# Existence of mature human CD4+ve T cells with genuine self class I restriction.

Goulmy, E.A.J.M.; Bueger, M.M. de; Bakker, A.

Short paper

Marleen De Bueger, Astrid Bakker and Els Goulmy

Department of Immunohematology and Bloodbank, University Hospital Leiden, Leiden

# Existence of mature human CD4+ T cells with genuine class I restriction

A human T cell receptor (TcR) α/β CD4+CD8− T cell clone (R416) is reactive with the minor histocompatibility antigen H-Y in the context of major histocompatibility complex (MHC) class I and not class II molecules. Therewith clone R416 violates the so-called specificity association of mature TcR α/β+ T cells. R416 displays H-Y-specific, HLA-A2-restricted proliferation as well as cytotoxicity *in vitro*. Its fine specificity is identical to that of a classical H-Y-reactive CD4−CD8+ MHC class I-restricted CTL clone, showing that CTL expressing either CD4 or CD8 can display identical antigenic specificities. Exploiting the MHC class I restriction of this CD4+ T cell clone, it was found that interaction of CD4 with non-TcR-bound MHC class II molecules does not contribute to antigen specific activation of these CD4+ T cells. This coreceptor-mismatched T cell clone was not generated *in vitro* but obtained by expansion of CD8-depleted peripheral blood mononuclear cells of a female who had been immunized against H-Y. The existence of such MHC class I-restricted mature TcR α/β+ T cells expressing CD4 and not CD8 is relevant because it indicates that the generally accepted model for thymic selection, in which the TcR specificity *alone* determines CD4/CD8 expression of mature thymocytes, may not be absolute.

## 1 Introduction

A rather strict association exists between the expression of the CD4/CD8 accessory molecules and the MHC restriction of mature TcR α/β-expressing T cells [1, 2]. The vast majority of CD8-expressing T cells recognize antigen presented by MHC class I, whereas virtually all CD4+ T cells react with MHC class II gene products. Still, numerous CTL lines and clones of the CD8 phenotype exhibiting class II restriction have been described both in man and in mouse [3–6]. In contrast, CD4-expressing T cells recognizing antigen in the context of MHC class I have only rarely been described [6–9]. Most CD4+ T cell populations observed in primary T cell responses against class I alloantigens do recognize MHC class I determinants but in a classical class II-restricted fashion [6, 9].

Here we report to our knowledge for the first time on an antigen-specific, MHC class I-restricted CD4+CD8− T cell clone generated by *in vivo* priming. Its phenotypic and functional characterization as well as the contribution of MHC class II and CD4 molecules to activation of this TcR/coreceptor-mismatched T cell clone are analyzed and discussed. Furthermore, we discuss the impact of the existence of a subset of mature CD4+ class I-restricted, alloantigen-reactive T cell subset on the mechanism of thymic selection.

[I 9983]

* This work was supported by the Macropa Foundation and the J.A. Cohen Institute for radiopathology and radiation protection (IRS).

**Correspondence:** Marleen De Bueger, Department of Immunohematology, Room L3-37, University Hospital Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

## 2 Materials and methods

### 2.1 T cell clones

PBMC of a female donor (HLA-A2, B44, -B60, -Cw3, -DR4, -DR6) were depleted of >95% CD8+ cells using anti-CD8 mAb-coated magnetic beads (Dynal A.S., Oslo, Norway). This female had been selected because of known immunization for H-Y as a result of multiple transfusion and unsuccessful transplantation of HLA-identical *male* bone marrow [10]. CD4-enriched PBMC ($4 \times 10^6$) were cocultured for 10 days with $4 \times 10^6$ irradiated (2000 rad) HLA-identical male PBMC in culture medium (RPMI 1640 supplemented with L-glutamine, antibiotics and 15% pooled human serum). After a second stimulation in the presence of highly purified IL 2 (Biotest, Dreieich, FRG), the generated T cell line was cloned at 0.5 cells/well in U-bottom microtiter plates containing $10^4$ PBMC (3000 rad) of the original stimulator cells in medium with 20 U/ml rIL 2. Out of 117 growing clones 30 were expanded and analyzed for H-Y specific proliferation. Clonality was confirmed by the presence of a single TcR gene rearrangement as measured by polymerase chain reaction (PCR). Other clones used in this study were the CD8+ clones 1R35, and H-Y-specific, HLA-A2-restricted CTL clone [10], and R26, an H-Y-specific, B60-restricted cytotoxic and proliferative T cell clone, both previously obtained from undepleted PBMC of the same female. The cytolytic clone 36 (anti-DRw13) and the proliferative clone 2616 (anti-DR2) class II alloreactive CD4+ control clones were kindly supplied by Dr. A. Termijtelen (University Hospital Leid-



0014-2980/92/0303-0875$3.50+.25/0

en). All T cell clones were maintained in medium with 20 U/ml rIL 2 by weekly restimulation using allogeneic PBMC.

### 2.2 Proliferation assay

Cells ($10^4$) were cultured with $10^5$ PBMC (2000 rad) or $0.25 \times 10^5$ EBV-lymphoblastoid cell line (LCL) (7500 rad) in a volume of 150 µl medium in 96-well flat-bottom plates for 48 h. Triplicate cultures were labeled with 1 µCi [$^3$H]dThd and after 16 h assayed for isotope incorporation in a liquid scintillation counter. When inhibition of proliferation by mAb was studied, responder or stimulator cells were preincubated for 30 min at 20 °C with 50 µl of ascites fluid at several dilutions.

### 2.3 $^{51}$Cr-release assays

$^{51}$Cr-release assays were performed without modifications. In blocking experiments either effector or target cells were preincubated in 50 µl ascites fluid at the indicated dilution for 20 min at 20 °C. Besides panels of EBV-LCL and PHA-induced T cell blasts, the male lymphoblastoid T cell line CCRF-HSB-2 expressing HLA-A1, -A2, -B12 and -Cw2 but no class II antigens were used as target cells [11].

### 2.4 Lymphokine measurement, mAb and immunofluorescence analysis

Using standard ELISA assays concentrations of TNF-α, IFN-γ, IL 4, IL 5 and IL 6 were measured in T cell supernatants after 24 h of antigen-specific or Con A-induced stimulation of the T cell clones.

HLA-reactive mAb used were W6/32, B9.12.1 (anti-class I framework), B1.1.G6 (anti-$\beta_2$-microglobulin), 7.5.10.1 (anti-class II framework) and B8.11.2 (anti-DR non-polymorphic). The FK18 anti-CD8 mAb and 8.3.14.1 anti-LFA-1 (CD11a) mAb were produced in this laboratory. CD4-reactive mAb used included OKT4, OKT4A, RIV6, RIV7 (RIVM, Utrecht, The Netherlands) and Leu-3A. The mAb 15E8 (anti-CD28 , anti-Leu-8/p80, UCHL1 (anti-CD45R0), WT31 (anti-TcR α/β) and 11F2 (anti-TcR γ/δ) were also used for cell surface phenotyping.

Cell surface densities were quantified using a series of FITC-coated beads as standards (Flow Cytometry Standard Corp. Res., Triangle Park, NC). Relative cell surface densities (represented as arbitrary unit per cell) were measured for CD4 and LFA-1α.

## 3 Results and discussion

### 3.1 Phenotypic characterization

A proliferative as well as cytotoxic CD4$^+$ T cell clone reactive with the male specific antigen H-Y was obtained from CD8-depleted female PBMC. The phenotype of this clone R416 as determined by FCM analysis was 100% CD4$^+$, TcR α/β$^+$, CD45R0$^+$, LFA-1α$^{++}$ and 0% CD8$^+$, TcR γ/δ$^+$, p80/Leu-8$^+$ and CD28$^+$. CD4 expression was not aberrant, as judged by CD4 cell surface density measured with five mAb known to bind to distinct epitopes of the CD4 molecule (not shown). CD8 α/α, but not functional CD8 α/β$^-$ dimer expression could be induced in the presence of IL 4. LFA-1α (CD11a) expression was found to be relatively high on R416 cells (37 U/cell) when compared to 20 other long term T cell clones (mean: 21 ± 6 U/cell). Upon Con A stimulation, R416 cells produced TNF-α, IFN-γ, but no detectable levels of IL 4, IL 5 or IL 6 (data not shown).

### 3.2 Antigenic specificity

The antigenic specificity and HLA restriction of clone R416 were determined using PBMC and EBV-LCL of HLA-typed donors of known sex ($n = 80$) as stimulator cells for proliferation, and EBV-LCL and PHA blasts ($n = 30$) as

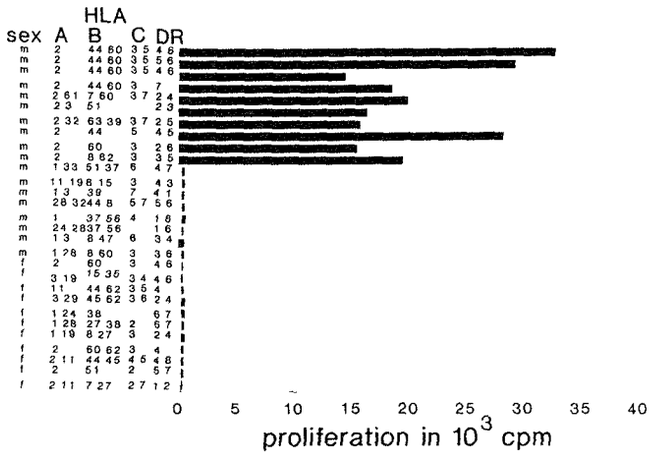


*Figure 1* Antigen-specific proliferation of CD4$^+$ clone R416 measured against PBMC of a panel of HLA-typed male and female donors.

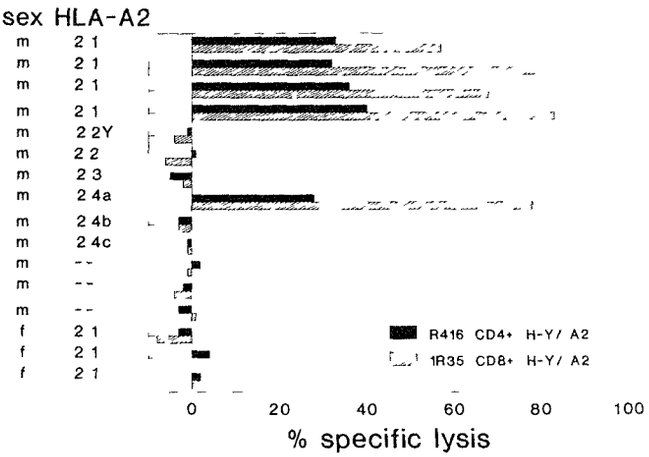


*Figure 2* Comparison of a CD4$^+$ and a CD8$^+$ H-Y/A2-specific CTL clone in their capacity to lyse male EBV-LCL expressing variant HLA-A2 molecules. Variant alleles correspond to the following sequence nomenclatures. A2 2Y: A*205, A2 4a: A*0206, A2 4b: A*0207, A2 4c. A*0208, A2.3 (Veef) and A2.2 (Thung) have not been sequenced. % Lysis at $E_{R416}$. T = 20 and $E_{1R35}$. T = 1 are presented.

target cells in $^{51}Cr$ release assays As shown in Fig 1 only male HLA A2+ cells induced proliferation The fine specificity of this CD4+ T cell clone was compared to that of a classical H-Y/A2 reactive CD8+ CTL clone previously obtained from the same donor [10] Upon assay of cytotoxicity towards EBV-LCL of male individuals expressing HLA A2 variant molecules, it was found that mutations in the HLA A2 molecule affected recognition of the H-Y/A2 complex by both the CD8+ and the CD4+ CTL clone in the same way (Fig 2) It is well established that co expression of the appropriate CD4/CD8 accessory molecule increases the avidity of a mature T cell to interact with an antigen-presenting cell (reviewed in [12]) The question, however, of whether the expression of the 'wrong' co-receptor could change the antigenic specificity displayed by the T cell was only recently brought up [13 14] Here, a CD8+ and a CD4+ T cell clone are shown to display undistinguishable fine antigenic specificities

### 3.3 Role of CD4 in MHC class I-restricted T cell activation

Antibody inhibition experiments were performed to confirm the MHC class I restriction and to examine the role of class II in activation of R416 As shown in Fig 4, anti-HLA class I mAb indeed inhibited proliferation of R416 to a similar extent as that of a CD8+ H-Y-specific HLA-B60-restricted T cell clone Anti-class II mAb did not affect H Y-induced proliferation of R416 at all, whereas proliferation of a control class II allospecific CD4+ clone was fully abrogated (Fig 4) Lysis of H-Y/A2+ target cells by R416 was also MHC class II independent The HLA A2+ male T cell line HSB-2 [11] devoid of cell surface class II expression was lysed to the same extent as class II-expressing PHA T cell blasts (Fig 3) Also, anti-class II mAb did not inhibit antigen specific lysis of any of the target cells (not shown)

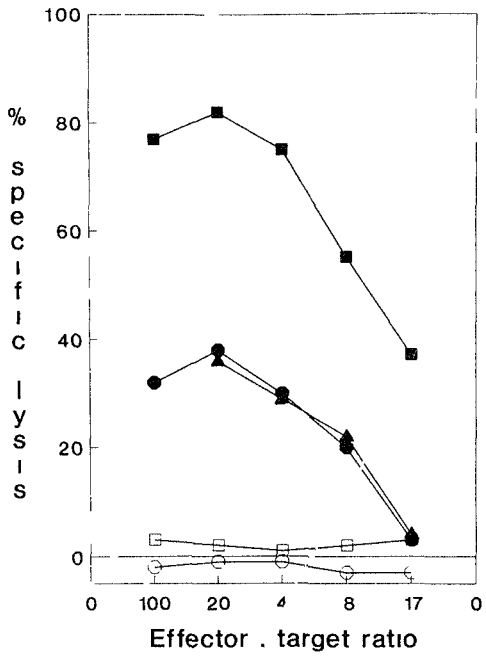


*Figure 3* Cytolytic response of the anti H-Y/A2 CD4+ clone R416 towards EBV-LCL (■) and PHA T cell line (●) of a male A2+ donor (HLA A2 -B12 -Cw5 DR4 DR5) and of a male A2+ T cell line devoid of class II (▲ HLA-A1 A2 -B12 -Cw2) measured in a 4-h $^{51}Cr$-release test Female control EBV-LCL (□) and PHA blasts (○) were not lysed

Taken together, class II molecules on APC are not involved in H-Y/A2-specific activation of R416 This suggests that the binding of CD4 to class II molecules, that do not at the same time serve as TcR ligand, does not augment TcR-mediated activation of these CD4+ T cells This contrasts with a previous study in which activation of a constructed class I-restricted murine T cell hybrid was augmented when CD4 was allowed to interact with class II on APC [15] Reports on CD4+CD8+ murine T cell clones, on the other hand, suggested that the relative contribution to T cell activation of the 'unmatching" CD4 or CD8 molecule, which is incapable of binding to the TcR ligand, may be small [16] or absent [17] as observed with R416

In an attempt to analyze the involvement of the CD4 molecule in H-Y/A2 specific activation of R416, anti-CD4 mAb were tested for their inhibitory effect on antigen-induced proliferation and cytotoxicity of R416 In Table 1 a representative experiment (out of four) is shown, indicating that all anti CD4 mAb inhibited proliferation of R416, though never completely as observed for a class II restricted CD4+ T cell clone This partial inhibition of R416 proliferation (Table 1) and cytotoxicity (not shown) in the presence of anti-CD4 mAb could be due to either (a) nonspecific inhibition, (b) steric hindrance, (c) socalled "direct negative signalling" via CD4 [18], or (d) reflect abrogation of a physical association between the CD4 and TcR/CD3 molecules in the absence of class II molecules [19] Whatever the mechanism underlying this inhibition, the availability of this naturally occurring CD4+, class I restricted T cell clone may be helpful to further dissect the binding *vs* signaling functions (reviewed in [12]) of the CD4 molecule in T cell activation

### 3.4 Relevance of a CD4+ MHC class I-restricted mature T cell subset

The detection of a T cell clone of such rare phenotype in this study could be the consequence of removal of the major population of class I-reactive T cell precursors by CD8 depletion of PBMC, thereby allowing the precursors of a low frequency CD4+ T cell subset to be expanded and detected As we did not screen other individuals for T cells of this specificity/phenotype, it cannot be exluded that detection might be related to the history of aplastic anemia of this donor We feel that selective *in vitro* expansion, which in numerous other studies has revealed T cell subsets of low frequency [20, 21] is most likely responsible for the visualization of CD4+, class I-restricted mature T cells in this study

It is an established view that the TcR dictates thymic development in such a way that only those thymocytes are allowed to mature whose accessory molecules match the class of MHC molecules recognized by their TcR [22] However, the developmental stage, as well as the molecular nature of the triggers underlying this phenomenon are still unclear [23, 24] The existence of mature CD4+CD8− T cells which *in vitro* display class I restriction, can only be

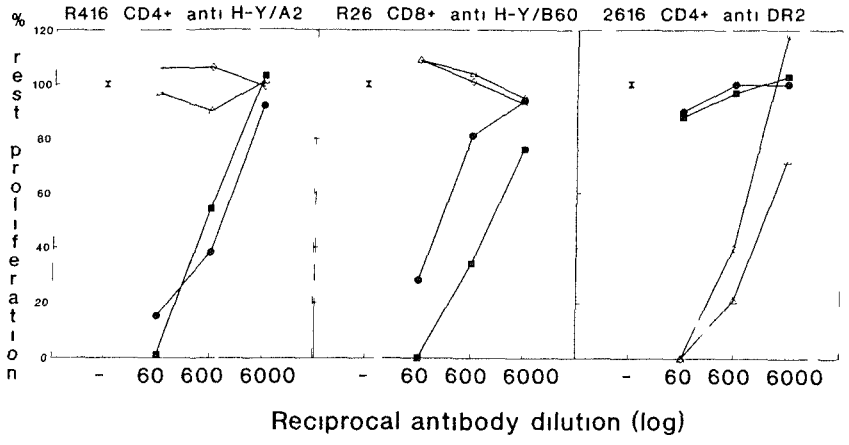


*Figure 4* Effect of anti HLA class I mAb [W6/32 (■) B9 12 1 (●)] and anti class II mAb [7 5 10 2 (◇) B8 11 2 (△)] on the proliferative responses of the anti H Y/A2 CD4+ clone R416 a class I restricted CD8+ and a class II specific CD4+ clone Mean values of triplicate cultures are given as percentages of the unblocked response (always >20 000 cpm) Indicated antibody dilution represents the final concentration in the wells

**Table 1.** Effect of antibodies against CD4 and CD8 on antigen specific proliferation of CD4+ H Y/A2 specific clone R416 and control clones

| | | % Specific inhibition in the presence of | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Clone | RIV6 | | | | RIV7 | | | | OKT4 | | | | OKT4A | | | | Leu-3A | | | | FK18 | | | |
| Code | CD4/CD8 specificity | 1 | 2 | 3 | 4[a] | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 2616 | CD4, anti-DR2 | 67 | 67 | 53 | 46 | 87 | 81 | 63 | 37 | 88 | 31 | 2 | 0 | – | – | 97 | 9 | – | 53 | 13 | – | 0 | 0 | 0 | 0 |
| R26 | CD8, anti-H-Y/B60 | 0 | 0 | 0 | 0 | 10 | 10 | 10 | 10 | 15 | 23 | 15 | 2 | – | – | 0 | 0 | – | 7 | 8 | – | 87 | 87 | 81 | 61 |
| R416 | CD4, anti-H-Y/A2 | 38 | 52 | 50 | 32 | 54 | 44 | 43 | 32 | 54 | 7 | 17 | 14 | – | – | 46 | 20 | – | 49 | 15 | – | 6 | 10 | 13 | 5 |

a) 1 2 3 and 4 represent 1/20 1/200 1/2000 and 1/20 000 dilutions respectively of dialyzed ascites fluid – ND

reconciled with this view if one assumes that their TcR cross-reacts with MHC class II molecules and thus maturation into CD4+ mature thymocytes could result from TcR interaction with thymic MHC class II [14] Such MHC class I and II cross-reactive TcR have been described for mature TcR $\alpha/\beta^+$ T cells [4, 13] The fact that the process of thymic selection yields at least some mature CD4+CD8 T cells whose TcR seems strictly MHC class I reactive could suggest that the MHC restriction of the TcR may not be the *only* factor to determine the fate of immature thymocytes, though we cannot exclude that cells with this receptor have been selected by self class II MHC molecules

*We thank Dr T Ottenhof and Prof C Melief for critical comments*

Received September 26, 1991, in final revised form November 27 1991

## 4 References

1 Meuer S C Schlossman S F and Reinherz E L *Proc Natl Acad Sci USA* 1982 *79* 4395
2 Swain S L *Immunol Rev* 1983 *74* 129
3 Spits H Yssel H Thompson A and De Vries J E *J Immunol* 1983 *131* 678
4 Schilham M W, Lang R Benner R Zinkernagel R M and Hengartner H *J Immunol* 1986 *137* 2748
5 Shinohara N Hozumi N Watanabe M Bluestone J A Johnson Leva R and Sachs D H *J Immunol* 1988 *140* 30
6 Golding H Mizuochi T McCarthy S A Cleveland C A and Singer A *J Immunol* 1987 *138* 10
7 Blomenberg N Russo C Ferrone S and Dupont B *Immunogenetics* 1984 *19* 39
8 Strassman G and Bach F H *J Immunol* 1984 *133* 1705
9 Mizuochi T Ono S Malek T K and Singer A *J Exp Med* 1986 *163* 603
10 Goulmy E Termijtelen A Bradley B A and Van Rood J J *Nature* 1977 *266* 544
11 Krangel M S *J Exp Med* 1986 *163* 1173
12 Parnes J R *Adv Immunol* 1989 *44* 265
13 Wallace V A Ohashi P S Hengartner H and Mak T W *J Immunol* 1990 *20* 2471
14 Teh H-S Garvin A M, Forbush K A Carlow D A Davis G B Littman D R and Perlmutter R M *Nature* 1991 *349* 241
15 Greenstein J L Malissen B and Burakoff S J *J Exp Med* 1985 *162* 369
16 Jones B Khavari P A Conrad P J and Janeway C A *J Immunol* 1987 *139* 380
17 Fazekas de St Groth B Gallagher P F and Miller J F A P *Proc Natl Acad Sci USA* 1986 *83* 2594
18 Fleischer B Schrezenmeier H and Wagner H *J Immunol* 1986 *136* 1625
19 Owens T and Fazekas de St Groth B *J Immunol* 1987 *138* 2402
20 Rivas A Koide J Laus B and Engleman E G *J Immunol* 1990 *145* 470
21 Bourgault I Gomez A Comard E Picard F and Levy J P *J Immunol* 1989 *142* 252
22 Teh H S Kisielow P Kishi H Scott B Uematsu Y Bluthmann H and Von Boehmer H *Nature* 1988 335 229
23 Robey E A Fowlkes B J Gordon J W Kioussis D Von Boehmer H Ramsdell F and Axel R *Cell* 1991 *64* 99
24 Guidos G J Danska J S Fathman G C and Weissman I L *J Exp Med* 1990 *172* 835