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Monoclonal antibodies raised against engineered soluble mouse T cell receptors and specific for $V_\alpha 8$-, $V_\beta 2$- or $V_\beta 10$-bearing T cells*

We have characterized a panel of monoclonal antibodies (mAb) produced by immunizing rats with two distinct soluble mouse α/β T cell receptor (TcR). Fifty mAb were found to react with the corresponding surface-bound TcR. Such observations suggest that the soluble TcR molecules used as immunogen are folded in a conformation similar to the native structure. Furthermore, the binding to T cells of four antibodies was found to correlate with the expression of the $V_\alpha 8$, $V_\beta 2$ or $V_\beta 10$ gene segments. Finally, staining of T lymphocytes from various mouse strains suggests that (a) the two anti-$V_\alpha 8$ antibodies recognize different epitopes, and each on only a fraction of $V_\alpha 8^+$ cells; (b) the anti-$V_\beta 10$ mAb identifies a $V_\beta 10$ polymorphism among mouse strains, and (c) T cells expressing the $V_\beta 2$ or $V_\beta 10$ gene segments are not subject to major clonal deletion events induced by the major histocompatibility complex class II and Mls products which were tested.

1 Introduction

The TcR is made up of two chains, α and β, each with a C region and a clonally variable V region. The genes coding for TcR V regions are formed by the somatic assembly of V, D (in the case of TcR β) and J gene segments. Operationally, the V gene segments can be grouped into distinct subfamilies based upon sequence similarities [1, 2]. For instance, the mouse V_β gene segments have been organized into at least 19 V_β subfamilies, most of which contain one member [3, 4], whereas the approximately 100 mouse V_α gene segments have been grouped into at least 16 predominantly multimembered V_α subfamilies [5].

Considerable progress has been made in the analysis of the mechanisms by which the V regions of the TcR α and β chains recognize peptides bound to the MHC class I and class II products [6]. However, a complete understanding of MHC-restricted antigen recognition will require the production of soluble TcR molecules amenable to X-ray crystallography. We have constructed soluble α/β TcR by shuffling the TcR V and C domains to the C region of Ig κ L chain ([7]; Necker, A. et al., unpublished results). To gain insight into the folding of these soluble molecules, we have raised a panel of mAb against two distinct soluble α/β TcR. Here, we show that most of these mAb recognize the corresponding surface-bound TcR and that the binding to T cells of four of these antibodies correlates with expression of the $V_\beta 2$, $V_\beta 10$ or $V_\alpha 8$ gene segments.

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2 Materials and methods

2.1 Cells

CW3/1.1. is a $CD8^+$ CTL clone isolated from a DBA/2 mouse and specific for H-$2K^d$ plus a peptide corresponding to region 170–182 of the HLA-CW3 molecule [8]. X63.Ag8.653 is a derivative of the X63 mouse myeloma that does not produce any Ig chains [9]. DO-11.10 is a $CD4^+$ T cell hyridoma [10]. DOIS19 and $58\alpha^-\beta^-$ are two variants of the DO-11.10.7 hybridoma which do not express functional TcR β or TcR α/β genes, respectively [11]. DC27.1 is a T cell transfectant expressing a K^b-specific α/β TcR derived from the KB5-C20 CTL clone [12]. The panel of T cell hybridomas used to characterize the specificity of the various mAb has been previously described [13].

2.2 Plasmid construction and transfection

Plasmids pCA212 and pAC1 encode soluble $V_\alpha C_\alpha C_\kappa$ and $V_\beta C_\beta C_\kappa$ chains, respectively [7]. The TcR V genes used in the pCA212 and pAC1 TcR/Ig chimeric constructions originate from the KB5-C20 CTL clone. An identical pair of plasmids was constructed using the TcR V genes expressed by the CW3/1.1 CTL clone. Briefly, the KB5-C20-derived V_β cassette of pAC1 has been replaced with a 6.5-kb Bam HI fragment derived from a genomic library made from the CW3/1.1 cells and corresponding to the productively rearranged CW3/1.1 V_β gene. Similarly, the KB5-C20-derived V_α cassette of plasmid pCA212 has been replaced with a 4-kb Eco RV fragment derived from the same genomic library and corresponding to the productively rearranged V_α gene. A plasmid containing the native (membrane-bound) TcR β CW3/1.1 gene was constructed as previously described for the KB5-C20 TcR β gene [12].

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The native CW3/1.1 TcR α chain was expressed as a full-length cDNA, after cloning in the vector pHβAPr-1 neo [14]. Transfections of B and T cell recipients with protoplasts and selection of the transfectants were as previously described [7, 12].

2.3 Cytofluorometric analysis and mAb

Cytofluorometric analysis was performed as previously described [15–17]. The following mAb were used : 145-2C11 (2C11), anti-CD3 ε [18]; GK1-5, anti-CD4 [19]; 53.6.72, anti-CD8 [20]; 44.22.1, anti-V_β6 [21]; RR3-15, anti-V_β11 [22]; H139.52.2, anti-mouse C_κ [23].

2.4 Metabolic labeling, immunoprecipitation and gel electrophoresis

Two million transfected B cells were washed twice in methionine-free medium, preincubated for 1 h in the same medium supplemented with 10% dialyzed FCS and then biosynthetically labeled with 0.3 mCi ^{35}[S]methionine. After 10 h SN were harvested, immunoprecipitated and analyzed by SDS-PAGE on a 10% gel [24].

2.5 Purification of TcR/Ig chimeric proteins

Since no antibody against the CW3/1.1 TcR was available at the initiation of these studies, soluble CW3/1.1 TcR molecules were purified on an anti-C_κ immunoadsorbent column made with the H139.52.2 mAb. After passage of culture SN, the column was extensively washed and then eluted with 0.1 M HCl glycine, pH 3.2. Eluted fractions were neutralized, dialyzed against PBS and concentrated on an Centricon 30 filtration unit (Amicon, Lexington, MA).

2.6 Derivation of mAb against TcR/Ig chimeric proteins

mAb against the CW3/1.1 soluble TcR were raised as previously described for the KB5-C20 soluble TcR [7]. The antibody-producing cell lines described in this report are freely available for non-commercial purposes.

2.7 Mice and RFLP analysis

The mouse strains derived from wild stocks were maintained as previously described [16]. V_β10 genotyping was performed as described [25].

3 Results

3.1 Characterization of a panel of mAb reactive with a soluble TcR expressing the V_α8.P71 and V_β10 gene segments

We have recently engineered a soluble form of a mouse α/β TcR by shuffling its V and C domains to the C region of an Ig κ L chain [7]. A similar strategy was used to construct a second soluble TcR containing the V_α (V_α8.P71-J_αpHDS58, nomenclature according to [26]) and V_β (V_β10-D_β1-J_β1.2, nomenclature according to [27–28]) genes from the CW3/1.1 CTL clone [8]. Three cell lines expressing V_αCW3/1.1$C_\alpha C_\kappa$ (AN 17.1), V_βCW3/1.1 $C_\beta C_\kappa$ (AN 9.8), or a combination of both genes (AN 19.4) were established after transfection of the mouse myeloma X63.Ag8.653. As previously observed for the KB5-C20 soluble TcR chains [7], both V_αCW3/1.1 $C_\alpha C_\kappa$ and V_βCW3/1.1 $C_\beta C_\kappa$ chains were secreted and found to migrate as single bands with an M_r of about 50 kDa (Fig. 1, panel anti-C_κ). To analyze if the soluble CW3/1.1 TcR chains were folded in a conformation similar to the one they adopt at the cell surface, the material secreted by the AN19.4 double-chain transfectant was purified on an anti-C_κ immunoadsorbent and used to derive a panel of rat mAb.

Fifty mAb were found to be capable of reacting with a transfectant derived from the 58α$^-$β$^-$ variant and expressing the native, surface-bound CW3/1.1 TcR (transfectant 58α$^-$β$^-$/TcR CW3/1.1, Fig. 2). None of these mAb stained the untransfected 58α$^-$β$^-$ recipient cells or unrelated TcR molecules expressed at the surface of the DO-11.10 hybridoma. Therefore, the epitopes recognized by this panel of mAb are probably encoded by the CW3/1.1 V_α/V_β module. When analyzed on two transfectants derived from DO-11.10 and expressing at their surface, after pairing with the endogeneous DO-11.10 TcR chains, either the CW3/1.1 TcR α (DO-11.10/TcRαCW3/1.1) or TcRβ (DO-11.10/TcRβ CW3/1.1) chains, most of the selected mAb were found to segregate into two distinct sets. One set of mAb, represented by the B21.13 (data not shown) and B21.14 (Fig. 2) clones, only reacted with the cell line expressing the product of the CW3/1.1 TcR α gene. The other set,

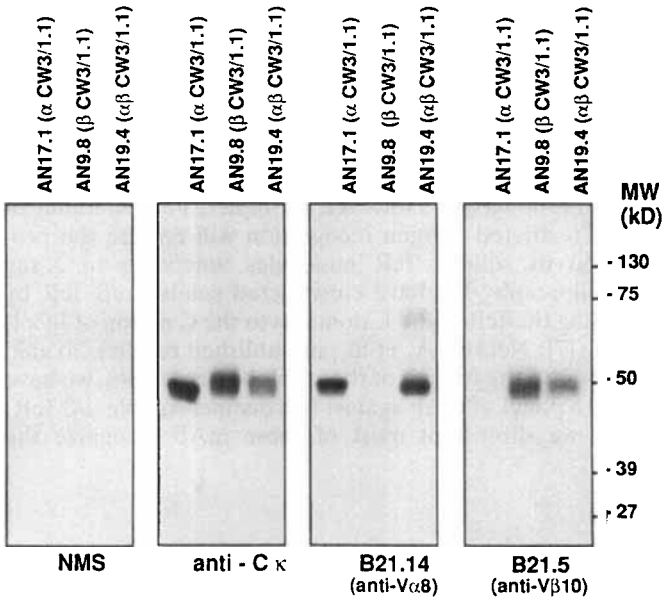

Figure 1. Immunoprecipitation of TcR/Ig chimeric molecules secreted by the AN17.1 (V_αCW3/1.1$C_\alpha C_\kappa$), AN9.8 (V_βCW3/1.1$C_\beta C_\kappa$) and AN19.4 (V_αCW3/1.1$C_\alpha C_\kappa$ + V_βCW 3/1.1$C_\beta C_\kappa$) transfectants. SN from cells biosynthetically labeled with ^{35}[S]methionine were incubated with normal mouse serum (NMS); H139.52.2, an anti-mouse C_κ mAb (anti-C_κ); B21.14, an anti-V_α8 mAb; or B21.5, an anti-V_β10 mAb. Immunoprecipitates were analyzed by SDS-PAGE on a 10% gel under reducing conditions. Position of the molecular weight markers are indicated on the right margin.

represented by the B21.5 clone (Fig. 2), only recognized the DO-11.10/TcRβCW3/1.1 transfectant. This reciprocal pattern of staining suggests that B21.13 and B21.14 recognize determinants expressed on the CW3/1.1 V_α domain irrespective of the nature of the associated V_β domain, whereas B21.5 recognize an epitope expressed on the CW3/1.1 V_β domain irrespective of the paired V_α domain. In support of this conclusion, B21.13 and B21.14, but not B21.5, were able to precipitate the soluble $V_\alpha CW3/1.1C_\alpha C_\kappa$ polypeptide secreted by the AN17.1 single-chain transfectant, whereas B21.5, but not B21.13 or B21.14, could precipitate the $V_\beta CW3/1.1C_\beta C_\kappa$ protein secreted by the AN9.8 single-chain transfectant (Fig. 1).

To determine if the epitope recognized by the B21.5 mAb on the CW3/1.1 V_β domain is unique to sequences encoded by the $V_\beta 10$ gene segment, we have analyzed a panel of T cell hybridomas of known V_α and V_β expression. As shown in Table 1, six independently derived cell lines that were shown [13] to express the $V_\beta 10$ gene segment in association with four different α chains reacted with the B21.5 mAb. Conversely, none of the tested cell lines expressing members of the $V_\beta 1$, $V_\beta 2$, $V_\beta 3$ or $V_\beta 8$ subfamilies are recognized by B21.5. Therefore, B21.5 appears to bind specifically to all the analyzed T cells that express the $V_\beta 10$ gene segment irrespective of D_β/J_β, V_α or J_α usage (see below).

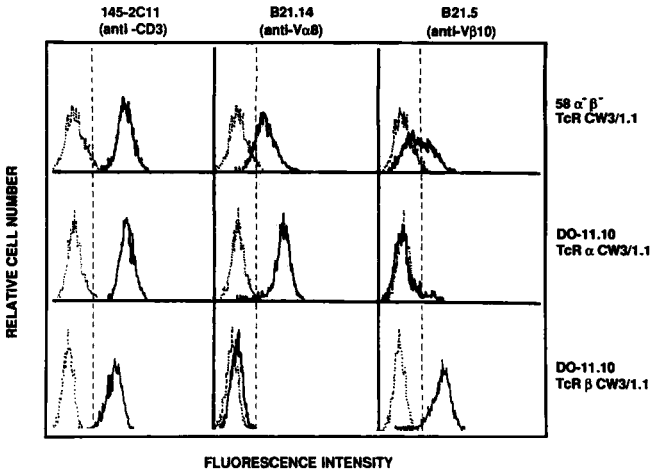

Figure 2. The B21.14 and B21.5 mAb raised against the soluble $V_\alpha CW3/1.1C_\alpha C_\kappa$ and $V_\beta CW3/1.1C_\beta C_\kappa$ polypeptides recognize two distinct epitopes on the native, surface-bound CW3/1.1 TcR. FCM analysis of the CW3/1.1 α (DO-11.10/TcRαCW3/1.1) and CW3/1.1 β (DO-11.10/TcR β CW3/1.1) DO-11.10 transfectants and of the 58 $\alpha^-\beta^-$ cells transfected with the CW3/1.1 α/β TcR combination (58$\alpha^-\beta^-$-TcR CW3/1.1) after staining with the mAb indicated at the top of each panel. Each fluorescence histogram is compared with a negative control histogram obtained by staining with B20.2, a rat anti-clonotypic mAb directed against the KB5-C20 α/β TcR.

Table 1. Relationship between V gene segment usage and expression of the B21.5, B21.14 and B21.13 determinants

Hybridomas and T cell transfectants[a]	V gene segment usage[b]		Reactivity with		
	V_α gene segment[c]	V_β gene segment[d]	B21.5[e]	B21.14[e]	B21.13[e]
58α⁻β⁻TcRCW3/1.1	8	10	+	+	+
1 HB 115	8	10	+	−	−
2 HB 16	8	10	+	−	−
3 HB 118	10	10	+	−	−
3 Q 48	4	10	+	−	−
4 BR 98	2	10	+	−	−
1 BR 124	8	3	−	−	+
3 Q 21	8	2	−	+	−
1 Q 74	8	2	−	+	−
DC 27.1	2	2	−	−	−
DO-11.10	1.13	1,8	−	−	−

a) T cell transfectants and hybridomas were established and characterized as described in Sects. 2.1 and 2.2
b) TcR V gene segment expression was determined by Northern blot analysis or cloning.
c) Nomenclature according to [27].
d) Nomenclature according to [27].
e) Cells were stained with the B21.5, B21.14 or B21.13 antibodies and analyzed on a FACScan analyzer.

Table 2. Percentage of B21.5 $(V_\beta 10)^+$ LN T cells in the CD4 and CD8 subsets of laboratory inbred mouse strains

Strain	H-2	$V_\beta 10$ genotype[b]	% B21.5$(V_\beta 10)^+$[a] CD4	CD8
C57BL/10(B10)	b	b	6.2 ± 0.8	5.3 ± 0.7
B10.S	s	b	5.4 ± 1.1	3.8 ± 0.5
B10.Q	q	b	4.8 ± 0.2	6.0 ± 0.2
B10.BR	k	b	8.9*	7.1*
B10.D2	d	b	8.8*	5.4*
CBA/J	k	b	10.1 ± 0.1	4.7 ± 0.3
DBA/2	d	b	9.8 ± 1.4	8.5 ± 0.8
BALB/c	d	b	7.9 ± 0.2	6.3*
SWR	g	a	<0.1	<0.1
SJL	s	a	<0.1	<0.1

a) LN cells were double stained with B21.5 (anti-$V_\beta 10$) and either GK-1.5 (anti-CD4) or 53-6.7 (anti-CD8). Data are expressed as mean percentages of three individuals ± SD except where indicated (*) in which cases only a single mouse was analyzed.
b) The TcR $V_\beta 10$ genotype was determined as described in [25].

When analyzed on the panel of hybridomas shown in Table 1, the B21.13 and B21.14 mAb recognized $V_\alpha 8$-bearing TcR unrelated to the one expressed on the CW3/1.1 CTL clone. However, both B21.13 and B21.14 did not react with all T cell hybridomas known to express $V_\alpha 8$ mRNA. Moreover, analysis of the pattern of staining of the 1BR124, 3Q21 or 1Q74 hybridomas indicates that the determinants recognized by B21.13 and B21.14 are distinct and may be expressed independently of each other (Table 1). Therefore, as previously observed for mAb with specificity for $V_\alpha 3.2$ [29], $V_\alpha 8$ [30] and $V_\alpha 11$ [31] products, B21.13 and B21.14 each react with only some members of the $V_\alpha 8$ subfamily. Such a restricted pattern of reactivity may account for the fact that B21.14 only stains from 2–4% of the LN cells found in the mouse strains shown in Table 2. Due to the presence of five functional members in the $V_\alpha 8$ subfamily [32] and to the existence of a high degree of interstrain V_α polymorphism [5, 33], analysis of transfectants which express each of the functional members of the $V_\alpha 8$ subfamily will be necessary to determine the structural relationship among the epitopes recognized by B21.13, B21.14 and the two anti-$V_\alpha 8$ mAb previously identified by Tomonari et al. [30].

3.2 The B21.5 mAb identifies an interstrain $V_\beta 10$ polymorphism

We next analyzed the percentage of $V_\beta 10$ (B21.5)$^+$ T cells in the CD4 and CD8 subsets of several laboratory mouse strains belonging to either $V_\beta{}^a$ or $V_\beta{}^b$ haplotypes. The $V_\beta{}^a$ or $V_\beta{}^b$ status of each strain has been previously determined by RFLP analysis of V_β gene segments [25, 33–35]. (Most of the laboratory inbred strains (*e.g.* BALB/c, C57BL/10...) display a unique combination of V_β RFLP denoted as $V_\beta{}^b$, whereas $V_\beta{}^a$ strains such as SJL or SWR display a distinct combination of V_β RLFP and a deletion of 10 contiguous V_β gene segments.) The results of the distribution of the B21.5$^+$ T cells are shown in Table 2 and can be summarized as follows. First, in all strains of $V_\beta 10^b$ genotype [34], the CD4 subset had slightly higher numbers of $V_\beta 10^+$ T cells (4.8–10.1%) than the CD8 subset (3.8–8.5%). Second, the MHC class II or Mls alleles expressed by the strains analyzed in Table 2 apparently did not influence $V_\beta 10$ usage. Third, we were unable to detect any T cells reactive with B21.5 in strains of $V_\beta{}^a$ genotype (SWR and SJL) in spite of the fact that Southern blot analysis of SWR or SJL DNA revealed the presence of a $V_\beta 10$-hybridizing band [25]. The absence of B21.5$^+$ T cells in SJL and SWR could be accounted for by one of the following possibilities. First, as previously observed for the $V_\beta 17^b$ gene segment [35], the $V_\beta 10^a$ gene segment may bear a mutation which interferes with its expression. Second, SJL and SWR B21.5$^+$ T cells may undergo major intrathymic deletion similar to the ones previously observed for $V_\beta 17^{a+}$ or $V_\beta 6^+$ T cells in I-E$^+$ or Mls 1^{a+} strains [36, 37]. Third, the rat mAb B21.5 was raised against the product of a $V_\beta 10^b$ gene segment and may detect an epitope which is absent from the $V_\beta 10^a$ product. Three observations support the latter possibility. First, no B21.5$^+$ thymocytes have been found in the SJL strain (data not shown). Second, analysis of a $V_\beta 10^a$ cDNA isolated from an SWR thymus does not reveal any obvious mutation that will prevent its expression, and indicates that the $V_\beta 10^a$ and $V_\beta 10^b$ coding sequences display an unusually high degree of interallelic polymorphism [34]. Third, a myelin basic protein-specific, I-A^s-restricted T cell clone derived from SJL and expressing a $V_\beta 10^+$ TcR was found to be negative upon staining with B21.5 (J. Fehling, personal communication). Therefore, these data suggest that the epitope recognized by the B21.5 mAb is absent from the product of the $V_\beta 10^a$ segment expressed in SWR and SJL. Interestingly, analysis of a number of strains derived from wild stocks of *Mus musculus musculus*, *Mus musculus domesticus* and *Mus musculus molossinus* indicates that the RFLP polymorphism defining the $V_\beta 10^a$ and $V_\beta 10^b$ alleles [25] does not always correlate with the absence or presence of the B21.5 epitope. For instance, as shown in Table 3, B21.5$^+$ T cells may be readily detected in the MPW, PWK and MAI strains in spite of the fact that they display a $V_\beta 10$ RFLP identical to the one found in other $V_\beta 10^a$ strains. Therefore, most but not all the $V_\beta 10^a$ strains have lost the epitope recognized by the B21.5 mAb.

Table 3. Percentage of B21.5 ($V_\beta 10$)$^+$ T cells in mouse strains derived from wild stocks

		Geographical origin	$V_\beta 10$ genotype[a]	% B21.5($V_\beta 10$)$^+$/CD3$^+$ cells[b]
BALB/c			b	8.0
SJL			a	0.0
M.m.domesticus				
BIK/g	(19)[c]	Israel	a	0.0
DJO	(10)	Italy	a	0.0
38CH	(31)	Italy	a	0.0
WLA	(30)	France	a	0.0
WMP	(21)	France	a	0.0
M.m.musculus				
MPW	(13)	Poland	a	4.8
PWK	(30)	Czechoslovakia	a	3.3
MAI	(20)	Austria	a	1.5
MBT	(10)	Bulgaria	a	0.0
MBB	(11)	Bulgaria	a	0.0
M.m.molossinus				
MOL	(20)	Japan	a	0.0

a) The TcR $V_\beta 10$ genotype was determined as described [25].
b) PBL were double stained with B21.5 (anti-$V_\beta 10$) and 145-2C11 (anti-CD3). Two animals have been independently analyzed for each strain.
c) The number of brother-sister mating generations is indicated in parentheses for each strain.

Table 4. Percentage of B20.6 $(V_\beta 2)^+$ T cells in inbred mouse strains[a)]

Strain	H-2	Mls	% positive T cells B20.6($V_\beta 2$)	44-22-1($V_\beta 6$)	RR3-15($V_\beta 11$)
C57BL/10(B10)	b	b	6.5	7.0	3.5
B10.D2	d	b	6.0	7.4	0.2
B10.BR	k	b	10.2	9.0	0.0
DBA/2	d	a	8.5	0.0	1.2
CBA/J	k	d(a/c)	8.8	0.0	0.5
SJL	s	c	18.3	11.3	0.0[b)]

a) Cortisone-resistant thymocytes from the indicated strains were stained with rat mAb directed against $V_\beta 2$, $V_\beta 6$ or $V_\beta 11$. Data are presented as % positive cells (following subtraction of background staining with the fluorescent conjugate alone).
b) Genomic deletion in SJL includes $V_\beta 11$.

3.3 Characterization of a $V_\beta 2$-specific mAb raised against a soluble α/β TcR module

We have extended the above approach to a second soluble TcR expressing the V_α ($V_\alpha 2$-$J_\alpha A10$) and V_β ($V_\beta 2$-$D_\beta 2$-$J_\beta 2.3$) genes from the KB5-C20 CTL clone, and shown that anti-clonotypic [7] or anti-$V_\alpha 2$ [H. Pircher et al., submitted for publication] mAb may be readily raised against this soluble α/β TcR. A third type of mAb, represented by the B20.6 clone, was analyzed on a panel of T cell clones and hybridomas whose V_α and V_β expression is known and found to recognize the product of the $V_\beta 2$ gene segment, irrespective of the D_β, J_β, V_α or J_α usage (data not shown). The expression of the $V_\beta 2$ (B20.6) determinant was analyzed on T cells from different mouse strains. As shown in Table 4, $V_\beta 2$-bearing T cells are found in all the tested strains and, therefore, appear not to be subject to major clonal deletion mechanisms similar to the one observed for $V_\beta 6$- or $V_\beta 11$-bearing T cells. Moreover, in contrast to the B21.5 (anti-$V_\beta 10$) mAb (see above) and in agreement with the fact that the $V_\beta 2^a$ and $V_\beta 2^b$ products display the same amino acid sequence [34], the B20.6 mAb was found to react with both $V_\beta 2^b$ (*e.g.* B10-derived) and $V_\beta 2^a$ (*e.g.* SJL-derived) products.

4 Discussion

We have produced and characterized a panel of V_α- and V_β-specific mAb by immunizing rats with soluble α/β TcR molecules. This approach, as illustrated here for two distinct α/β combinations, appears particularly efficient. Out of two fusions, we have isolated a panel of mAb specific for the $V_\alpha 2$, $V_\alpha 8$, $V_\beta 2$ and $V_\beta 10$ products and the KB5-C20 α/β TcR combination ([7], this report and H. Pircher et al., submitted for publication). While the present report was under review, a report appeared by Devaux et al. [38] supporting the view that the utilization of soluble TcR polypeptides constitutes a powerful way of generating anti-TcR mAb. Moreover, the present data extend the ones obtained for the soluble KB5-C20 TcR [7] and indicate that at least some segments of these soluble α/β TcR molecules are folded in a conformation indistinguishable from that which the native molecules assume on the cell surface.

Among the previously identified anti-V_β mAb, some were found to behave like the B21.5 mAb in the sense that they do not stain T cells originating from certain strains of mouse. In most of the cases, the lack of staining has been accounted for by the absence of the corresponding V_β structural gene. For instance, mice of the V_β^a genotype have deleted ten contiguous V_β gene segments [25, 39], and consequently do not express T cells reacting with mAb specific for the $V_\beta 5$, $V_\beta 8$, $V_\beta 9$ and $V_\beta 11$ subfamilies [40–44]. In addition, the anti-$V_\beta 17^a$ mAb KJ23a did not react with T cells from V_β^b mouse strains because of a mutation leading to a termination codon within the $V_\beta 17^b$ coding sequence [35]. Here, we have shown that the anti-$V_\beta 10$ mAb B21.5 does not stain T cells originating from most of the V_β^a-bearing strains because of the loss of the epitope it recognizes. Such an observation reflects the unusually high degree of polymorphism existing between the $V_\beta 10^a$ and $V_\beta 10^b$ products [34]. For instance, six mostly non-conservative amino acid differences have been identified between $V_\beta 10^a$ and $V_\beta 10^b$, and postulated to be located at the surface of the $V_\beta 10$ domain [34]. As exemplified by the $V_\beta 8.2$ [45] and $V_\beta 17$ [16] gene segments, the analysis of the sequences of $V_\beta 10$ gene segments derived from wild mice (see Table 3) should allow us to delineate the polymorphic amino acids involved in the constitution of the B21.5 epitope.

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