Table 1. Effects of anti DR moAbs on specific T cell clones

moAb	Number of clones with relative response > 50%	Number of clones with relative response 20% 50%	Number of clones with relative response < 20%
BM Mag 8	11	0	0
BM 50	11	0	0
141	10	1	0
135	9	2	0
206	7	2	2
157	4	4	3
VI 15 C	1	0	10
D1 12	2	4	5

data suggest (a) an epitopic restriction by class II antigens of specific human T cell clone proliferation, (b) the recognition of functional epitopes on the human Ia by some but not all moAbs studied, and (c) the participation of more than a single functional Ia determinant in class II restricted proliferative responses to soluble antigens

Variation in the Epitopes on the HLA-A2 Molecule as Recognized by HLA-A2-Restricted and Alloimmune HLA-A2-Specific Cytotoxic T Lymphocytes*

J J van der Poel, E Goulmy, M J Giphart, and J J van Rood

Department of Immunohaematology University Medical Center, 2333 AA Leiden The Netherlands

Introduction

Serologically defined HLA specificities can be subdivided by biochemical and immunobiological techniques Especially the use of cytotoxic lymphocytes (CTLs) has been shown to be effective in this regard Polymorphism within the serologically defined HLA A2 antigen was demonstrated by means of HLA-A2-restricted influenza virus-specific CTLs [1], Epstein-Barr virus (EBV)-specific CTLs [2], HLA A2-restricted H-Y-specific CTLs [3], and HLA-A2-specific alloimmune CTLs [4, 5] Analysis of the heterogeneity of the HLA A2 antigen in a combined biochemical and alloimmune CTL study revealed the existence of four distinct HLA-A2 subtypes The major HLA-A2 subtype, designated HLA-A2 1, includes 89% of the serologically defined HLA-A2 antigen The remaining 11% could be divided into three minor HLA-A2 subtypes, designated HLA-A2 2, HLA-A2 3, and HLA-A2 4 [6] Previously, we have shown that HLA-A2-restricted anti-H-Y CTLs failed to recognize lymphocytes of the male HLA-A2 variant "M7" [3] Recently, we analyzed the reaction patterns of HLA-A2-restricted minor histocompatibility (minor H) antigen-specific CTLs against lymphocytes from individuals who carried the HLA-A2 2, HLA-A2 3, or HLA-A2 4 subtypes The results indicated that the minor HLA-A2 subtype antigens have, generally speaking, lost the relevant epitope(s) for associative recognition of minor H antigens by minor H antigen-specific CTLs [7]

Nevertheless, one exception was found An HLA-A2 4 subtype positive individual was recognized by HLA-A2-restricted anti-H-Y CTLs [7] Subsequently, the HLA-A2 4 subgroup was analyzed with HLA-A2-restricted minor H antigen-specific CTLs and alloimmune HLA-A2 4 subtype-specific CTLs The results reported here show that using different types of HLA-A2-specific CTLs, ad-

* Supported in part by the Dutch Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Organisation for the Advancement of Pure Research (ZWO) the J A Cohen Institute for Radiopathology and Radiation Protection (IRS), and the Dutch Kidney Foundation

Table 1 HLA A and B phenotypes of the anti HLA A2 4 CTL combinations

Responder cells	Stimulator cells
CTL 4 A1 A9 B8 Bw50	Individual 3 A1 A2 4 B8 Bw50
CTL 5 A1 B8 B35	Individual 4 A2 4 A24 B8 B35
CTL 6 A1 Aw19 B8 B27	Individual 1 A1 A2 4 B8 B27
CTL 7 A2 1 A24 B8 B35	Individual 4 A2 4 A24 B8 B35

ditional polymorphism can be demonstrated within the heterogeneous HLA-A2 specificity The relationship between the recognition patterns of both types of CTLs at the level of the HLA-A2 target molecule will be discussed

Materials and Methods

The HLA-A2-restricted minor H antigen-specific CTLs used were as follows
CTL 1 HLA-A2-restricted anti-H-Y CTLs
CTL 2 HLA-A2- and B7-restricted anti-H-Y CTLs
CTL 3 HLA-A2-, B27-, and Bw62-restricted anti-minor HA CTLs
These CTLs have been described in detail [8 – 10]
Alloimmune CTLs directed against the HLA-A2 4 subtype were made using the cell donors listed in Table 1 CTLs were established by standard mixed lymphocyte cultures for 6 days, followed by T cell growth factor expansion [5]
Cell-mediated lympholysis (CML) was performed as described in detail [4, 5] Experimental results for the alloimmune CTLs were normalized to a percent relative cytotoxic response (RCR), based on the specific response of a given CTL by the formula

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

RCR values up to 25% were considered as negative cytotoxic reactions Values greater than 60% were considered as positive, while values ranging from 25% to 40% were considered as weak cytotoxic reactions [5]

Results

Analysis of HLA-A2-Restricted Anti-Minor H Antigen-Specific CTLs Against the HLA-A2 1, A2 2, and A2 3, Subtypes

The anti minor H CTLs themselves carried the HLA A2 1 major subtype antigen and are therefore HLA-A2 1 restricted As shown in Table 2, the three anti minor H CTLs recognized, as expected, the HLA-A2 1 antigen when the appropriate minor H antigens (i e H-Y or minor HA) were present The reactivity pattern of the HLA A2-restricted minor H-specific CTLs was analyzed on lymphocytes from individuals carrying the HLA-A2 2 and A2 3 minor subtypes No reactivity was found on the A2 2 and A2 3 subtype-positive individuals (Table 2) These data confirmed and extended the observation made with the HLA-A2-restricted anti H-Y CTL 1 on the male HLA-A2 variant "M7" [3] The results indicated that the absence of the target determinant, as expressed on the HLA-A2 1 molecule, led to the loss of the epitope(s) necessary for the associative recognition of the minor H antigens by minor H-specific CTLs [7]

Table 2 Reactivity pattern of HLA A2 1 restricted anti minor H antigen specific CTLs against the HLA A2 1 A2 2 and A2 3 subtypes

Target cell specificity	Sex/no of target cell tested	Reactivity pattern with		
		CTL 1	CTL 2	CTL 3
HLA A2 1	Male/50	+	+	+
	Female/20			+
HLA A2 2[a]	Male/3	–		–
	Female/4	–		
HLA A2 3	Male/2			
	Female/2	–	–	–

Individuals carrying other antigens (i e HLA B7 Bw62 and B27) which can function as restricting molecules for associative recognition of minor H Y or minor HA were excluded

Table 3 Reactivity patterns of the minor H antigen specific CTLs on the HLA A2 4 subtype positive individuals

Target cell individual	Presence (+) or absence (–) of restricting molecule				Reactivity pattern with		
	A2 4	B7	B27	Bw62	CTL 1	CTL 2	CTL3
2	+		+	+			+
3	+						
4	+				+	+	
5	+	+		–	+	+	
6	+				+	+	+

All target cells were obtained from males

Reactivity of HLA-A2-Restricted Anti-Minor H Antigen-Specific CTLs on the HLA-A2 4 Subtype

The reactivity pattern obtained with the HLA-A2-restricted minor H CTLs against the HLA A2 4 subtype-positive individuals, as shown in Table 3, is more complex As expected, negative reactions were found on several HLA-A2 4-positive individuals However, positive reactions were also observed against the lymphocytes from some individuals carrying the HLA-A2 4 subtype Apparently, the restricting epitope(s) necessary for the recognition by the minor H CTLs, as present on the HLA-A2 1 molecule, were retained in those individuals

When the reactivity pattern of the two anti-H-Y CTLs 1 and 2 was analyzed, positive reactions were observed on lymphocytes from individuals 4, 5, and 6 The lymphocytes from individuals 2 and 3 were not recognized Thus, by using the anti-H-Y CTLs, the HLA-A2 4 subgroup can be subdivided When the reactivity pattern of the HLA-A2-, B27-, and Bw62-restricted anti-minor HA CTL 3 was analyzed, fewer positive reactions were observed

Only the lymphocytes from individuals 2 and 6 were recognized by CTL 3 The positive reaction of CTL 3 on individual 2 was caused by the presence of another restricting molecule, namely HLA-Bw62, in addition to the HLA-A2 4 molecule This is supported by segregation studies (data not shown) As far as the recognition of the HLA-A2 4 subtype antigen is concerned, only one (i e , individual 6) out of the five individuals tested, were recognized by the minor HA-specific CTL 3 Individuals 4 and 5, lysed by the anti-H-Y CTLs 1 and 2, have lost the epitope(s) necessary for the recognition by the anti-minor HA CTL 3

Thus, the A2 4 subgroup can also be subdivided by using the anti-minor HA CTL 3 Apparently, the anti-minor H-Y CTLs and the anti-minor HA CTL do not always use the same restricting epitope(s) on the HLA-A2 molecule The cytotoxic reactions of the HLA-A2-restricted anti minor H antigen-specific CTLs against the A2 4 subtype can be divided into three patterns

1 Absence of recognition by anti-H-Y- and anti-minor HA specific CTLs, thus loss of the epitope(s) for associative recognition of minor H-Y and minor HA
2 Recognition by anti-H-Y-specific CTLs only, thus retention of epitope(s) for associative recognition of minor H-Y, but loss of epitope(s) for associative recognition of minor HA
3 Recognition by anti-H-Y- and anti-minor HA-specific CTLs, indicating, despite the presence of the A2 4 subtype molecule, the presence of epitope(s) for the associative recognition of both minor H antigens, H-Y and HA

The latter situation is in fact identical with the reaction pattern obtained in the HLA-A2 1 subtype-positive individuals (see previous section)

Alloimmune HLA-A2 4 Subtype-Specific CTLs

We have previously shown that the HLA-A2 4 subtype was defined by a lack of recognition by HLA-A2 1 and A2 2 subtype-specific CTLs [6]

We have been able to show that also HLA-A2 3-specific CTLs failed to lyse HLA-A2 4 subtype-positive individuals (van der Poel et al , manuscript in preparation) It should be emphasized that the HLA-A2 1-, A2 2-, and A2 3-specific CTLs clearly identified individuals carrying the relevant subtype antigen Since this approach was successful, CTLs were made against A2 4 subtype-positive individuals in order to positively select for the A2 4 antigen Four CTLs could be made against three of the A2 4 subtype-positive individuals identified (Table 1)

The alloimmune CTLs 4 to 7 were tested against six HLA-A2 4 subtype-positive individuals As shown in Table 4, the reactivity patterns of CTL 4 to 7 within the HLA-A2 4 subgroup were complex CTL 4 reacted with all individuals carrying the HLA-A2 4 subtype antigen

CTL 5 recognized individuals 4 (specific stimulator), 5, and 6 Lymphocytes from individuals 1, 2, and 3 were only weakly lysed CTL 7, which was made against the same stimulator cell (i e , individual 4) as CTL 5, showed the same reactivity pattern as CTL 5 Thus, using CTL 5 and CTL 7, the A2 4 subgroup could be subdivided

CTL 6 recognized individuals 1 (specific stimulator), 2, 4, and 5 Lymphocytes from individual 3 were not recognized at all, while targets from individual 6 were weakly lysed by CTL 6 Thus, the A2 4 subgroup could also be subdivided by using

Table 4 Reactivity patterns of HLA A2 4 subtype specific alloimmune CTLs on HLA A2 4 subtype-positive individuals

Target cell individual	CTL 4	CTL 5	CTL 6	CTL 7
1	87[a]	25	100	17
2	97	40	98	21
3	100	32	7	20
4	78	100	77	100
5	71	90	82	87
6	66	85	35	74

[a] Results are expressed as mean % RCR obtained with the alloimmune CTL indicated

CTL 6 CML analysis of the HLA-A2 4 subtype antigen with the alloimmune HLA-A2 4 subtype-specific CTLs demonstrated that further heterogeneity in the A2 4 subgroup existed

Discussion

The reaction patterns of HLA-A2-restricted minor H antigen (minor H-Y and minor HA)-specific CTLs and alloimmune HLA-A2 subtype-specific CTLs against lymphocytes from individuals carrying different HLA-A2 subtypes have been analyzed The presence of the minor H-Y antigen, a prerequisite for recognition by CTL 1 and 2, is easily controllable since it is sex-linked The minor HA antigen, recognized by CTL 3, is present in 95% of the HLA-A2 1 subtype-positive individuals and can, furthermore, be verified by family studies in most cases [7] The minor H-specific CTLs 1 and 2 (anti-minor H-Y) and CTL 3 (anti-minor HA), which were HLA-A2 1 restricted, recognized A2 1 subtype-positive individuals carrying the relevant minor H antigen Absence of lysis was observed when lymphocytes from individuals carrying the HLA-A2 2 and A2 3 subtype were tested with the two types of HLA-A2-restricted anti-minor H CTLs The A2 2 and A2 3 subtype molecules must have lost the epitope(s) necessary for associative recognition by the minor H antigen-specific CTLs The cytotoxic reactions of the anti-minor H CTLs on the HLA-A2 4 subtype can be divided into three patterns

1 Loss of epitope(s) necessary for recognition by both minor H antigen-specific CTLs
2 Loss of epitope(s) for minor HA recognition but retention of epitope(s) for minor H-Y recognition
3 Retention of epitope(s) for recognition by both anti-minor H CTLs despite the presence of an HLA-A2 4 subtype antigen

For the A2 4-positive individuals 4 and 5, belonging to the second category, the absence of the minor H antigen HA could not be excluded This might explain the lack of recognition by CTL 3 However, since the minor H antigen HA is present in 95% of the HLA-A2 1 positive individuals tested, we favor the interpretation that the epitope(s) necessary for associative recognition were absent One explanation would be that the minor H-Y and minor HA CTLs use different epitope(s) on the HLA A2 4 molecule for associative recognition
An alternative explanation would be that the A2 4 subgroup can be divided into three subtypes In order to prove the latter assumption alloimmune HLA-A2 4 subtype-specific CTLs were generated

It should be noted that the definition of the A2 4 subtype was essentially based on the lack of recognition by HLA-A2 1-, A2 2-, and A2 3-specific alloimmune CTLs The finding that the A2 4 subtype is not lysed by A2 1 subtype-specific CTLs was confirmed independently by Brenner and Strominger using a cytotoxic T cell clone which was A2 1 specific (M Brenner and J L Strominger, personal communication) This confirmation reinforces the notion that the A2 4 subtype is genuine

Further heterogeneity was found within the A2 4 subtype using A2 4-specific alloimmune CTLs Three types of reaction patterns were observed (see Table 4) In fact, it seems that the four unrelated A2 4-positive individuals 1, 3, 4, and 6 differ from each other with respect to the epitopes recognized by the anti-A2 4 subtype-specific CTLs tested When the relationship between the alloimmune CTLs and the anti-minor H CTLs within the A2 4 subgroup is compared, it is seen that the reaction pattern of alloimmune CTLs 4 and 6 do not correlate with the reaction pattern of the anti minor H CTLs However, the reaction pattern of alloimmune CTLs 5 and 7 are identical to that of the anti-H-Y CTLs 1 and 2, since these CTLs recognized the A2 4-positive individuals 4, 5, and 6 The epitope(s) which these CTLs recognize on the A2 molecule may be identical, but this can probably only be stated with certainty when the reactivity pattern is analyzed at the clonal level Biochemical analyses may eventually bring answers to this problem Amino acid differences have been shown to exist in the tryptic peptide spanning residues 147–157 in the A2 heavy polypeptide chains of the A2 1, A2 2 and A2 3 subtype [11, 12] (J L Strominger, personal communication) These differences have been postulated to be of functional relevance for the recognition by CTLs [12] The results obtained with CML analysis of the HLA-A2 4 subtype predict that differences in amino acid sequence would have to be found within the A2 4 subgroup and between the A2 4 and the other A2 subtypes Amino acid sequence data should clarify this issue

One other point should be taken in consideration Additional polymorphism might be generated by differences in folding of the A2 molecule, resulting in conformational changes The conformation of molecules seems to be important for the recogniton by CTLs [13]

Therefore conformational changes in the different HLA-A2 subtype molecules may explain the reaction patterns obtained with the minor H antigen-specific CTLs and the alloimmune HLA-A2 4-specific CTLs

References

1 Biddison WE, Ward FE, Shearer GM, Shaw S (1980) The self determinants recognized by human virus immune T cells can be distinguished from the serologically defined HLA antigens J Immunol 124 548–552
2 Gaston JSH, Rickinson AB, Epstein MA (1983) Epstein-Barr virus specific cytotoxic T lymphocytes as probes of HLA polymorphism J Exp Med 158 280–293
3 Goulmy E, Van Leeuwen A, Blokland E, Van Rood JJ, Biddison WE (1982) MHC restricted H-Y specific antibodies and cytotoxic T lymphocytes can recognize different self determinants J Exp Med 155 1567–1572
4 Horai S, Van der Poel JJ, Goulmy E (1982) Differential recognition of the serologically defined HLA-A2 antigen by allogeneic cytotoxic T cells I Population studies Immunogenetics 16 135–142
5 Van der Poel JJ, Pool J, Goulmy E, Van Rood JJ (1983) Differential recognition of the serologically defined HLA-A2 antigen by allogeneic cytotoxic T cells II Definition of three HLA-A2 subtypes by CTLs Immunogenetics 17 599–608
6 Van der Poel JJ, Molders H, Thompson A, Ploegh HL (1983) Definition of four HLA-A2 subtypes by CML typing and biochemical analysis Immunogenetics 17 609–621
7 Goulmy E, Van der Poel JJ, Giphart MJ, Van Rood JJ (1984) Analysis of the functional epitopes on different HLA A2 molecules Immunogenetics 20 13–21
8 Goulmy E, Termijtelen A, Bradley BA, Van Rood JJ (1977) Y antigen killing by women is restricted by HLA Nature 266 544–545
9 Goulmy E, Hamilton JD, Bradley BA (1979) Anti-self HLA may be clonally expressed J Exp Med 149 545–550
10 Goulmy E, Gratama JW, Blokland E, Zwaan FE, Van Rood JJ (1983) A minor transplantation antigen detected by MHC restricted cytotoxic T lymphocytes during graft versus host disease Nature 302 159–161
11 Krangel MS, Taketani S, Biddison WE, Strong DM, Strominger JL (1982) Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes Variants M7 and DR1 Biochemistry 21 6313–6321
12 Krangel MS, Biddison WE, Strominger JL (1983) Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes II Variant DK1 evidence for a discrete CTL recognition region J Immunol 130 1856–1862
13 De Waal LP, Nathenson SG, Melief CJM (1984) Direct demonstration that cytotoxic T lymphocytes recognize conformational determinants and not primary amino acid sequences J Exp Med 158 1720–1726

Cloned Cytotoxic T Cells Which May Define a Minor Transplantation Antigen and a Variant of the HLA-B8 Molecule

P.F. Pfeffer, E. Qvigstad, and E. Thorsby

Institute of Transplantation Immunology, The National Hospital, 0027 Oslo 1, Norway

A male patient (HLA-A3,9, B8,15, DR3,w6) first rejected a female cadaveric kidney (HLA-A1,10, B8,40, DR3,8) and then a kidney from his HLA identical sister. The recipient and his sister typed identically with Ninth Workshop antisera and pretransplant MLC tests were reciprocally negative Six weeks after the rejection the recipient responded weakly to the sister in MLC tests and he formed cytotoxic T cells (CTLs) reactive with his sibling donor These T cells were then cloned.

Two clones with different specificities (clones 20 and 23) were tested (Table 1) Clone 20 lysed target cells from the HLA identical donor, from some family members, and from approximately 50% of unrelated individuals carrying HLA-B15. This clone probably recognizes a minor transplantation antigen and is restricted by B15

Table 1. Specificity of donor specific cytotoxic T cell clones

Target cells	HLA-A,B specificities shared with the recipient	Clones tested	
		20	23
Donor	3, 9, 8, 15	81[a]	73
Mother	9, 8	0	37
Father	3, , 15	64	5
Sibling 1	9, 8	1	32
Sibling 2	3, 9, 8, 15	0	37
Sibling 3	3, , 15	65	0
Unrelated ($U_1 - U_{10}$)	, 8	0–10	22–44
Unrelated ($U_{11} - U_{15}$)	, 15	50–87	0–14
Unrelated ($U_{16} - U_{20}$)	, 15	0–8	0–2
Unrelated ($U_{21} - U_{29}$)	3, 9,	0–14	0–7

[a] % Cytotoxicity

Clone 23 lysed target cells from the HLA identical donor and all family and unrelated individuals carrying HLA-B8 This clone may recognize an almost ubiquitous minor transplantation antigen (which the recipient lacks), and be restricted by B8 Alternatively, the recipient may have inherited a "variant" B8 molecule from his mother (by point mutation or gene conversion), thereby being able to recognize the nonvariant B8 antigen of the sibling donor and most others (including the first cadaveric donor) as foreign When CTLs were generated toward ("non-variant") B8 using third-party responding and stimulating cells, they lysed target cells from all B8 family members, except those from the recipient This suggests, but does not prove, that the recipient may have inherited a "variant" of B8, which may be recognized by CTLs but not by available antisera Biochemical studies are under way

Construction and Expansion of CTLs for HLA Typing: Definition of B44 Subtypes by Cellular Typing

P W Hansen, S E Christiansen, F Kissmeyer-Nielsen, and T Kristensen

The Tissue Typing Laboratory, University Hospital of Aarhus, 8000 Aarhus, Denmark

Introduction

The polymorphic gene products of the human HLA region can be identified both by serological and cellular typing techniques Although the definition of the system, its loci, and their antigenic determinants is in essence based on serological studies and analyses, it is now evident that a determinant primarily described by one technique, e g , serology, may be detected similarly by the other, viz cellular typing (MLC, PLT, or CML) Thus, serological and cellular typing studies are equally precise with regard to specificity and must supplement each other

This paper concerns the definition of the serologically defined HLA A, B, C antigens by cytotoxic T lymphocytes (CTLs) and focuses on the definition of antigenic subtypes not as yet defined by serology Such observations are not new Mawas et al [1], Kristensen et al [2], Schendel et al [3], and Goulmy et al [4] all published similar results, but tended to interpret them as evidence for new loci in the HLA region Recent studies extending our biochemical knowledge of the HLA molecules suggest that a majority of the discrepancies observed earlier can in all probability be attributed to CTL recognition of epitopes on classical HLA molecules not as yet defined by antibodies [5–11]

Cellular typing research in this area, however, has until recently been impeded by the fact that only small numbers of CTLs could be produced at a time, resulting in considerable experimental variance due to the use of different CTL batches Further, the relatively low numbers of active CTLs in classical CML effector combinations called for high effector-to-target (E/T) ratios or extensive E/T titrations

Initiated by our participation in the European CML Study Group, we have developed methods for production of interleukin-2 (IL-2)-conditioned media and expansion of classical CML effector combinations into T cell lines based on the studies of Gillis et al [12] Consequently, large batches of CTL-enriched effector combinations may be ready at any time, enabling cellular typing of selected panels, populations, and families with identical CTLs We used this approach to study the serological HLA-B44 determinant (For an extensive review on the CTL approach to HLA typing, see [13])

Material and Methods

Cells were prepared from defibrinated blood using Isopaque-Ficoll separation Donors were selected from our files of HLA-typed healthy individuals, in some special cases including their family

CTL Generation

Primary cultures were set up according to the protocol adopted by the European CML Study Group [14] In brief, equal numbers of responding and 4000-rad-irradiated stimulating cells were mixed in

 Histocompatibility Testing 1984
Edited by P I Terasaki et al
Springer Verlag Berlin Heidelberg 1984