suggest that he is a B/D recombinant (S. Shaw and W. E. Biddison, unpublished observations). However, the pattern of recognition of this donor's effectors is similar to that observed with effectors from his HLA-A and -B-identical siblings (FB6, FB9, FB10), and thus provides no evidence for HLA-D-linked control of recognition of HLA-A and -B-linked determinants.

Thus, the family studies do not demonstrate any HLA-linked regulatory genes which are distinct from the structural genes whose products are recognized in association with virus. However, preliminary studies of unrelated donors suggest that such regulatory genes may exist. Donor FB7 consistently does not generate a strong cytotoxic response to influenza in association with HLA-B7, despite a strong response to influenza in association with other determinants. In contrast, other donors' T cells generate strong cytotoxic activity against influenza in association with HLA-B7 (W. E. Biddison and S. Shaw, unpublished observations). Such effectors lyse virus-infected target cells from FB7 and other family members with whom they share only HLA-B7. The existence of such donor-dependent differences in the magnitude of cytotoxicity

specific for HLA-B7 in association with influenza suggests that the magnitude of these responses may be controlled by genes distinct from those which code for the HLA-B7 antigen.

The findings in this study of human cytotoxic responses to influenza are strikingly similar to the findings in murine studies, that cytotoxic T-cell responses to certain foreign antigens are only observed in association with specific *H-2K* and/or *H-2D* region products. For example, H-2K^b antigens are not recognized in association with influenza by T cells from any mouse strain that has been tested (5). Furthermore, in selected situations, control of cytotoxic T-cell responses to an *H-2K* or *H-2D* region product has been mapped to genes which are located outside of that *H-2K* or *H-2D* region. For example, murine influenza-immune cytotoxic T-cell responses to virus in association with H-2D^b are apparently subject to Ir gene control by *K* and/or *I-A* region genes (5). Similar observations have been made for the murine T-cell response to TNP (3), H-Y (4), SV 40 virus (15), vaccinia virus (6), and Sendai virus (6).

The patterns of selective recognition of HLA-linked determinants by cytotoxic T cells appear to be dependent on which foreign antigen is used for immunization. Investigations of the same family members with respect to their cytotoxic T-cell responses to TNP-modified autologous cells revealed that a component of the cytotoxic activity was specific for TNP in association with HLA-linked determinants (16). However, the patterns of haplotype preference for the TNP responses of certain siblings differed from those observed in influenza responses, indicating an element of antigen-specificity. Antigen-specific selective recognition of MHC-linked determinants by cytotoxic T cells could readily account for the extensive polymorphism of these determinants in the following manner (17). Possession of certain alleles of MHC loci would have survival value because their gene products permit a strong cytotoxic response in association with some foreign antigens, while possession of other alleles would have similar advantages with respect to other antigens. An individual's chance of survival would be enhanced by heterozygosity at the MHC loci, which would increase the probability that his MHC determinants would include one which was immunogenic in association with any particular pathogen.

Our findings in family studies and population studies of human virus-immune T-cell responses are consistent with the following model for differential responsiveness. Cytotoxic T cells recognize influenza virus in association with self determinants coded by the HLA-A and -B loci. The magnitude of the response to influenza in association with each self determinant is controlled in large part by the molecular structure of that determinant. In family studies, this control results in a hierarchy in the strength of the responses to influenza in association with determinants coded by each haplotype. However, population studies suggest that Ir-like genes distinct from the structural genes coding for HLA-A and -B-cell surface determinants may modulate this hierarchy of response.

Summary

We have investigated elements of the genetic control of human in vitro cytotoxic T-cell responses to influenza virus-infected autologous cells by studies of a large family. The pattern of virus-immune cytotoxicity among siblings demonstrated T-cell recognition of influenza virus predominantly (>90%) in association with determinants which are coded by genes linked to HLA ($P < 0.0002$). Many family members consistently generated cytotoxic activity against influenza predominantly in associa-

tion with antigens coded by genes of only one of their HLA haplotypes. Such haplotype preferences were consistent among HLA-identical siblings, indicating that the specificity of the T-cell response to influenza virus in association with HLA-A and -B antigens is controlled by genes linked to HLA.

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