Immunogenetics 20 13–21, 1984

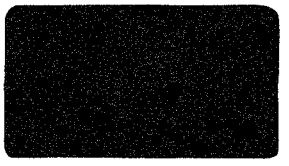
Immuno-genetics

Analysis of the Functional Epitopes on Different HLA-A2 Molecules

Els Goulmy, Jan van der Poel, Marius Giphart, and Jon J van Rood

Department of Immunohaematology, Universital Medical Center Leiden, The Netherlands

Abstract. Recent studies show that the serologically defined HLA-A2 molecule can be subdivided according to functional and biochemical characteristics By the use of various HLA-A2-specific cytotoxic T lymphocytes (CTLs) and isoelectric focusing, the serologically homogeneous HLA-A2 molecule can be divided into four subtypes The polymorphism of the serologically defined HLA-A2 molecule has also been demonstrated by the use of HLA-A2-restricted CTLs This study was designed to analyze the functional epitopes on different HLA-A2 molecules with special regard to the recognition patterns of different types of HLA-A2-restricted CTLs directed against minor histocompatibility (minor H) antigens Fifteen so-called HLA-A2 variants belonging to distinct HLA-A2 subtypes were tested as target cells in the cell-mediated lympholysis (CML) assay against (1) HLA-A2-restricted antiminor H-Y CTLs, (2) HLA-A2 and -B7-restricted antiminor H-Y CTLs, and (3) HLA-A2, -Bw62 and -B27-restricted antiminor "HA" CTLs We found that those three CTLs recognized only *one* of those HLA-A2 variants Furthermore, positive reactions by the antiminor H CTLs were only observed on those variant cells which carried, in addition to the HLA-A2 variant, either another "normal" HLA-A2 molecule or another required restricting class I molecule necessary for associative recognition These results indicate that the absence of HLA-A2 normal allotypic target determinant(s) leads to the loss of epitope(s) necessary for recognition of minor H-Y and minor "HA" transplantation antigens by HLA-restricted CTLs We can conclude from the present study that HLA-A2-restricted antiminor H CTLs use, in general, the same epitope (or cluster of epitopes) for cellular recognition as alloimmune HLA-A2-specific CTLs



Introduction

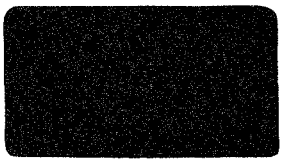
The genetic fine structure of the HLA specificities can now be studied by the use of a variety of biochemical and immunobiological techniques One of these techniques, namely, the use of cytotoxic T lymphocytes (CTLs), has been shown to be particularly effective for that purpose The results of numerous studies indicate that serologically defined HLA-molecules can be subdivided by cellular reagents (Goulmy et al 1976, 1982a, Bradley et al 1978, Biddison et al 1980, Kato et al 1982, Breuning et al 1982, Horai et al 1982, Spits et al 1982, Pfeffer and Thorsby 1982, van der Poel et al 1983a, Gaston et al 1983)

Determination of polymorphism within the serologically defined HLA-A2 molecule has been demonstrated by the use of HLA-A2-restricted CTLs, namely, HLA-restricted influenza virus immune T cells (Biddison et al 1980), Epstein-Barr virus-specific CTLs (Gaston et al 1983), HLA-restricted H-Y-specific cytotoxic T cells (Goulmy et al 1982a, Pfeffer and Thorsby 1982), and by the use of HLA-A2-specific alloimmune CTLs (Horai et al 1982, van der Poel et al 1983a)

Comprehensive analyses of the heterogeneity of HLA-A2 molecules using HLA-restricted virus-specific CTLs as well as alloimmune CTLs revealed more variability of recognition sites than anticipated (Biddison et al 1982, van der Poel et al 1983a) In a combined biochemical and immune CTL analysis reported earlier (van der Poel et al 1983b), we described *four* distinct HLA-A2 subtypes, of which the major HLA-A2 subtype (i e , HLA-A2 1) includes 89% of the serologically defined HLA-A2 antigen (Horai et al 1982, van der Poel et al 1983a)

Human CTL responses to the male-specific H-Y antigen (Goulmy et al 1977, 1979, Singal et al 1981, Pfeffer and Thorsby 1982) and to minor transplantation antigens (Goulmy et al 1982b, 1983a, Elkins et al 1982, Tekolf and Shaw 1983) have shown to be restricted by self HLA-A and -B molecules The HLA-restricted anti-H-Y and antiminor H antigen (designated HA) CTLs, available in our laboratory, recognize the HLA-A2 1 major subtype defined by HLA-A2-specific alloimmune CTLs Thus, HLA-A2-restricted anti-H-Y CTLs failed to recognize lymphocytes of the male HLA-A2 variant "M7" (Goulmy et al 1982a), originally detected as an HLA-A2 *variant* by HLA-A2-specific influenza virus immune T cells (Biddison et al 1980)

The aim of this study was to investigate the relationship between the recognition patterns of our two types of CTLs (i e , HLA-A2-restricted anti-H-Y and antiminor HA) at the level of the restricting HLA-A2 molecule To that end, we analyzed the reaction patterns and discriminatory capacity of the anti-H-Y and antiminor HA CTLs on a series of HLA-A2 variants belonging to four distinct HLA-A2 subtypes We report here that HLA-A2-restricted anti-H-Y *and* HLA-A2-restricted antiminor "HA" CTLs, in all except one case studied so far, failed to recognize HLA-A2 subtypes as defined by alloimmune CTLs These results lead us to the conclusion that in most *but not all* cases HLA-restricted antiminor H CTLs and alloimmune HLA-A2 major subtype CTLs both make use of the same epitope(s) on class I molecules for cellular recognition We will discuss the possibility that MHC-restricted CTLs directed to two different minor transplantation antigens may use different epitopes for recognition



Materials and Methods

Alloimmune HLA-A2-specific CTLs Four cytotoxic reagents were generated against the HLA-A2 antigen using unrelated individuals which shared all but the sensitizing HLA-A2 antigen (van der Poel et al 1983a) Eleven percent of the HLA-A2-seropositive lymphocytes used as target cells were identified as HLA-A2 variants The HLA-A2 serologically defined specificity could be divided into four subtypes based on the reactivity patterns in cell-mediated lympholysis (CML) using (a) alloimmune major subtype HLA-A2-specific CTLs and alloimmune minor subtype HLA A2-specific CTLs, (b) cold target competition experiments, and (c) biochemical analyses (van der Poel et al 1983a, b) We refer to them as HLA-A2 1 (major subtype, 89% of the serologically defined HLA-A2 specificity), HLA A2 2 (minor subtype), HLA-A2 3 (minor subtype), and HLA-A2 4 type (minor subtype) Lymphocytes were obtained from 15 individuals who carried the subtypes A2 2, A2 3 or A2 4 (see Table 1)

CTLs 1 HLA-A2-restricted anti-H Y CTLs These CTLs were prepared from the lymphocytes from a multitransfused woman suffering from aplastic anemia in partial remission (HLA-phenotypes A2, Bw44, B40, Cw3, Cw5, DR4, DRw6) This patient received a bone marrow graft, which was subsequently rejected, from an HLA-identical male sibling donor We have previously shown that her cells (after a 6 day in vitro sensitization period against irradiated peripheral blood lymphocytes from an HLA-A, B, C and -DR identical but mixed lymphocyte reaction positive unrelated male donor) were able to show preferential lysis of male target cells carrying the HLA-A2 antigen (Goulmy et al 1977) We refer to these CTLs as CTLs 1 or anti-H-Y CTLS

CTLs 2 HLA-A2 and -B7-restricted anti-H-Y CTLs These CTLs were obtained from a multitransfused female aplastic anemia patient (HLA phenotype A2, A28, B7, Bw62, Cw3, DR1, DR2) CTLs which specifically lysed HLA-A2 *and* HLA-B7 male target cells (Goulmy et al 1979) were generated in vitro similarly to the method described and used for the generation of CTLs 1 We refer to these CTLs as CTLs 2 or anti-H-Y CTLs

CTLs 3 HLA-A2, B27, Bw62 restricted antiminor HA CTLs Recently we demonstrated that posttransplant lymphocytes from a bone marrow transplanted acute myeloid leukemia patient suffering from severe graft-versus-host disease, exhibited strong cytotoxicity in CML against his own pre-transplant lymphocytes (Goulmy et al 1982b) Additional studies showed that the patient's post-transplant cytotoxic effector cells recognize one (or more) minor transplantation antigen(s) in association with three self class I HLA molecules, namely, HLA A2, -B27, and -Bw62 (Goulmy et al 1983a) We refer to these CTLs as CTLs 3 or anti minor "HA" CTLS

Cell-mediated lympholysis assay (CML) The CML assay has been described in detail previously (Goulmy 1982) The HLA restricted anti-H Y and HLA-restricted antiminor HA CTLs as described above and designated as CTLs 1, 2 and 3 were mixed on the day of assay with ^{51}Cr-labeled target cells in various CTL to-target cell ratios in round-bottomed microtiter plates Cytotoxicity (i e , the amount of isotope released from ^{51}Cr-labeled target cells) was determined and calculated according to the method described previously (Goulmy 1982) All experiments were repeated at least twice at six effector to-target ratios Standard errors of the mean of triplicate determinations were less than 5% When only one CTL-to-target ratio was used, lysis levels equal to or less than 10% were considered negative, 11–15% weakly positive, 16–40% positive and greater than 40% strongly positive

Results

Panel studies Lymphocytes from 15 individuals, with the HLA-A2 subtypes A2 2, A2 3 and A2 4 and most of their relatives were tested with CTLs 1, 2 and 3 (see *Materials and Methods*) Table 1 shows the results of individuals 1–15 tested as target cells in CML against the three HLA-A2-restricted antiminor -H CTLs The positive reactions that were observed were confined to the lymphocytes of

individuals 10–15. The presence of the additional "normal" HLA-A2.1 major subtype (in individuals 11, 14 and 15) resulted in positive reactions with CTLs 1 and 2 against the lymphocytes of the male individuals 14 and 15, and a positive reaction with CTLs 3 against the lymphocytes of female individual 11. The presence of additional restricting elements, i. e., HLA-B27 and/or Bw62 (in individual 10 and 12), resulted in positive reactions with CTLs 3. The assumption that the positive reactions seen on target cells 10–15 are caused by the presence of either an additional normal HLA-A2.1 major subtype or other additional restricting elements (necessary for the associative recognition of the minor HA) is supported by segregation studies (see below). Anti-H-Y and antiminor HA CTLs showed no lysis against target cells carrying one of the HLA-A2 minor subtypes (except those from individual 13).

Family studies. Six families with different HLA-A2 subtypes were tested with CTLs 1, 2 and 3. Three informative families will be shown. We showed earlier that the HLA-A2 antigen subtypes are inherited codominantly (van der Poel et al. 1983a). Investigation of the relatives of some individuals with the antiminor H-Y and antiminor HA CTLs revealed that those which carried haplotypes that included an HLA-A2 variant also lost their restricting epitopes for minor H-Y and minor HA. One such example is shown in Figure 1. The father (01) of family I carries the HLA-A2 major subtype on the *b* haplotype; the mother (00) carries one HLA-A2.1 major subtype (haplotype *c*), and one HLA-A2 minor subtype on haplotype *d*. As is shown in Figure 1, child 02 (male) with the maternal *d* haplotype (carrying the HLA-A2.2 subtype) is neither lysed by the two anti-H-Y CTLs nor by antiminor HA CTLs. However, child 03 (male) with the maternal *c* haplotype with the HLA-A2.1 (i. e., "normal") subtype was recognized normally by CTLs 1, 2, and 3.

The minor H antigen HA is recognized in association with the class I molecules A2, Bw62 and B27. Therefore we investigated the influence of HLA-A2 variant molecules on the recognition pattern of those antiminor HA CTLs. We chose family II in which the HLA-A2.2 subtype and one of the restricting elements (i. e., Bw62) were expressed. Figure 2 shows that the paternal lymphocytes carried the HLA-A2.1 major subtype; the maternal lymphocytes carried the Bw62 antigen in addition to the HLA-A2.2. subtype. As expected, the two types of antiminor H CTLs (H-Y and HA) lysed the lymphocytes of the father (01). Child 03 with the paternal *b* haplotype showed a similar pattern of lysis. When the mother's lymphocytes (00) were used as target cells, a positive reaction occurred only with the antiminor HA CTLs 3. The question of whether the positive reaction seen by CTLs 3 is due to associative recognition with the HLA-A2.2 antigenic determinant or with the HLA-Bw62 antigen with the minor HA CTLs was investigated by analyzing the offspring. Child 02 inherited the maternal *c* haplotype. As shown in Figure 2, no lysis was observed by the antiminor HA CTLs, indicating that the positive reaction on the maternal lymphocytes was not due to associative recognition with the HLA-A2.2 antigen but was caused by the recognition of the restricting molecule HLA-Bw62.

Next, the offspring of the "exception to the rule" (i. e., individual 13, whose lymphocytes were lysed by CTLs 1 and 2, see Table 1) was investigated with CTLs 1, 2 and 3. The results of the CTL analyses are shown in Figure 3. Despite the fact that the father (individual 13 from the panel is No. 21 in the pedigree) carried an HLA-

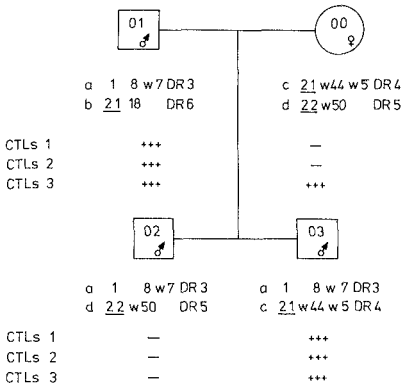


Fig. 1. The characters *a*, *b*, *c*, and *d* refer to the *HLA* haplotypes of family I The origin of CTLs, 1, 2, and 3 as well as the positive (+++) and negative (−) assignments are described in *Materials and Methods*

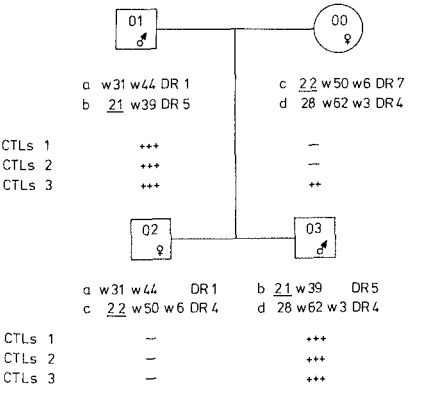


Fig. 2. The characters *a*, *b*, *c*, and *d* refer to the *HLA* haplotypes of family II The amount of lysis observed against HLA-Bw62-positive target cells (such as the mother of family II) by CTLs 3 is always lower than that observed against HLA-A2-positive target cells (Goulmy et al 1983a) See legend to Figure 1

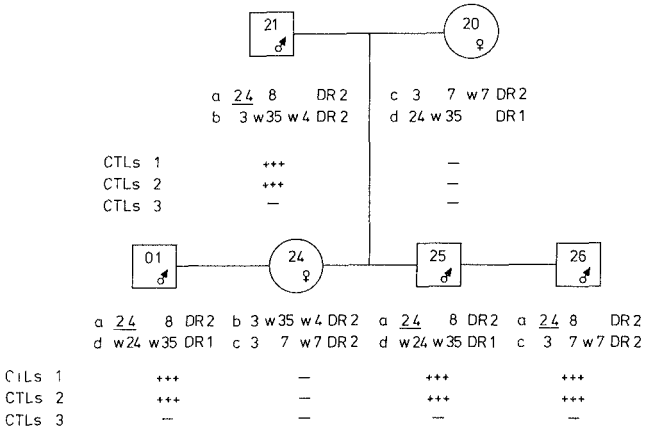


Fig. 3. The characters *a*, *b*, *c*, and *d* refer to the *HLA* haplotypes of family III The father, i e , 21, is equivalent to individual 13 in the panel analysis See legend to Figure 1

A2.4 minor subtype on his *a* haplotype, the two anti-H-Y CTLs were able to lyse these target cells. The lymphocytes of his children who had inherited the HLA-A2.4 determinant (i e., 01, 25, 26) were also lysed by *both* anti-H-Y CTLs. On the contrary, no lysis occurred when the antiminor HA CTLs were tested against their target cells. The results obtained in family III indicate strongly that the subtype HLA-A2.4 antigen lost its restricting epitope for associative recognition of the minor HA but retained its restricting epitope for minor H-Y

Discussion

Several subtypes of the serologically defined HLA-A and -B molecules have been determined by the use of cytotoxic T lymphocytes: HLA-A2 (Biddison et al. 1980, Horai et al. 1982, Spits et al. 1982, van der Poel et al. 1983a), HLA-A3 (Biddison et al. 1981), B7 (Spits et al. 1982), B27 (Breuning et al. 1982), Bw35 (Goulmy et al. 1976, Bradley et al. 1978), B40 (Malissen et al. 1981), and Bw44 (Kato et al. 1982). The polymorphism of the serologically defined HLA-A2 antigen was first established by Biddison and co-workers (1980) by means of HLA-A2-restricted influenza virus-specific CTLs, later confirmed by HLA-A2-restricted anti-H-Y specific CTLs (Goulmy et al. 1982a) and also by Epstein-Barr virus-specific CTLs (Gaston et al. 1983).

HLA-A2 variants can also be detected easily by means of alloimmune HLA-A2-specific CTLs (Horai et al. 1982, van der Poel et al. 1983a). The functional as well as biochemical analyses of the serological defined HLA-A2 molecules (Biddison et al. 1982, van der Poel et al. 1983b) have revealed considerable additional polymorphism in the determinants of the HLA-A2 serotype at the population level. This report presents the results of the analyses of HLA-A2 variants in 15 individuals using HLA-restricted antiminor H CTLs, i.e., minor H-Y CTLs and minor HA CTLs. They show that these antiminor H CTLs recognize, in general, the same epitope (or cluster of epitopes) as do the allo-CTLs to the HLA-A2.1 major subtype CTLs, the so-called "normal" HLA-A2 (Table 1). This conclusion is based on the identical reaction patterns obtained by "normal" alloimmune HLA-A2-specific CTLs and HLA-A2-restricted antiminor H CTLs against all (except one) variants classified as minor A2 subtypes, i.e., HLA-A2.2, A2.3 and A2.4. Consequently, our results suggest that associative recognition of minor H antigens requires an epitope (or epitopes) that is selectively present on the HLA-A2.1 molecule. Our conclusions may be homologous to the observations described by Weynand and co-workers (1981), who reported a striking preference of alloreactive and H-2-restricted CTLs for the same domain of the H-2 molecule.

We reported earlier that HLA-A2 subtypes are inherited codominantly (van der Poel et al. 1983a). Reaction patterns in six families with variant HLA-A2 determinants, obtained with our two types of HLA-restricted antiminor H CTLs, confirmed the latter observation. The three most informative families are described in this paper. The analysis of the lymphocytes of the family members of family I with the HLA-restricted antiminor H-Y and minor HA CTLs showed indeed that the HLA-A2 subtypes are inherited in a codominant way. Family 2 (Fig. 2) shows that the presence of the minor transplantation antigen HA can be demonstrated by MHC-restricted CTLs only through the presence of one of the structurally "correct" class I molecule. The lysis obtained against target cells of the mother of family II is derived through the presence of the Bw62 restricting element, as can be seen by the absence of lysis against target cells from child 02 who inherited the HLA-A2.2 subtype antigen and not Bw62. These results are concordant with the positive reactions in the panel study (Table 1).

Finally, we discuss the exception to the rule which is demonstrated by individual 13. Target cells of individual 13 carried the HLA-A2.4 subtype as demonstrated earlier by functional analysis (Horai et al. 1982, van der Poel et al. 1983a). Those

Table 1. Analysis of the HLA A2 variants by HLA restricted CTLs

Target cells Ind ‡	Presence (+) or absence (−) of restricting elements			Sex	HLA A2* subtype	Reactivity pattern with		
	A2	Bw62	B27			CTLs 1†	CTLs 2	CTLs 3
1	+	−	−	Male	A2 4	−	−	−
2	+	−	−	Female	A2 2	−	−	−
3	+	−	−	Female	A2 2	−	−	−
4	+	−	−	Male	A2 2	−	−	−
5	+	−	+	Female	A2 4	−	−	−
6	+	−	−	Female	A2 3	−	−	−
7	+	−	−	Male	A2 3	−	−	−
8	+	−	−	Male	A2 2	−	−	−
9	+‖	−	−	Female	A2 3, A2 3	−	−	−
10	+	−	+	Female	A2 2	−	−	+
11	+‖	−	−	Female	A2 1 A2 2	−	−	+
12	+	+	+	Male	A2 4	−	−	+
13	+	−	−	Male	A2 4	+	+	−
14	+‖	−	−	Male	A2 1, A2 2	+	+	+
15	+‖	−	−	Male	A2 1, A2 3	+	+	+

* HLA A2 subtypes are described in *Materials and Methods*
† CTLs 1 2 and 3 are described in *Materials and Methods*
‡ Ind , individual
‖ HLA A2 homozygous

authors showed that the lymphocytes from individual 13 [individual 13 is equivalent to LV4 in van der Poel et al (1983 a)] were clearly identified as HLA-A2 variants The serologically HLA-A2-positive individuals 1, 5, 12, 13 all carried the HLA-A2 4 subtype, which was essentially defined by the absence of lysis by HLA-A2 1 major subtype, HLA-A2 2 minor subtype, and HLA-A2 3 minor subtype CTLs as well as by the absence of specific inhibitory capacity Nevertheless, individual 13 is clearly different from individual 1, 5 and 12 (who also have the *same* HLA-2 4 subtype) with respect to its reaction pattern obtained with antiminor H-Y CTLs (see Table 1)

The analysis of the offspring of individual 13 (family III) revealed that the same reaction patterns were obtained with the antiminor H-Y CTLs on the lymphocytes of only those children which inherited the paternal HLA-A2 4 subtype Thus, despite the presence of an HLA-A2 variant molecule, individual 13 and his offspring apparently retained the restricting epitope for recognition of the minor H-Y Nevertheless, the lymphocytes of individual 13 and his children 01, 25 and 26 were not lysed by antiminor HA CTLs (Fig 3) Therefore, the latter target cells lack the restricting epitope for the recognition of the minor HA, a situation which, in fact, is comparable with reaction patterns against all other HLA-A2 variants studied so far with the antiminor HA CTLs

We cannot answer the question as to whether the lymphocytes of individual 13 carry the minor transplantation antigen HA The possibility that all variants are negative for minor HA is very slight, since in a random population study only 5% of the HLA-A2 1-positive individuals were found to be negative for minor HA

(Goulmy et al 1983 a, b). Other HLA-A2 subtypes can only be typed for minor HA when they carry the restricting molecules Bw62 and/or B27 (Fig 2 and Table 1) Unfortunately, individual 13 does not carry one of those molecules and therefore cannot be typed for minor HA. Another explanation may be that the minor H-Y and minor HA antigen use two different epitopes for associative recognition. Both epitopes are present on HLA-A2 1 type molecules, but absent on A2 2 and A2 3 molecules The A2 4 type molecules can be divided into two subtypes, one lacking both epitopes, as in individuals 1, 5 and 12, and one subtype containing the epitope for H-Y associative recognition, as in individual 13. Peptide mapping and amino acid sequencing of the HLA-A2 heavy chains of individual 1, 5, 12 and 13 may eventually resolve this issue The results of analyses of some HLA-A2 variants by Krangel and co-workers (1982, 1983) suggest that a tryptic peptide spanning residues 147 to 157 of the HLA-A2 heavy chain may play an important role in the recognition of the HLA-A2 molecule by CTLs

In conclusion, the results of this study show that HLA-restricted antiminor H-Y and antiminor HA CTLs use, in general, the same epitope on the HLA-A2 molecule for associative cellular recognition

Acknowledgments We thank Messrs Els Blokland, Jos Pool, and Allan Thompson for excellent technical assistance and Dr J D'Amaro for reading the manuscript and for his critical comments This work was supported in part by the Dutch Foundation for Medical Research (FUNGO) which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO), the J A Cohen Institute for Radiopathology and Radiation Protection (IRS) and Dutch Kidney Foundation

References

Biddison, W E , Ward, F E , Shearer, G M , and Shaw, S The self determinant recognized by human virus immune T cells can be distinguished from the serologically defined HLA antigens *J Immunol 124* 548–552, 1980

Biddison, W E , Shearer, G M , and Shaw, S Influenza virus specific cytotoxic T cells are restricted by multiple HLA-A3 related self antigens Evidence for recognition of distinct self structures in conjunction with different foreign antigens *J Immunol 127* 2231–2235, 1981

Biddison, W E , Kostyu, D D , Strominger, J L and Krangel, M S Delineation of immunologically and biochemically distinct HLA-A2 antigens *J Immunol 129* 730–734, 1982

Bradley, B A , Goulmy, E , Schreuder, G M T , and Van Rood, J J Targets for killer T cells in human lymphocyte differentiation Its application to cancer *In* N Serrow and C Rosenfeld (eds) *Inserm Symposium No 8*, pp 231–240, Elsevier North Holland Biochemical Press, Amsterdam, 1978

Breuning, M H , Lucas, C J , Breur, B , Engelsman, M Y , De Lange, G G , Dekker, A J , Biddison, W E , and Ivanyi, P Subtypes of HLA-B27 detected by cytotoxic T lymphocytes and their role in self-recognition *Hum Immunol 5* 259–268, 1982

Elkins, W L , Pierson, G , Negendonk, W , and Zier, K S Recognition of human minor alloantigen(s) by cytotoxic lymphocytes in vitro *Immunogenetics 15* 485–499, 1982

Gaston, J S H , Rickinson, A B , and Epstein M A Epstein-Barr virus specific cytotoxic T lymphocytes as probes of HLA polymorphism Heterogeneity of T cell restricting determinants associated with the serologically defined HLA-A2 antigen *J Exp Med 158* 280–293, 1983

Goulmy, E HLA-A, B restriction of cytotoxic T cells *In* S Ferrone and B G Solheim (eds) *HLA-Typing, Methodology and Clinical Relevance*, Volume 2, pp 105–122, CRC Press Inc , West Palm Beach 1982

Goulmy, E , Termijtelen, A , Bradley, B A , and Van Rood, J J HLA restriction of non HLA A, B -C and -D cell mediated lympholysis (CML) *Tissue Antigens* 8 317–326, 1976

Goulmy, E , Termijtelen, A , Bradley, B A , and Van Rood, J J Y-antigen killing by women is restricted by HLA *Nature 266* 544–545, 1977

Goulmy, E , Hamilton, J D , and Bradley, B A Anti-self HLA may be clonally expressed *J Exp Med 149* 545–550, 1979

Goulmy, E , Van Leeuwen, A , Blokland, E , Van Rood, J J , and Biddison, W E MHC restricted H-Y specific antibodies and cytotoxic T lymphocytes an recognize different self determinants *J Exp Med 155* 1567–1572, 1982a

Goulmy, E , Gratama, J W , Blokland, E , Zwaan, F E , and Van Rood, J J Recognition of an – as yet unknown – minor transplantation antigen by post-transplant lymphocytes from an A M L patient *Exp Hematol 10, suppl 10* 127–129, 1982b

Goulmy, E , Gratama, J W , Blokland, E , Zwaan, F E , and Van Rood, J J A minor transplantation antigen detected by MHC restricted cytotoxic T lymphocytes during graft versus host disease *Nature 302* 159–161, 1983a

Goulmy, E , Gratama, J W , Blokland, E , Zwaan, F E , and Van Rood, J J MHC restricted cytotoxic T lymphocytes recognize a human minor transplantation antigen *Exp Hematol 1, suppl 13* 67–69, 1983b

Horai, S , Van der Poel, J J , and Goulmy, E Differential recognition of the serologically defined HLA-A2 antigen by allogeneic cytotoxic T cells I Population studies *Immunogenetics 16* 135–142, 1982

Kato, S , Ivanyi, P , Lacko, E , Breur, B , Du Bois, R , and Eysvoogel, V P Identification of human CML targets HLA-B locus (B12) antigen variants defined by CTL generated between B locus identical (B12) responder-stimulator pairs *J Immunol 128* 949–955, 1982

Krangel, M S , Taketani, S , Biddison, W E , Strong, D M , and Strominger J L Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes Variants M7 und DR1 *Biochemistry 21* 6313–6321, 1982

Krangel, M S , Biddison, W E , and Strominger, J L 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, 1983

Malissen, B , Charmot, D , and Mawas, C Expansion of human lymphocyte populations expressing specific immune reactivities III Specific colonies, either cytotoxic or proliferative obtained from a population of responder cells primed in vitro Preliminary immunogenetic analysis *Hum Immunol 2* 1–13, 1981

Pfeffer, P F and Thorsby, E HLA restricted cytotoxicity against male specific (H-Y) antigen after acute rejection of an HLA identical sibling kidney *Transplantation 33* 52–56, 1982

Singal, D P , Wadia, Y J , and Naipal, N In vitro cell-mediated lymphocytotoxicity to the male specific (H-Y) antigen in man *Hum Immunol 2* 45–53, 1981

Spits, H , Breuning, M H , Ivanyi, P , Russo, C , and De Vries, J E In vitro-isolated human cytotoxic T-lymphocyte clones detect variations in serologically defined HLA antigens *Immunogenetics 16* 503–512, 1982

Tekolf, W A and Shaw, S In vitro generation of cytotoxic cells specific for human minor histocompatibility antigens by lymphocytes from a normal donor potentially primed during pregnancy *J Exp Med 157* 2172–2177, 1983

Van der Poel, J J , Pool, J , Goulmy, E , and Van Rood, J J Differential recognition of the serologically defined HLA-A2 antigen by allogeneic cytotoxic T cells II Definition of three HLA-A2 antigen by allogeneic cytotoxic T cells II Definition of three HLA-A2 subtypes by CTLs *Immunogenetics 17* 599–608, 1983a

Van der Poel, J J , Molders, H , Thompson, A , and Ploegh, H L Definition of four HLA-A2 subtypes by CML typing and biochemical analysis *Immunogenetics 17* 609–621, 1983b

Weynand, B , Hammerling, G J , and Goronzy, J Recognition of H-2 domains by cytotoxic T lymphocytes *Nature 292* 627–629, 1981

Received January 9, 1984 revised version received April 9, 1984