Brief Definitive Report

MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED H-Y-SPECIFIC ANTIBODIES AND CYTOTOXIC T LYMPHOCYTES MAY RECOGNIZE DIFFERENT SELF DETERMINANTS

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Many lines of evidence from several species have demonstrated that gene products of the major histocompatibility complex (MHC) play a crucial role in immune responses. In man, influenza virus-immune cytotoxic T lymphocytes (CTL) recognize virus in conjunction with self antigens that are highly associated with the serologically-defined HLA-A and -B antigens (HLA restriction) (1, 2). However, previous studies had shown that the association between the serologically-defined antigens and the CTL restriction antigens is not absolute (3). Those studies demonstrated that influenza-immune CTL from a large number of HLA-A2 positive individuals lysed 14 out 15 virus-infected targets obtained from unrelated individuals matched only for HLA-A2. They consistently failed to lyse the virus-infected target lymphocytes from donor M7. Extensive serological analyses of the HLA-A2 antigen of donor M7 have not revealed any detectable differences from the HLA-A2 antigens of the other unrelated donors (3, and G. M. Th. Schreuder, personal communication). However, isoelectric focusing of the HLA-A2 molecule from donor M7 revealed a clear difference in the heavy polypeptide chain when compared with the HLA-A2 molecules of other donors (4). These findings were interpreted as evidence for the absence of determinants on the M7 HLA-A2 molecule, which are recognized by the influenza-immune CTL of "normal" HLA-A2-positive donors.

Human CTL reponses to the male-specific antigen, H-Y, have also been shown to require recognition of self HLA-A and -B specificities (5, 6). Goulmy et al. (5) and Van Leeuwen et al. (7) have demonstrated that both HLA-A2 restricted anti-H-Y specific CTL and an antiserum with specificity for HLA-A2 plus the H-Y antigen could be obtained from a female aplastic anemia patient after multiple transfusions. In the present study, the antiserum and CTL specific for HLA-A2 plus anti H-Y were examined for reactivity with the cells of the HLA-A2 "variant" male donor M7. The results show that the HLA-A2-restricted anti-H-Y CTL fail to lyse HLA-A2-matched M7 male target cells. In contrast, the HLA-A2 plus H-Y-specific antiserum lysed the M7 cells to an extent comparable to that of other "nonvariant" HLA-A2-positive

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TABLE I

Specific Lysis by HLA-A2-restricted α H-Y CTL

		Seriological typing for HLA-A2		Sex of HLA-A2-positive target cells	
		A2+	A2−	Male	Female
CML	+	45	0	43	2*
	−	33	47	1‡	32

* We have shown previously (5) that among the HLA-A2-positive male donors, two HLA-A2-positive females were killed marginally by the HLA-A2-restricted anti-H-Y CTL.

‡ The variant HLA-A2 (M7) male target cells demonstrate the only exception that could be detected to this point in a panel of randomly selected HLA-A2-positive male target cells.

male cells. These results suggest that in systems involving HLA restriction, recognition by CTL and antibody are regulated by separate epitopes that are preferentially recognized by T and B cells, or that different receptor repertoires are used by the MHC-restricted T and B cells for the recognition of foreign antigens such as H-Y.

Materials and Methods

Peripheral blood lymphocytes were obtained from a female patient with aplastic anemia, in partial remission (HLA phenotype A2, Bw44, B40, Cw3, Cw5, Dw4, Dw6, DR4, DRw6) (5). The lymphocytes were separated from her peripheral blood by Ficoll-Isopaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) -gradient centrifugation. We have shown previously (9) that her cells (after a 6-d in vitro sensitization against the irradiated PBL from an HLA-A, -B, -C, and -DR-identical but mixed lymphocyte reaction [MLR] -positive unrelated male donor) were able to lyse cells from HLA-A2 positive male donors but not from other donors. These HLA-A2 restricted anti-H-Y CTL were, on the day of assay, mixed with the target cells in different effector/target cell ratios. The target cells were ^{51}Cr labeled or nonlabeled (i.e., cold inhibitor cells) PHA-stimulated lymphoblasts.

The indirect immunofluorescence method used for detection of HLA-A2 restricted anti-H-Y antibody and the cell-mediated lympholysis (CML) assay were performed as described previously (7, 8). Continuous growing of the 6-d specific cytotoxic effector cells was carried out, and cytotoxic T cell lines were obtained with specific HLA-A2-restricted anti-H-Y cytotoxic activity stronger than that seen with the bulk cultures (10).

Results

The results of testing the HLA-A2-restricted anti-H-Y CTL against a panel of phytohemagglutinin (PHA) blast target cells from males and females (HLA-A2 positive or negative) are summarized in Table I. They show that, with the exception of cells from the male donor M7, all male target cells that expressed HLA-A2 and H-Y antigens were lysed by CTL.

In cold target inhibition experiments, the lysis of ^{51}Cr-labeled HLA-A2-positive male target cells by the HLA-A2-restricted H-Y-specific CTL was inhibited by a panel of unlabeled cold inhibitor cells (Fig. 1). No significant inhibition of cytotoxicity was obtained by the addition of cold M7 inhibitor cells, whereas normal HLA-A2-positive male cells strongly inhibited cytotoxicity. The level of inhibition obtained with cold M7 cells is comparable to the level of inhibition obtained by the addition of HLA-A2-negative male cold target cells.

These experiments with CTL generated in bulk culture were repeated using a cytotoxic T cell line established from this same patient. The results (Table II)

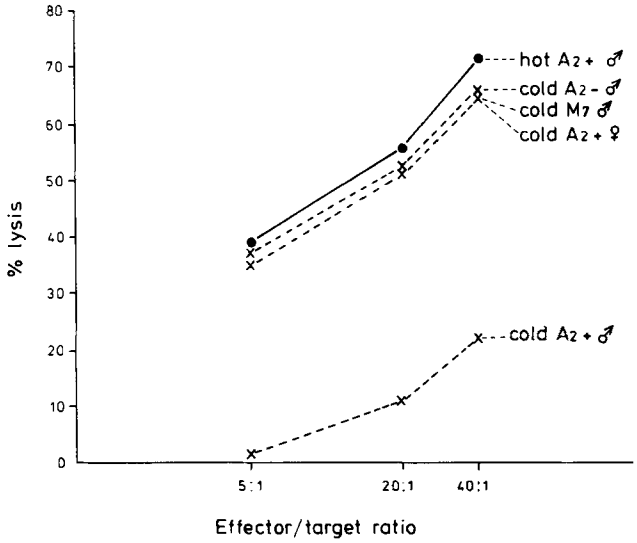

FIG. 1. Inhibition of HLA-A2-restricted H-Y-specific lysis by cold target inhibitor cells. CTL generated in bulk culture (see Materials and Methods) was tested against ^{51}Cr-labeled PHA-stimulated target cells at different effector/target cell ratios. Several PHA-stimulated unlabeled inhibitor cells were added in a 1:10 hot/cold target cell ratio. The lower line in the figure represents the amount of inhibition obtained by three normal HLA-A2-positive male cold target inhibitor cells.

TABLE II

Lysis Pattern of the HLA-A2-restricted Anti-H-Y Cytotoxic T Cell Line

^{51}Cr-labeled target cells	Percent specific lysis	Cold inhibitors added	Percent specific lysis
M7 male "A2"*	+1	ND‡	
Female A2+	−4	ND	
Male A2+	+64	ND	
Male A2+	+67	None	+67
		Male A2−	+61§
		Male M7	+61
		Male A2+	+20

* The HLA-A2-restricted anti-H-Y cytotoxic T cell line has been used as effector cells at a 40:1 effector/target cell ratio.

‡ Not done.

§ Both hot target cells and cold inhibitor cells were PHA-stimulated blast cells and were used at a 1:10 hot/cold target cell ratio.

demonstrate (*a*) that the cytotoxic T cell line could not lyse M7 targets, and (*b*) that M7 cold targets could not inhibit the cytotoxic activity of the cell line assayed on normal male HLA-A2-positive target cells. Taken together, these results indicate that the HLA-A2-associated H-Y determinant, recognized by anti-H-Y-specific CTL and cytotoxic T cell line on normal HLA-A2-positive male cells, are not detectable on the male M7 target cells. These findings are comparable to those previously reported for the absence of recognition of HLA-A2-positive M7 target cells by influenza-immune CTL from HLA-A2 donors (3).

In previous studies of this female aplastic anaemia patient (7) (whose lymphocytes generate anti-H-Y, HLA-A2-restricted CTL), we found a serum IgM antibody that reacted only with HLA-A2-positive male cells. The specificity of this antiserum

TABLE III

*Reactivity Pattern of the HLA-2-restricted Anti-H-Y Antiserum**

Donor	Sex	Percentage of cytotoxicity on	
		B cells‡	T cells
1. M7§	Male	36	7
2. Z	Male	45	8
3. G	Male	41	5
4. A	Female	11	8
control AB serum		4	6

Results are the means of three experiments.

* The presence of lympholytic antibodies, directed against a subset of the B cells from male HLA-A2 positive males, have been described earlier (7).

‡ It was found that complement-dependent cytolytic antibodies react with part of the B cells stained with anti-Ig fluorescein isothiocyanate (6). The monocytes were differentiated from the B cells by treatment of the blood with latex.

§ Donors 1–4 carry the serologically defined HLA-A2 antigen.

(designated serum R) was virtually indistinguishable from that of the anti-H-Y CTL obtained from the same patient. Serum R specifically detects an antigen that consists of components contributed by both HLA-A2 and H-Y. Cells from donor M7 were tested for reactivity with serum R to determine if such an HLA-A2 plus H-Y association could be demonstrated on the cell surface. The results in Table III demonstrate that the HLA-A2 plus H-Y-specific antiserum did react with M7 cells, and that the level of cytotoxicity was comparable to that obtained with control normal HLA-A2 positive male cells. The antibody activity appeared to be directed mainly against B cells, which is consistent with our earlier observations (7). These results indicate that the association of HLA-A2 and H-Y detected by serum R is present on the surface of M7 B cells.

Discussion

A crucial point in this study is whether or not M7 carries the male Y chromosome and expresses the H-Y antigen on the cell surface. Karotype analyses of M7 cells that were Q-banded (11) and G-banded (12) were performed on air-dried chromosome preparations of 72-h stimulated lymphocyte cultures. All cells examined, in 15 metaphases, showed a normal male karotype. The cell-surface expression of the H-Y antigen on M7 cells was examined by the use of a rat anti-H-Y antiserum (13), which is cytotoxic for human male peripheral blood lymphocytes. This anti-H-Y antiserum lysed M7 cells to the same extent as other male cells, indicating a normal expression of the H-Y antigen on the surface of M7 cells.

The failure of the HLA-A2 plus H-Y-specific CTL to lyse male M7 target cells could be explained by the inability of the structurally distinct M7 HLA-A2 molecule to form a physical association with the H-Y antigen on the cell surface. However, the finding that the anti-HLA-A2 plus H-Y-specific antiserum reacted normally with the M7 targets suggests that at least some degree of HLA-A2 H-Y association exists on the surface of the M7 cells. Therefore, a structural variation in the M7 HLA-A2 molecule may have produced a loss of the restricting antigenic determinants recognized as self by normal HLA-A2-restricted H-Y-specific CTL, but has not affected the HLA-A2 H-Y structural epitope(s) recognized by the HLA-A2 plus H-Y-specific antibody. Another possible explanation for the failure of HLA-A2-restricted anti-H-Y

CTL to lyse M7 target cells could be that there has been a qualitative or quantitative alteration in the expression of the cell surface membrane HLA-A2 and H-Y determinants, resulting in interference with CTL but not with antibody recognition. In this context, it should be remembered that the antibody recognizes a MHC H-Y complex on a part of the B cells (7), and the CTL, a similar complex on PHA-stimulated T cells. However, the CTL were also tested against unstimulated T cells and B cell lines of the donor M7 with negative results (data not shown). Taken together, the most likely interpretation of our findings appears to be that MHC-restricted anti-H-Y CTL and antibody can recognize different self HLA-A2 determinants. The dichotomy observed between the self specificity of the anti-H-Y CTL and the antibody responses of the same individual further supports the concept that the HLA-A2 plus H-Y-specific receptor repertoire expressed by CTL and B cells may be different.

Summary

Previous studies have shown that influenza virus-immune cytotoxic T lymphocytes can recognize virus in conjunction with self HLA-A2 antigens. Nevertheless, the virus-infected target cells from one HLA-A2-positive male donor (designated M7) could not be lysed by the virus-immune cytotoxic lymphocytes from any HLA-A2-matched unrelated donors. Although extensive serological analyses showed no difference between the HLA-A2 antigens of donor M7 and other HLA-A2-positive donors, isoelectric focusing of the HLA-A2 molecule from donor M7 revealed a clear difference in the heavy polypeptide chains when compared with the HLA-A2 molecules of other donors.

The present study demonstrates that the HLA-A2-restricted anti-H-Y cytotoxic T lymphocytes obtained from a female aplastic anaemia patient fail to lyse the male M7 target cells, whereas the HLA-A2-restricted anti-H-Y antibodies from the same patient react with the cells of donor M7. These results suggest that: (*a*) HLA-A2-restricted anti-H-Y antibodies can recognize self determinants on the HLA-A2 molecule that are distinct from those that are recognized by HLA-A2-restricted anti-H-Y cytotoxic T cells; and (*b*) HLA-restricted T and B cells may use different receptor repertoires for the recognition of foreign antigens such as H-Y.

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