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Activation of cytotoxic T lymphocytes in HLA-A, -B and -C-identical responder-stimulator pairs II

New subtypes of HLA-Bw35

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We compared five cytotoxic T lymphocytes raised by primary mixed lymphocyte cultures of HLA A, -B and C serologically identical Bw35 positive responder stimulator combinations When tested on a panel of third party target cells, the reactivity pattern of these cytotoxic T lymphocytes allowed the distinction of three subtypes of HLA-Bw35 Cold-target inhibition experiments and analysis of CTL activity at the clonal level showed the existence of subsets of CTLs directed against distinct antigenic determinants associated with HLA Bw35

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Cytotoxic T lymphocytes (CTLs) can be used to discriminate subtypes of serologically defined HLA A and -B antigens (Bradley et al 1978, Breuning et al 1982, Goulmy et al 1976, Horai et al 1982, Kato et al 1980, Spits et al 1982, Tekolf et al 1982) According to these reports, CTLs can define subtypes of HLA antigens on HLA molecules that are at present not recognized by currently available (monospecific) HLA-typing sera HLA-antigen subtypes can also be recognized by virus-specific and H Y-specific, HLA-restricted CTLs (Biddison et al 1980a, Biddison et al 1981, Breuning et al 1982, Goulmy et al 1982) and may be relevant for studies of the role of HLA antigens in tissue transplantation and in HLA-associated diseases The repertoire of the allogeneic CTLs can be used to unravel the complexity of the HLA antigens

We have generated CTLs in a series of responder stimulator pairs matched for serologically defined HLA-A, -B and -C antigens (Breuning et al 1984 I) In this report, we describe three CTLs from this series which seem to detect subtypes of the HLA-Bw35 antigen In addition, the specificity of these CTLs is compared with Bw35 subtype-specific CTLs previously described (Bradley et al 1978, Goulmy et al 1976) The results indicate that at least three distinct types of HLA-Bw35 exist

Material and methods

Lymphocytes were obtained from HLA-A, -B, -C and -DR-typed individuals from the Dutch population. Isolation, cryopreservation and thawing of the peripheral blood lymphocytes (PBL) have been described by Breuning et al. (1982).

HLA phenotypes of the cells used for the generation of CTLs are shown in Table 1. Typing for CTL-defined subtypes of HLA-B27 was performed as described by Breuning et al. (1982) and Breuning et al. (1983). In all combinations, there was an HLA-DR difference and a significant proliferation in mixed lymphocyte culture (MLC), as measured by the incorporation of ^{3}H-thymidine after 5 days of culture (Breuning et al. 1984 I).

One of the donors (cell no. 345) was a patient with ankylosing spondylitis from Spanish origin. Cells from one pair of homozygous twins (cells no. 265 and 266) were used alternatively. In several experiments, the latter cells were found to be interchangeable (data not shown). Responder cells as well as stimulator cells (irradiated with 2000 rad from a ^{137}Cs source) were cultured for 6 days at a 1:1 ratio in Iscove's modified Dulbecco's medium (IMDM; Gibco) with 100 IU penicillin and 100 µg streptomycin/ml, supplemented with 20% pooled human serum. PBL, cultured for 6 days in the same medium without any stimulant, were used as target cells. After 6 days, 2.10^{5}, 10^{5} and 5.10^{4} effector cells were incubated with 2.10^{4} ^{51}Cr-labelled target cells for 8 h in IMDM with 100 IU penicillin and 100 µg streptomycin/ml, supplemented with 10% heat-inactivated foetal calf serum (FCS; Gibco). The percentage of cytotoxicity was calculated according to the formula $100 \times (ER - SR)/(MR - SR)$, counts per minute of the median of triplicate experiments, where ER is the experimental release of chromium, SR the spontaneous release from target cells incubated in medium alone, and MR the re-

Table 1.
HLA phenotypes of individuals whose cells were used for the generation of CTL.

CTL designation	Responder cell no.	HLA A	HLA B	HLA C	HLA DR	Bw35 subtype	Stimulator cell no.	HLA A	HLA B	HLA C	HLA DR	Bw35 subtype	Sensitizing DR antigen	^{51}Cr release, %
10	436	2,3	w60, w35	3,4	6,7	C	202	2,3	w60, w35	3,4	1,6	B	1	46.4
9	202	2,3	w60, w35	3,4	1,6	B	436	2,3	w60, w35	3,4	6,7	C	7	58.4
17	265	2,3	27[a], w35	1,4	1,–	B	345	2,3	27[a], w35	1,4	1,4	A	4	31.7
18[c]	Rc8	2,11	w44, w35	4,5	8,LB5[b]	A	Rc3	2,11	w44, w35	4,5	8, LB5.8[b]	B	LB5.8[b]	35.5
19[c]	Rc3	2,11	w44, w35	4,5	8, LB5.8	B	Rc8	2,11	w44, w35	4,5	8, LB5	A	LB5	45.0

[a] These cells carry the same CTL-defined subtype of HLA-B27.
[b] LB5 and LB5.8 are Leiden local assignments for DR5 splits.
[c] CTLs 18 and 19 have been described in detail (Bradley et al. 1978, Goulmy et al. 1976). Rc1 and Rc2 are the mother and the father, respectively, of Rc3 and Rc8, the pedigree of the family has been described (Bradley et al. 1978, Goulmy et al. 1976). The effector:target-cell ratio in these experiments was 10:1.

lease from cells lysed with saponin, the maximal release

Cold-target inhibition experiments were performed as described by Breuning et al (1983) Unlabelled PBL, cultured for 3 days without any stimulant, were used as cold target cells Unlabelled lymphocytes, $2\,5 \times 10^5$ or $5\,10^5$, were added to wells containing 10^5 effector cells and $5\,10^3$ ^{51}Cr-labelled target cells corresponding to cold/hot-target ratios of 50 1 or 100 1 and to an effector/hot-target-cell ratio of 20 1 The release time was 5½ h The percentage ^{51}Cr release was calculated as described above Most CML tests were repeated two to three times, and the mean value is shown in this work

Results

Panel studies

The CTLs used are listed in Table 1 CTLs 9 and 10 were raised by reciprocal one-way-mixed lymphocyte cultures of HLA-A, -B and -C-identical cells obtained from two unrelated blood donors The CTLs were tested on a large panel of third-party target cells from unrelated individuals (Figure 1) Although the responder cells used for the generation of CTL 10 themselves expressed HLA-Bw35, CTL 10 lysed all samples of Bw35 positive target cells except two One of the latter two samples were the autologous target cells CTL 9, on the contrary, strongly lysed only the target cells from the stimulator as well as the one exceptional sample of third-party Bw35 target cells that were not lysed by CTL 10 (Figure 1) These results suggest that the two target cells not recognized by CTL 10 carry a variant HLA-Bw35 antigen The antigenic determinant recognized by CTL 10 was found to segregate with an HLA-Bw35-positive haplotype in several families (results not shown)

CTL 17 was also raised by MLC of

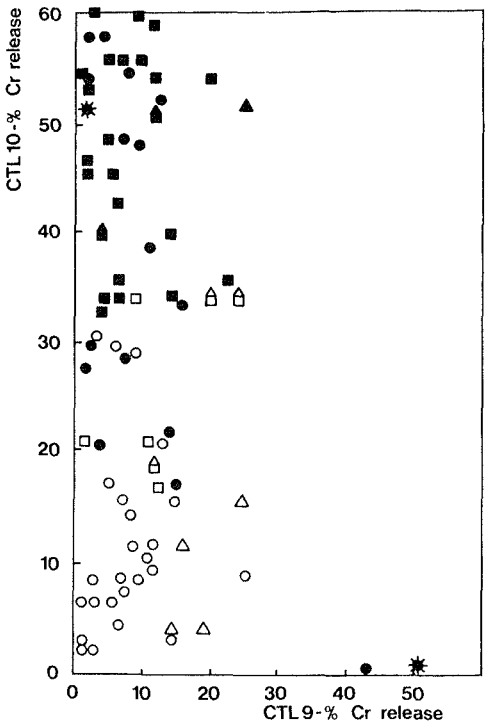

Figure 1 Cytolytic activity of CTLs 9 and 10 against target cells from 79 unrelated individuals ★, responder/stimulator cells, ○, Bw35–, ●, Bw35+, □, Bw35– DR1+, ■ Bw35+ DR1+, △, Bw35– DR7+, ▲, Bw35+ DR7+ The effector/target-cell ratio was 10 1

serologically HLA-A, -B, -C-identical, D/DR-different responder-stimulator combinations (Table 1, Breuning et al 1984 I) Initially, we tested CTL 17 on a panel of 60 samples of target cells from unrelated individuals CTL 17 showed cytotoxicity on 12 of these target cells, all of which expressed the HLA antigen Bw35 However, 28 HLA-Bw35-positive target cells were not lysed by CTL 17, neither were any of the remaining 20 Bw35 negative target cells (results not shown) Thus, there being no serologically defined HLA antigen significantly associated with the cytotoxic action of CTL 17, the antigenic determinant(s) recognized by this CTL

could at first not be determined. CTLs 18 and 19 were raised by reciprocal one-way MLC of cells from two HLA-A, -B, -C serologically identical, D/DR-different siblings. These CTLs have been shown to be able to subdivide a panel of HLA-Bw35-positive target cells (Goulmy et al. 1976).

To further investigate the complexity of the HLA-Bw35 antigen and the specificity of CTLs 9, 10, 17, 18 and 19, we compared their cytotoxic activity against a selected panel of HLA-Bw35-positive target cells (Figure 2). Each of the 5 CTLs reacted with a part of this panel. The subdivision of HLA-Bw35 by CTLs 18 and 19 revealed that CTL 17 recognized a particular subset of HLA-Bw35-positive target cells. However, CTLs 17 and 18 showed a similar, but not identical reaction pattern. We concluded that, by using these 5 CTLs, the HLA-Bw35 antigen can be subdivided into at least 3 subtypes. A fourth subtype of Bw35 (type D) may be represented by the cells that were only weakly lysed by CTLs 10 and 19. A preliminary designation of the 4 subtypes is given in Figure 2.

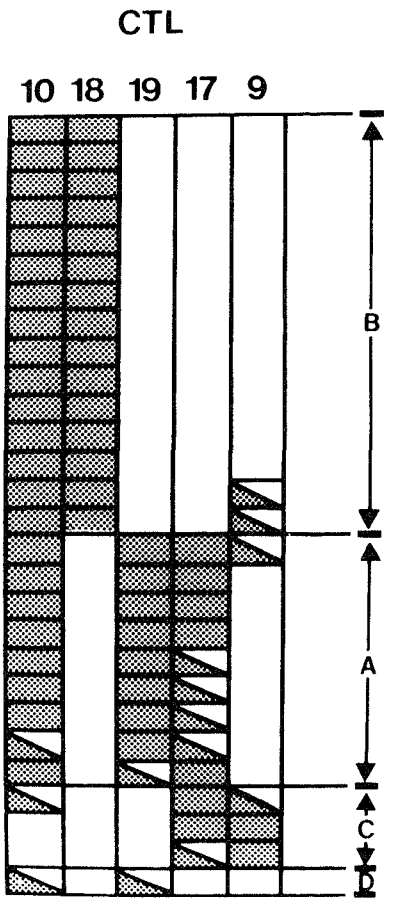

Figure 2. Cytolytic activity of five CTLs raised in HLA-A, -B and -C serologically identical responder-stimulator pairs and tested against HLA-Bw35-positive target cells from 28 unrelated individuals. ▩, more than 20% ^{51}Cr release; ▨, between 15 and 20% ^{51}Cr release; □, less than 15% ^{51}Cr release. A–D, arbitrary designation of CTL-defined subtypes of HLA-Bw35.The effector/target-cell ratio was 10:1.

Cold-target inhibition experiments

CTLs from primary MLCs are polyclonal and may contain different population of CTLs directed against distinct antigenic determinants. Such CTLs can be subdivided into subsets of CTL specific either for shared determinants or for HLA-antigen subtypes (Breuning et al. 1983, Spits et al. 1982, Tekolf et al. 1982). Because it was already known that HLA-Bw35 can be subdivided into 2 subtypes (Goulmy et al. 1976), it was particularly interesting to test whether all third-party Bw35-positive target cells lysed by CTL 10 could inhibit the cytotoxic activity of these CTLs. The results of cold-target inhibition experiments are shown in Table 2. The stimulator cells completely inhibited the cytotoxic action of CTL 10. The addition of cold-target cells from the responder (cell no. 436) caused a reduction of the lysis of 'hot'-target cells.

Such a non-specific reduction of cytotoxic

Table 2.
Cold-target inhibition of CTL 10 by Bw35-positive cells of 6 donors.

Cold target cells added	HLA types A	B	C	DR	Subtype of Bw35	% Specific 202[a]	436[b]	^{51}Cr release of hot target cell 191	181	345	016	144	152
	no cold cells added					50.0	0.0	41.8	9.6	31.5	38.6	37.8	41.4
202	2,3	35,60	3,4	1,6	B	9.0		7.9					
436	2,3	35,60	3,4	6,7	C	22.7	0.1	20.3					
191	2,33	40,35	3,4	4,6	B	7.9		3.3					
181	24,32	40,35	3,4	4,6	C	16.3		15.2	6.7				
345	2,3	27,35	1,4	1,4	A	21.6		24.3		4.4			
016	11,32	51,35		4,6	A	30.5		22.4			14.7		
144	3,28	44,35	4,5	1,4	B	10.4		9.2				10.3	
152	2,3	62,35	3,4	1,3	B	7.0		2.6					7.0

The effector/'hot-target-cell ratio was 10:1. The cold/hot target-cell ratio was 50:1.
[a] Cells no. 202 are the stimulator cells of CTL 10 (Table 1).
[b] Cells no. 436 are the responder cells of CTL 10 (Table 1).

activity was seen in many different cold-target inhibition experiments (results not shown). Target cells not lysed by CTL 10 (cell no. 181) reduced the cytotoxic activity on 'hot'-target cells of the simulator to a similar degree. However, of 5 third-party HLA-Bw35-positive target cells lysed by CTL 10, only 3 (nos. 191, 144, 152; Bw35-'B' type) completely inhibited the lytic action of CTL 10. Two target cells (nos. 345, 016; Bw35-'A' type) did not inhibit the activity of CTL 10 against stimulator cells more than cold cells of the responder did. These results indicate that CTL 10 consists of at least 2 different sets of CTLs directed against distinct antigenic determinants associated with HLA-Bw35.

As for CTL 9, the one third-party cell lysed by this CTL (Figure 1) (cell no. 181) was able to completely inhibit the cytotoxic activity of CTL 9 against 'hot'-target cells of the stimulator (results not shown).

Cold-target inhibition of CTL 17 by Bw35-positive cells of 5 donors is shown in Table 3. Addition of cold stimulator cells (cell no. 345) completely inhibited the cytotoxic activity of CTL 17 against stimulator target cells and 2 third-party cells. The addition of cold responder cells (no. 265) caused some reduction of the kill, but a major part of the cytotoxic activity remained. Although CTL 17 showed cytotoxic activity on hot-target cells nos. 436, 181, 016, 334 and Rc3, none of these third-party Bw35-positive target cells was able to completely inhibit the cytotoxic activity of CTL 17 against stimulator cells. Moreover, when tested against third-party 'hot'-target cells of subtype 'C' (no. 436), the cytotoxicity of CTL 17 was more reduced by cold 'C-type' cells than by 'A-type' cells. Conversely, when tested on 'hot'-target cells of Bw35 subtype 'A' (no. 016), the cytotoxicity was more reduced by cold 'A-type' cells than by cold 'C-type' cells. These results indicate that CTL 17 consists of at least 2 subpopulations of CTLs, one of which reacts with HLA-Bw35 'A', the other with -Bw35 'C' type. However, the percentage of cytotoxicity obtained during these experiments is rather low (Table 3). A more detailed analysis of the various determinants recognized by the CTL 17 would require cloning of the subsets of CTL involved.

Discussion

In this paper, we report on the analysis of the

Table 3
Cold target inhibition of CTL 17 by Bw35-positive cells of 5 donors

Cold target cells added	HLA types A	B	C	DR	Subtype of Bw35	% Specific ^{51}Cr release of hot target cell 345[1]	265	436	181	016	334	Rc3
	no cold cells added					46 5	4 1	18 2	21 8	22 5	21 3	21 0
345	2,3	27[b],35	1,4	1,–	A	10 7		4 9		7 1		
265	2,3	27[b],35	1,4	1,4	B	31 2	–1 4	13 7		16 1		
436	2,3	35,60	3,4	6,7	C	28 8		4 5		14 0		
181	24,32	35,40	3,4	4,6	C	34 2		4 6	5 5	13 8		
016	11,32	35,51	–,–	4,6	A	35 4		8 1		8 2		
334	3,24	35,27[b]	1,4	4,–	A	18 0		9 7		4 5	4 9	
Rc3	2,2	35,44	4,5	5 8[c],8	A	24 5		9 2		11 3		4 2

The effector/'hot'-target cell ratio was 10 1 The cold/hot target-cell ratio was 100 1
[1] Cells no 345 are the stimulator cells of CTL 17 (Table 1)
[b] These cells carry the same CTL-defined subtype of B27, "W type" (Breuning et al 1982, Breuning et al 1983)
[c] LB5 8 is a Leiden local assignment of a DR5 split

specificity of 5 CTLs raised in combination of HLA-A, -B and -C-identical, -D/DR-different responder and stimulator cells CTL 9 can be used to define a rare variant of HLA-Bw35, as contrasted with CTL 10 that lysed almost all Bw35-positive target cells tested (Figure 1) However, by cold-target inhibition experiments we found that CTL 10 consisted of at least 2 populations of CTLs directed against different antigenic determinants associated with HLA-Bw35 (Table 2) This can be explained by the observation that 2 additional CTLs (CTLs 18 and 19) subdivide the panel of Bw35-positive cells recognized by CTL 10 in 2 groups (Figure 2) Apparently, one subset of CTL 10 recognized an antigenic determinant shared between almost all HLA-Bw35-positive cells ('A' as well as 'B' types), the other subset recognized HLA-Bw35 B-type cells only The results of cold-target inhibition experiments support this interpretation because cold-target cells of Bw35-B subtype completely inhibited the cytotoxic activity of CTL 10, whereas cells of subtype A did not (Table 2) By the use of 4 CTLs, the panel of HLA-Bw35-positive target cells can be subdivided into 3 groups (A, B and C in Figure 2) Cells that were only weakly lysed by CTLs 10 and 19 may represent a fourth variant of HLA-Bw35 Until now, these subtypes have not been recognized by HLA-typing sera (Bradley et al 1978, and unpublished observations)

Still further complexity is introduced by the

Table 4
Analysis of two HLA Bw35 subtypes at the clonal level

'Clone no	E/T[1] ratio	Target cells Rc 3 (Bw35 'B")	Rc 8[c] (Bw35 "A")
A22	40 1	– 5[b]	+13
B11	10 1	+ 4	+28
C7	40 1	+36	– 3
D3	7 1	+23	+ 4
D22	20 1	+23	+21
A12	7 1	+ 8	+12

[1] Effector/target cell ratio
[b] Percentage lysis
[c] For HLA phenotypes of target cells Rc3 and Rc8, see Table 1
The 34 "clones" that showed lysis lower than 10% are not listed in this Table

fifth CTL, CTL 17 HLA-Bw35 emerged as the target antigen for this CTL after subdividing the panel of target cells using CTLs 18 and 19 In a comparable situation, we have been able to identify, thanks to the subdivision of HLA-B27 by CTLs (Breuning et al 1982), the target antigen for a CTL clone which was cross-reactive between subsets of HLA-B7-, -B27 and -B40-positive target cells (Breuning et al 1984) The responder used for the generation of CTL 17 was Bw35 subtype 'B', the stimulator Bw35 subtype 'A' However, CTL 17 were cytotoxic for both Bw35-'A' and -'C' target cells (Figure 2) Does this mean that the stimulator cells of CTL 17 expressed a separate type of Bw35, including both 'A' and 'C'? This is unlikely, because the stimulator cells (no 345) were not lysed by CTL 9 which defined Bw35 subtype 'C' Therefore, the antigenic determinants recognized by CTL 17 may be different from Bw35 'A', 'B' or 'C' Furthermore, cold-target inhibition experiments showed that CTL 17 comprise at least 2 populations of CTLs with different specificities

We can conclude that the CTLs showed a series of antigenic determinants hitherto not detected by antisera Although closely associated with HLA-Bw35, these antigenic determinants are as yet not precisely defined More accurate typing for such CTL-defined antigenic determinants can be achieved in two ways First, several sets of CTLs from different combinations of HLA-A, -B and -C-identical donors, which recognize the same antigenic determinant, can be used in parallel This approach has been applied to the typing of CTL-defined subtypes of HLA-B27 (Breuning et al 1983) Second, the CTLs may be expanded, and cloned by limiting dilution, a technique that has already provided us with exquisitely specific, highly cytotoxic CTL clones recognizing subtypes of HLA-A2 and B7 (Spits et al 1982)

The results presented here do not formally prove that the antigenic determinants recognized by the CTLs and the HLA-Bw35 antigen are carried by one and the same molecule However, two subtypes of HLA-Bw35 have also been observed by immunoprecipitation and subsequent electrophoresis of HLA molecules (H Ploegh, personal communication) Analogous conclusions drawn from CML data have been confirmed by biochemical analysis of HLA-A2 (Biddison et al 1980a, Biddison et al 1980b, Krangel et al 1983, Spits et al 1982, Vasilov et al 1983) of which 4 subtypes have now been delineated (van der Poel et al 1983) Similarly, CTL-defined subtypes of HLA-B27 (Breuning et al 1982) have been confirmed by biochemical studies (Molders et al 1983, Vasilov et al 1983)

Little is known about CTL-defined subtypes of HLA antigens and their association with various diseases Subtypes of HLA-B27 have been investigated in patients with ankylosing spondylitis (Breuning et al 1982) Both subtypes were found among such patients, indicating that the antigenic determinant recognized by the subtype-specific CTLs, although functional in HLA-restricted recognition of virus-infected cells (Breuning et al 1982), may not be relevant for the occurrence of the disease However, subtypes of HLA-Bw35 may become interesting in this regard, because a clear association has been found between Bw35 and De Quervain's thyroiditis, a disease probably caused by a virus (Nyulassy et al 1975, Ryder et al 1979)

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References

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

Biddison, W E , Krangel, M S , Strominger, J L , Ward, F E , Shearer, G M & Shaw, S (1980b) Virus immune cytotoxic T cells recognize structural differences between serologically indistinguishable HLA A2 molecules *Hum Immunol* **1**, 225–232

Biddison, W E , Shearer, G M & Shaw, S (1981) 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

Bradley, B A , Goulmy E , Screuder, I & van Rood, J J (1978) Targets for killer T cells In *Human Lymphocyte differentiation,* eds B Serrou & C Rosenfield, Elsevier/North-Holland, New York, pp 231–240

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

Breuning, M H , Breur B S , Engelsma, M Y , Huis, B & Ivanyi, P (1983) Specificity of anti HLA B27 CTL *Tissue Antigens* **22**, 267–282

Breuning, M H , Spits, H , De Vries, J E & Ivanyi, P (1984) A cloned cytotoxic T lymphocyte (CTL) line recognizing a subtype of HLA B27 *Hum Immunol* **9**, 231–242

Breuning, M H , Breur, B S , Engelsma, M Y & Ivanyi, P (1984 I) Activation of cytotoxic T lymphocytes in HLA A , B and -C identical responder stimulator pairs I Variations in the generation of anti-class II CTL in primary MLC *Tissue Antigens* **24**, 81–89

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

Goulmy, E , van Leeuwen, A , Blokland, E , van Rood, J J & Biddison, W E (1982) Major histocompatibility complex restricted H Y-specific antibodies and cytotoxic T lymphocytes may recognize different self determinants *J Exp Med* **155**, 1567–1579

Goulmy E (1982) Methodology and clinical relevance In *HLA Typing,* eds S Ferrone & B G Solheim, CRC Press, pp 105–109

Horai, S , van der Poel, J J & Goulmy, E (1982) Differential recognition of the serologically defined HLA A2 antigen by allogeneic cytotoxic T cells *Immunogenetics* **16**, 135–142

Kato, S , Ivanyi, P , Lacko, E , Breur, B , Du Bois, R & Eijsvoogel, V P (1980) 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

Krangel, M S , Biddison, W E & Strominger, J J (1983) Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes II Variant DK 1 Evidence for a discrete CTL recognition region *J Immunol* **130**, 1856–1862

Molders, H H , Breuning, M H , Ivanyi, P & Ploegh, H (1983) Biochemical analysis of variant HLA B27 antigens *Hum Immunol* **6**, 111–117

Nyulassy, S , Hnilica, P & Stefanovic, J (1975) The HLA system and subacute thyroiditis A preliminary report *Tissue Antigens* **6**, 105–106

Ryder, L P , Andersen, E & Svejgaard, A (1979) *HLA and disease registry,* 3rd report Munksgaard, Copenhagen

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

Tekolf, W A , Biddison, W E , Aster, R D & Shaw S (1982) Two subgroups of HLA-Bw44 defined by cell mediated lympholysis that differ in Bw44 expression on platelets and in patterns of genetic linkage disequilibrium *J Immunol* **129**, 1474–1478

Vasilov, R G , Hahn, A , Molders, H H , van Rood, J J , Breuning, M H & Ploegh, H (1983) Analysis of class I antigens by two dimensional gelelectrophoresis *Immunogenetics* **17**, 333–356

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

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