Brief Definitive Report

IDENTIFICATION OF A SPECIFIC HLA DR2 Ia MOLECULE AS A RESTRICTION ELEMENT FOR MEASLES VIRUS-SPECIFIC HLA CLASS II-RESTRICTED CYTOTOXIC T CELL CLONES

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The HLA D/DR region of the human major histocompatibility complex encodes a family of highly polymorphic, human "Ia-like" molecules that function as genetic restriction elements in a variety of immune phenomena (1, 2). Correlations have been demonstrated between disease susceptibility and particular HLA D/DR antigens (1, 3, 4). Recent evidence (5–7) has demonstrated a considerable heterogeneity within serologically defined clusters of HLA-DR antigens. Individuals who are identical serologically can be shown to be distinct by the mixed lymphocyte reaction (MLR), thus defining unique HLA-D antigens. The view that HLA-DR is supertypic to the MLR-defined HLA-D specificities is supported by biochemical studies using two-dimensional polyacrylamide gels of immunoprecipitated Ia-like molecules from cell lines derived from phenotypically homozygous typing cell donors (homozygous cell lines, HCL). An analysis of the DR β chain patterns representative of different HLA-D clusters demonstrated a precise correlation with the HLA-D phenotype of the homozygous typing cell (HTC) donor; i.e., HLA-D specificities reflected structural variations in the DR β chains (6, 7). This heterogeneity within a single DR haplotype is central to the concept that Ia structural diversity may account for those polymorphic determinants that are important in Ia-controlled immune phenomena and HLA D/DR disease susceptibility.

We have used (8) HLA-DR2, OKT4$^+$ cytotoxic T lymphocytes (CTL) specific for measles virus as the immunologically reactive cell population to investigate which HLA-DR structural epitopes can serve as genetic restriction elements for these HLA class II–restricted CTL clones. The HLA-DR2 serotype includes up to three separate sets of Ia-like molecules that are expressed on HLA-DR2 HTC lines (7). Two distinct β chains (DR β1 and DR β2) were present on most HLA-DR2 cell lines (7). DR β1 did not vary among eight HLA-DR2 HCL tested, while DR β2 was electrophoretically variable among the DR2 cells examined (7). The

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variability of DR β2 correlated with the MLR-defined HLA-D type of each HTC line tested (7). We report here that the molecules which are apparently recognized by T cells in the MLR (DR β2) may also serve as the restriction element in the Ia-restricted immune response of OKT^+, HLA class II–restricted measles, virus-specific CTL clones derived from a patient with multiple sclerosis (MS).

Materials and Methods

Homozygous Lymphoblastoid Cell Lines. Cell lines CMcG, PAL, and SHI represent the common DR2-associated, HLA-Dw2 specificity; lines BGE, WJR, AZH, and WT18 were derived from HTC representing other non-Dw2 HLA-D specificities as described (7).

Monoclonal Anti-Ia Antibodies. Monoclonal murine antibodies P4.1 and S1.19 are specific for determinants on DR (IE-like) molecules, as described (5). Neither antibody reacts with non-DR, Ia-like molecules on these cells, such as the DQ (DS/DC) molecules. Both P4.1 and S1.19 react with framework determinants on DR molecules independent of haplotype; identical gel patterns were obtained with both antibodies (7).

Radiolabeling, Immunoprecipitation, and Two-dimensional Gel Electrophoresis. Lactoperoxidase-catalyzed iodinations were performed as described previously (6, 7). Immunoprecipitation using antibodies P4.1 and S.19 followed by Cowan 1 strain *Staphylococcus aureus* (SaCl) (Bethesda Research Laboratories, Gaithersburg, MD). This was followed by electrophoresis using nonequilibrium pH, polyacrylamide tube gels with pH 3.5–10 ampholines in the first dimension and sodium dodecyl sulfate–10% polyacrylamide slab gels with a discontinuous pH buffer system in the second dimension (7). Gels were dried and autoradiographed at −70°C with Kodak XR film and a Rarex intensifying screen.

T Cell Clones. The measles virus–specific CTL clones CL34 and CL14 have been described previously and shown to lyse measles virus–infected B cell lines in association with HLA-DR2 and HLA-DR4, respectively (8). Clone E4-4 has been shown (8) to proliferate specifically to measles virus in association with HLA-DR2 and to have a cell surface phenotype that was $OKT3^+$, $OKT4^+$, $OKT8^-$.

Cytotoxic Assay. Clones were incubated at a ratio of 10:1 with a panel of ^{51}Cr-labeled, measles virus–infected lymphoblastoid cell lines. B cell lines were infected with the Edmonston strain of measles virus at a multiplicity of infection of 1.0 and then incubated for 3 d. CTL assays were performed as described previously (8). Percent specific lysis was calculated as described previously (9). Values are expressed as means of triplicate cultures. Uninfected B cell lines gave <1% specific lysis when incubated with the CTL clones. Spontaneous release of infected and uninfected B cell lines was 10–15%. CTL were also derived from bulk cultures by a primary, in vitro 7-d measles virus stimulation of M15 peripheral blood lymphocytes, (PBL) as described previously (8).

Results

Nine Epstein-Barr virus–transformed lymphoblastoid cell lines were used in this study (Table I). Seven homozygous cell lines (HCL) have been described previously (7) and are known to be homozygous for one of the HLA-DR2-associated D locus antigens Dw2, Dw12, LD "tb24", LD "AZH", or LD "WJR". One cell line (E3B) was derived from a donor who is HLA-DR2,4 and is HLA-D typed as tb24 (Dr. David Eckels, personal communication). The M15B cell line, which is autologous to the measles virus–specific CTL clones used in this study, is HLA-DR2,4 and has an HLA-DR2-associated Dw2 type. Effector cells consisted of T cell clones CL34, CL14, and E4-4.

All nine lymphoblastoid cell lines infected with measles virus were lysed by CTL generated in bulk culture (Table I). Clone CL34 (DR2-restricted), CL14 (DR4-restricted), and E4-4 (DR2-restricted) lysed the autologous infected cell line M15B (DR2,4). However, clones CL34 and E4-4 could only lyse those HLA-

TABLE I

Lysis of Measles Virus–infected B Cell Lines by CTL Clones

	Measles virus–infected lymphoblastoid cell line	HLA-DR	HLA-DR2 D type	Percent specific lysis*			
				T cell clones			M15 PBL anti-MV
				CL34	E4-4	CL14	
1	M15B	2,4	Dw2	55.3	31.9	61.6	41.8
2	E3B	2,4	"tb24"	−2.5	3.5	61.5	19.4
3	WJR	2,2	LD"WJR"	−2.6	−2.1	0.6	35.7
4	AZH	2,2	LD"AZH"	−8.5	0.2	−6.6	26.6
5	WT18	2,2	"tb24"	0.7	2.1	0.3	27.8
6	CMcG	2,2	Dw2	31.8	NT	−5.6	43.4
7	PAL	2,2	Dw2	59.9	43.2	6.6	35.6
8	SHI	2,2	Dw2	37.0	42.3	−2.7	29.2
9	BGE	2,2	Dw12	37.8	44.0	1.8	37.8

* T cell clones were used at an effector/target ratio of 10:1. Autologous PBL (M15) were stimulated with measles virus (MV) as described in Material and Methods and used at an effector/target ratio of 40:1. Values are expressed as means of triplicate cultures.

DR2 targets that expressed HLA-D specificities Dw2 or Dw12 (cell lines 6–9, Table I). Both CL34 and E4-4 failed to lyse the HLA-DR2 HCL that express the HLA-D specificities LD WJR, LD AZH, or tb24 (targets 3–5, Table I). None of the DR2 HCL lines could be lysed by the DR4-restricted clone CL14.

The pattern of lysis by clones CL34 and E4-4 correlated precisely with the β chain gel profiles of immunoprecipitated DR molecules from these DR2 HCL (Fig. 1). Gel patterns are those of β chains immunoprecipitated with monoclonal antibodies directed at products of the DR locus from six DR2 HCL. β chain profiles from WJR, AZH, and WT18 (Fig. 1, *left*) contain a series of spots that represent a single polypeptide chain (gels made after neuraminidase treatment to reduce electrophoretic heterogeneity associated with glycosylation [7 and below]). DR β chain profiles from cells CMcG, BGE, and SHI are also shown (Fig. 1, *right*). In addition to a series of β chain spots similar to those in the other cell lines, each of these three HCL contain additional, more acidic β chain spots precipitated by this same antibody (Fig. 1, *β2*). These consist of a series of spots of variable intensity, some of which overlap the more acidic β1 spots. The electrophoretic relationship between the β1 and β2 spots is clarified in the lower panel in Fig. 1. Separate immunoprecipitates from HCL, AZH, and CMcG were treated with neuraminidase, mixed, and coelectrophoresed. Neuraminidase treatment, as reported (7), reduces the electrophoretic complexity of the β1 and β2 series of spots, and clearly separates them under nonequilibrium electrofocusing gel conditions. The β1 spots from AZH and CMcG overlap in the mixed immunoprecipitates (Fig. 1, *bottom*), whereas the β2 spots from CMcG appear as an additional acidic polypeptide. The β2 polypeptide is absent from cell lines WJR, AZH, and WT18 (above, and reference 7), which correlates with the lack of killing of these cell lines by clones CL34 and E4-4 (Table I).

To verify this apparent restriction to the HLA-DR2 β2 molecule, we have identified an individual who is heterozygous HLA-DR2,4 (*E3B*, Table I) whose HLA-DR2-associated D specificity was typed LD tb24. The measles virus–infected E3B line was efficiently lysed by clone CL14, which recognizes measles virus in the context of HLA-DR4. However, neither clones CL34 nor E4-4 could kill this measles virus–infected B cell line, confirming the lack of the required restriction element on DR2, LD tb24 haplotypes.

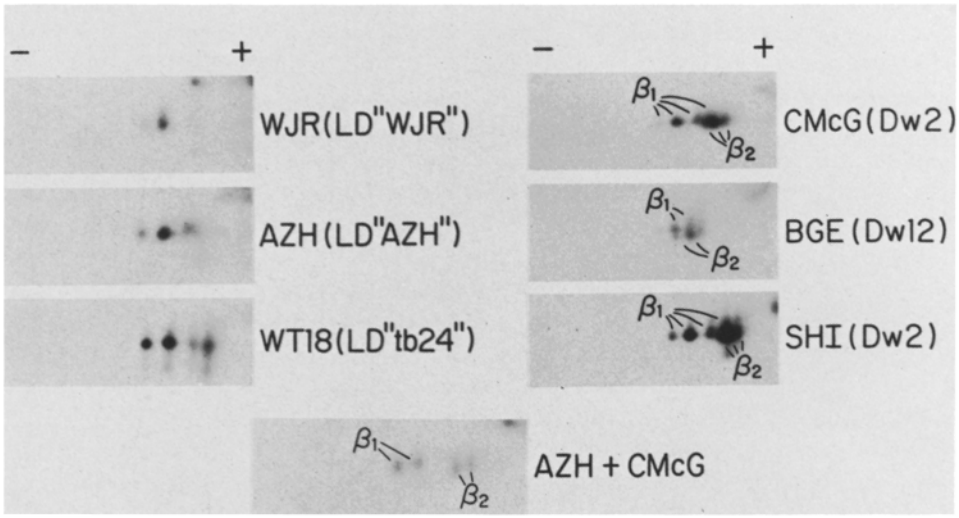

FIGURE 1. Two-dimensional polyacrylamide gel electrophoresis patterns of immunoprecipitated β chains from DR2 homozygous cell lines. (*Upper* panels) Separate immunoprecipitated β chains from DR2 homozygous cell lines (separate immunoprecipitates from six HLA-DR2-associated HCL, using monoclonal anti-DR framework antibodies). Each anode is aligned on the right; β chain region autoradiographs, exposed for 7–10 d, are shown for comparison. Additional acidic polypeptide spots (*β2*) are present in the three panels on the *right* which are not present in the *left* panels. The relationship between the β1 and β2 polypeptides is clarified in the lower gel panel, in which a mixture of neuraminidase-treated immunoprecipitates from two HCL are shown. After neuraminidase digestion, the variable series of β1-associated spots resolves into two, more basic spots, clearly distinct from the more acidic β2 polypeptide, as previously reported (7).

Discussion

It has been demonstrated (6, 7) that at least three Ia-like molecules, each of which may be a product of a separate gene, are expressed on HLA-DR HCL. There is considerable allelic variability for each of these molecules, even among cells expressing the same serologic HLA-DR specificities. It has been suggested (6, 7, 10) that this variability could account for the Ia structural polymorphisms fundamental to HLA-controlled immune responses and to HLA D/DR–associated disease susceptibility. An important question, therefore, is to identify which Ia-like molecules have a functional role in recognition by antigen-specific, immunologically reactive cell populations.

The seven HLA-DR2 HCL used in this study represent the five HLA-D clusters defined within HLA-DR2. In a previous study (7), two distinct β chains (DR β1 and DR β2) were found on HLA-DR2 HCL that express the HLA-D-associated specificities Dw2 and Dw12. The β1 polypeptide chain was invariant in all DR2 HCL tested, regardless of HLA-D type, while the β2 chain varied in a manner that correlated with the HLA-D specificity. HLA-DR2 HCL that express HLA-D LD tb24, LD WJR, or LD AZH did not have an immunoprecipitated DR β2 chain (Fig. 1). The presence of the β2 chain correlated precisely with the susceptibility of measles virus–infected HLA-DR2 HCL to lysis by measles virus–specific, $OKT4^+$, class II–restricted CTL clones (Table I). Measles virus–infected HLA-DR2 HCL in which the DR β2 polypeptide cannot be

detected (cell lines AZH, WJR, and WT18) could not be lysed by these HLA class II–restricted CTL clones.

In this study, the presence of a DR $\beta 2$ polypeptide is inferred from immunoprecipitation with anti-DR monoclonal antibodies. The possibility exists that lines AZH, WJR, WT18, and E3B might indeed possess a variant DR $\beta 2$ molecule sufficiently different from "normal" Dw2 and Dw12 DR $\beta 2$ molecules that it does not react with the anti-DR monoclonal antibodies. If this is the case, our interpretation linking T cell response to DR $\beta 2$ is still valid, since such variant molecules have apparently also lost epitopes governing T cell responses. In essence, such variants would be naturally occurring mutants that permit mapping of the restriction element to this chain. A more likely possibility, however, is that the Dw2- and Dw12-typed DR2 HCL (and not the WJR, AZH, or tb24 HCL) contain a separate gene encoding DR $\beta 2$ that can act as the restriction element for these measles virus–specific CTL clones.

These studies demonstrate that delineation of HLA-DR2 into various subgroups can have a functional significance that parallels the structural differences within the HLA-D region. Therefore, restriction elements that potentially function as disease-associated genetic markers may not correspond to broad, serologically defined, public HLA specificities (HLA-DR serotypes) but rather with products of individual loci, as has been suggested for HLA class II molecules present in juvenile rheumatoid arthritis (11).

It is of interest that both HLA-DR2 and Dw2 occur with increased frequency in patients with MS (12–14). This study links the DR $\beta 2$ polypeptide present in the HLA-Dw2 haplotype (which is more prevalent in MS patient populations) with the susceptibility of these cells to lysis by measles virus–specific, HLA class II–restricted CTL clones derived from a patient with MS. Measles virus has been considered as a possible etiological factor in the MS disease process (15, 16), and $OKT4^+$, measles virus–specific CTL could contribute to development of MS plaque lesions (8, 17). We do not yet know whether such a direct implication of specific restriction elements forms a mechanistic basis for the genetic associations with disease susceptibility. However, the identification of the DR2-associated molecule that appears to be a preferential restriction element for measles virus–specific CTL could provide a valuable clue for molecular genetic investigations of an HLA-linked influence on susceptibility to MS.

Summary

By using a panel of HLA-D-defined subtypes of HLA-DR2 HCL with known β chain structural variabilities, we have demonstrated that HLA-DR2, $OKT4^+$ cytotoxic T lymphocyte (CTL) clones specific for measles virus are apparently restricted to a distinct DR β chain. The presence of this DR $\beta 2$ molecule correlated precisely with the susceptibility of measles virus–infected HLA-DR2 HCL to lysis by these CTL clones. These studies demonstrate that delineation of HLA-DR2 into various subgroups can have a functional significance that parallels the structural differences within the HLA-D region. These results are discussed in the context of the possible association of HLA class II–restricted, measles virus–specific CTL and multiple sclerosis.

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