MHC-restricted H-Y specific antibodies and cytotoxic T lymphocytes may recognize different self determinants.

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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

* Supported in part by the Dutch Organization for Health Research (TNO) the Dutch Foundation for Medical Research (FUNGO subsidized by the Dutch Foundation for the Advancement of Pure Research [ZWO]), and the J A Cohen Institute for Radiopathology and Radiation Protection (IRS)

TABLE I

Specific Lysis by HLA A2-restricted α H Y CTL

		Serological 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 previ ously (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)

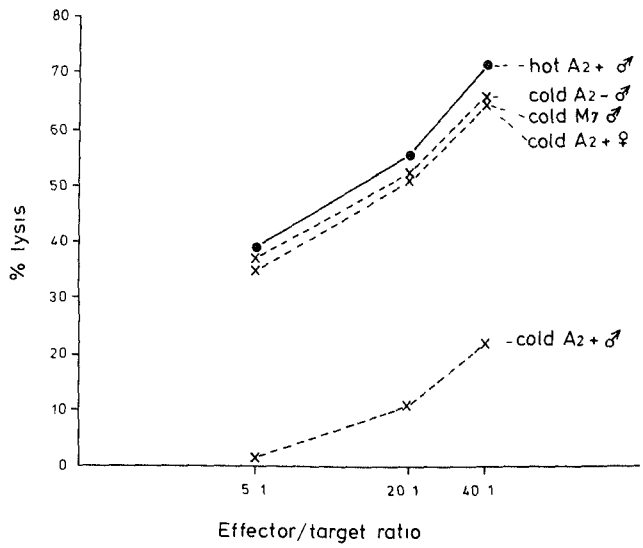

FIG 1 Inhibition of HLA A2-restricted H-Y-specific lysis by cold target inhibitor cells CTL generated in bulk culture (see Materials and Methods) was tested against ^{51}Cr-labeled PHA-stimulated target cells at different effector/target cell ratios Several PHA-stimulated unlabeled inhibitor cells were 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.

The authors wish to thank Dr. M. Fellous for providing the rat anti-H-Y serum; Dr. J. P. M. Geraedts for the karyotype analyses; Dr. J D'Amaro for critical reading of the manuscript; Dr. M. J. Giphart, Dr. F. H. Bach, Dr. G. Shearer, and Dr. R. Levy for informative discussions; and Mrs. A. Pesant for typing the manuscript. This study could not have been performed without the generous blood donations of Mrs. Reefhuis, Mrs. Wamelink, and Mr. Van Delft, and the technical assistance of Ms. T. Goekoop.

Received for publication 17 November 1981 and in revised form 17 February 1982.

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