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Differential Recognition of the Serologically Defined HLA-A2 Antigen by Allogeneic Cytotoxic T Cells

I. Population Studies

Satoshi Horai[1], Jan J. van der Poel[2]*, and Els Goulmy[2]

[1] Department of Anthropology, Faculty of Science, The University of Tokyo, Tokyo, Japan
[2] Department of Immunohaematology and Blood Bank, University Hospital, 2333 AA Leiden, the Netherlands

Abstract. Human alloimmune cytotoxic T cells, sensitized selectively against the HLA-A2 antigen, were tested on a panel of selected target cells. Five HLA-A2 positive outlier cells could be identified. These outlier cells were only weakly lysed by HLA-A2 specific CTLs, although they were serologically indistinguishable from the other HLA-A2 positive, strongly lysed target cells. Furthermore, it was found that the outlier cells were poor cold target inhibitors in contrast to the other HLA-A2 positive target cells, which showed adequate inhibition of specific lysis of HLA-A2 positive target cells. Population studies indicate that the frequency of such HLA-A2 outlier cells may be approximately 10%.

Introduction

It is generally accepted that gene products of the major histocompatibility complex (MHC) play an important role in cell mediated lympholysis (CML) both in the induction phase and in the recognition phase of allogeneic cytotoxic T lymphocytes (CTLs). The antigens recognized by allogeneic CTLs were initially shown to be the serologically defined HLA-A, -B and -C antigens (Miggiano et al. 1972, Eijsvoogel et al. 1976, Grunnet et al. 1976), but also the HLA-D region products were proven to be target antigens (Mawas et al. 1975, Albrechtsen et al. 1979, Feighery and Stastny 1979, Johnsen 1980). Apart from the serologically defined antigens, CTLs were shown to recognize other determinants (Kristensen et al. 1974, Schapira and Jeannet 1974, Willumsen and Heron 1974, Sondel et al. 1975). One group of determinants recognized by allogeneic CTLs comprises the splits or subtypes of serologically defined antigens (Goulmy et al. 1976, Long et al. 1976, Bradley et al. 1978, Schendel et al. 1978, Kato et al. 1982) for which there is not always as yet a

* *Address correspondence to* J J van der Poel, Department of Immunohaematology and Blood Bank, University Hospital, Rijnsburgerweg 10, 2333 AA Leiden, the Netherlands

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serologically defined counterpart Biddison and co-workers (1980a) described an HLA-A2 variant, which by virus immune CTLs and an HLA-A2 restricted anti-H-Y CTL (Goulmy et al 1982), was shown to be recognized differently from most other HLA-A2 positive cells Another possible HLA-A2 variant, detected by an HLA-A2 restricted anti-H-Y CTL, was recently described by Pfeffer and Thorsby (1982)

We report here the results of a systematic study on the occurrence of serologically HLA-A2 positive cells, which are not or only minimally lysed by alloimmune HLA-A2 specific CTLs Approximately 10% of a randomly selected panel of HLA-A2 positive individuals could be identified as outlier cells Furthermore, the outlier cells can be divided into different subsets

Materials and Methods

Cell donors Cell donors were selected from our files of HLA-A B, C and DR typed, healthy blood donors Selection was either performed randomly or according to HLA phenotypes in order to obtain CTLs directed against the serologically defined HLA A2 antigen

CML technique CML was performed according to the European standard technique (European CML-group report III 1980) In brief inducer cultures (i e standard mixed lymphocyte cultures) were established for 6 days followed by CML testing (4 h) at four different CTL dilutions against 10^4 ^{51}Cr labeled PHA-stimulated (3 days) lymphoblasts

Cold target inhibition The CML inhibition capacity of selected cells was tested by addition of non-^{51}Cr labeled (cold) PHA stimulated cells to the specific combination (e g effector AB_X against ^{51}Cr labeled target cells B) A fixed number of cold targets (10^5) was added to 10^4 hot targets at different CTL/hot target cell ratios Control values were established by adding cold competitors autologous either to the responder or the stimulator cells

Calculation of results Cytotoxicity was calculated for each CTL/target ratio according to the formula

$$\frac{\text{(experimental spontaneous) cpm}}{\text{(maximum spontaneous) cpm}} \times 100 = \text{percent release}$$

The experimental results from different experiments were normalized to a percent relative cytotoxic response (percent RCR) based on the specific response for a given CTL and calculated by the formula

$$\frac{\text{percent release of experimental target}}{\text{percent release of specific target}} \times 100 = \text{percent RCR}$$

In all experiments described the percent RCR was calculated based on the percent release observed at a CTL/target ratio of 40 1

Absorption of HLA A2 antisera HLA-A2 specific antisera were absorbed using 2×10^8 cells per ml of serum for 30 min at room temperature Unstimulated peripheral blood lymphocytes PHA stimulated lymphocytes and Epstein Barr virus transformed cells were used for absorptions The absorbed antisera were tested on selected cells in the National Institutes of Health technique

Results

HLA-A2 specific CTLs fail to lyse some serologically defined HLA-A2 positive target cells

In the experiments that initiated this study, it was noted that the percent lysis of an HLA-A2 specific CTL against three HLA-A2 positive target cells was marginal

compared with the percent lysis against the specific target cells and most other HLA-A2 positive target cells Since this phenomenon was reproducible, an expanded panel of HLA-A2 positive target cells was subsequently studied using a number of allogeneic CTLs

Four different CTLs were generated against the HLA-A2 antigen between unrelated individuals The HLA-A, -B, -C and -DR genotypes of the responder and stimulator cells used and the percent lysis of each CTL against autologous and specific target are listed in Table 1 Cytotoxicity of the CTLs against the corresponding specific target cells ranged from 51–78% lysis at CTL to target ratio 40 1 Marginal cytotoxicity ranging from 0–4% lysis was observed against the corresponding autologous targets

The results of the panel study in which 58 HLA-A2 positive and 28 HLA-A2 negative target cells have been tested are presented in Figure 1 A clearcut bimodal distribution of positive and negative targets was observed As expected, all CTLs lysed most of the HLA-A2 positive target cells strongly (60% RCR or more), while the HLA-A2 negative targets, with only a few exceptions, had RCR of 20% or less The three target cells mentioned above (designated LV1, LV2 and LV3) were recognized by all HI A-A2 specific CTLs tested, i e , as outher cells their RCR was well below 60% Furthermore,two additional outher cells (designated LV4 and LV5) were identified, which were consistently lysed less efficiently by two of the CTLs (i e , CTL1 and 2) However, LV4 and LV5 were not recognized as outlier cells by CTL3 and 4

Inhibition of lysis by outher cold competitor cells

The HLA-A2 outher cells were also tested as cold competitors for cytotoxicity against "normal" HLA-A2 ^{51}Cr-labeled target cells As shown for one HLA-A2 specific CTL in Figure 2, none of the outher cells was capable of inhibiting the specific lysis In this respect they behaved the same as cold competitors, which were

Table 1 HLA genotypes of responder stimulator combinations and percent CML against autologous and specific target cells

Effector cells	Responder cells				Stimulator cells				Percent lysis*	
									Autologous†	Specific
CTL1	A3	Bw35	Cw4	DR1	A2	B5	Cw2	DR4	4	78
	A11	B5		DR4	A2	B5	Cw4	DR7		
CTL2‡	A1	B8		DR1	A1	B8		DR3	0	67
	A1	Bw44		DRw6	A2	B44	Cw5	DR3		
CTL3‡	A1	B8		DR1	A1	B8		DR3	2	51
	A25	B44	Cw5	DR3	A2	B44	Cw5	DR3		
CTL4‡	A1	B8		DR1	A1	B8		DR3	3	56
	A25	B44	Cw5	DR3	A2	B44		DR4		

* Percent lysis at effector to target ratio 40 1

† *Target cells from responder cell donor* (autologous) and stimulator cell donor (specific)

‡ CTL2 and 3 were generated against the same stimulator cell while CTL3 and 4 were the same responder cell directed against two different stimulator cells

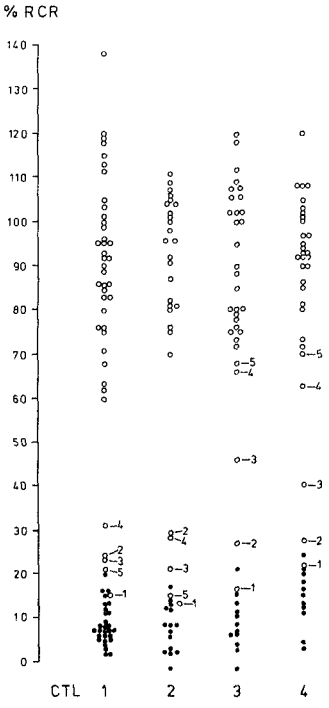

Fig 1 Percent relative cytotoxic responses of HLA A2 specific CTLs Open circles represent HLA A2 positive target cells Closed circles represent HLA A2 negative target cells The outher HLA A2 positive target cells LV1 LV5 are numbered 1 2 3 4 and 5 respectively HLA phenotypes of LV1 to LV5

LV1	A1	A2	B8	Bw50	
	Bw6	Cw6	Cw7	DR3	DR7
LV2	A2	Aw29	B7	Bw58	Bw4
	Bw6	–	–	DRw6	–
LV3	A2	A26	B27	B37	Bw4
	–	Cw2	Cw6	DRw6	DRw9
LV4	A2	A3	B8	Bw35	–
	Bw6	Cw4	–	DR2	–
LV5	A2	Aw32	Bw44	Bw50	Bw4
	Bw6	Cw6	–	DR1	DR7

HLA-A2 negative or as the autologous cells On the other hand, all strongly lysed HLA-A2 positive target cells tested were able to block specific lysis although the amount of blocking showed some variation

Serological evaluation

Serological analysis using alloimmune sera with proven specificity for the HLA A2 antigen was performed to confirm the presence of the HLA A2 antigen on the five outher cells and on randomly chosen strongly lysed target cells As expected, all cells carried the serologically defined HLA-A2 specificity As shown in Table 2 positivity in CML and the presence of the serological HLA A2 antigen were highly associated for all four CTLs ($p<0.0001$ by chi-square analysis with Yates correction)

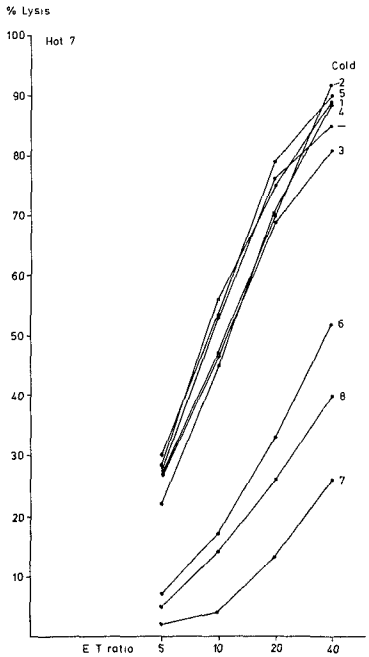

Fig 2 *Cold target inhibition of an HLA A2 specific CTL* CTL 1 was tested on specific stimulator target cells (target no 7) Cold target 1 2, 3 4, and 5 correspond with the outher cells I V1 LV5 while cold target 6 and 8 are two normal HLA A2 positive target cells The percent lysis without addition of cold target cells is indicated by –
HLA phenotypes cell 6
A2 Aw31 Bw38 Bw44
Bw4 Cw2 DR2 DRw6
HI A phenotypes cell 8
A2 A3 Bw62 –
Bw6 Cw3 DR1 DRw6

Table 2 Correlation serology and CML

	HLA A2*			HLA A2*		
		+	–		+	–
CML[1]	+	53	0	CML[1]	+	55	0
CTL1 2	–	5	28	CTL3 4	–	3	28

* The presence of the serologically defined HLA A2 antigen was tested with the alloantisera VR46316 and VR49484, which are used as typing sera in our department
[1] Susceptibility of target cells to lysis by CTLs directed against the HLA-A2 antigen

Two sera were absorbed with three of the outher cells and three control cells The results in Table 3 show that all HLA-A2 positive cells absorbed the anti-HLA-A2 activity One absorption was sufficient to remove the anti-HLA-A2 activity

Table 3 Absorption of HLA A2 antiserum by outlier and control cells

Absorbing cell*	Serum VR46316	Serum VR49484†
None	+ ‡	+
LV1	–	
LV2		–
LV3	–	–
Control 1†	–	
Control 2	–	
Control 3	+	+

* The absorbing cells shown in this table were EBV transformed cells However no differences in absorbing capacity were observed when unstimulated lymphocytes or PHA stimulated lymphocytes were used for absorption

‡ The titer of the serum before absorption was 1 16 No residual reactivity was observed after absorption with HLA A2 positive cells

† Reactivity of the sera was tested back on the five outlier cells and five randomly chosen other HLA A2 positive cells

The HLA phenotypes of control cells are

1	A2	B15	Bw6	Cw3	DR4
2	A2	Bw16	Bw4		DRw6
3	A9	B7	Bw4		DR2

completely No differences in absorbing capacity was observed between unstimulated peripheral blood lymphocytes PHA stimulated lymphocytes, and EBV transformed cells

Discussion

In this study evidence is presented that CTLs directed against the serologically defined HLA A2 antigen lysed the majority of HLA A2 positive target cells strongly However in a systematic study comprising a panel of 58 HLA A2 positive target cells five outlier cells were identified which were lysed consistently with low efficiency All outlier cells were positive for the HLA A2 antigen as analyzed with well defined alloimmune antisera specific for the A2 antigen When the outlier cells were tested as cold competitors, cytotoxicity against the specific target was hardly blocked if at all All other HLA A2 positive target cells could block the specific lysis Since the CTLs were highly specific for the serologically defined HLA A2 antigen the simplest interpretation is that the target antigen recognized by the cytotoxic T cells is the HLA A2 antigen itself Our data would then indicate that around 10% of the serologically defined HLA A2 antigens are variant or subtype

A difference was observed in cytotoxic capacity between CTLs 1 and 2 as compared with CTLs 3 and 4 The latter CTLs lysed the outlier cells LV4 and LV5 strongly (RCR above 60%) To clarify these differences in cytotoxic capacity several possibilities can be considered First cytotoxic responses in bulk cultures are polyclonal thus several cytotoxic determinants might be recognized by clones present in the bulk population of CTLs tested Since responder and stimulator cells

share HLA-A1, B8 and -B12 antigens, cytotoxic determinants associated with HLA B8 and -B12 (as described by Christiansen et al 1981 and Kato et al 1982) may play a role Interestingly, CTL2, which was generated against the same stimulator cell as CTL3, apparently did not recognize those extra specificities

Second, CTL3 and CTL4 recognize different epitopes of the HLA-A2 antigen as cytotoxic determinant, thereby defining a heterogeneity within a heterogeneity Preliminary results show that CTLs directed against the HLA-A2 antigens of the outlier cells indeed subdivide HLA-A2 into at least three subtypes (J J van der Poel, J Pool and E Goulmy, *manuscript in preparation*) Biddison and co-workers (1980b) documented chemical differences in the heavy polypeptide chain of an HLA-A2 variant that was detected by virus immune CTL Based on these observations, biochemical studies are in progress to document a molecular basis for the differential recognition of the HLA-A2 antigen using allogeneic CTLs as reagents

Population studies of CTLs between genotypically (Goulmy et al 1976) or phenotypically HLA identical individuals (Schapira and Jeannet 1974, Robinson et al 1978, Kato et al 1982) have demonstrated the existence of cytotoxic determinants that are serologically indistinguishable Recently, Biddison and coworkers (1981) reported that virus immune CTLs recognized different epitopes of HLA-A 3 antigens in conjunction with different types of influenza virus Since five independent HLA A2 outlier cells could be documented in this study, the same could hold true for the outlier cells in an influenza virus restricted system

The present study supports the notion that the polymorphism of HLA gene products is greater than anticipated The picture that arises for a number of HLA gene products resembles the complexity of target determinants described in mouse *H-2-K* mutants (Melief et al 1980, Sherman 1981)

Finally, it seems of importance to document the influence of the variation of MHC antigens which is serologically indistinguishable but CTL distinguishable, on the occurrence of kidney graft rejection This may document the biological relevance of variations in MHC antigens in transplantation biology and infectious diseases, e g viral infections

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