# Expression of Two T Cell Receptor α Chains: Dual Receptor T Cells

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Although many T cells carry two in-frame $V_\alpha$ rearrangements, the products of both $V_\alpha$ rearrangements have never been shown simultaneously on the surface of normal cells. With the use of monoclonal antibodies to $V_\alpha 2$, $V_\alpha 12$, and $V_\alpha 24$, up to one-third of mature T cells expressed two $V_\alpha$ chains as part of two functional and independent T cell receptors (TCRs). Thus, the "one cell, one receptor" rule does not apply to a large subset of αβ T cells. Cells that belong to this dual TCR subset may be specific for a broader range of antigens than cells with a single receptor, which may be important for autoimmunity and alloreactivity.

According to Burnet's clonal selection theory, each lymphocyte should bear a single receptor or at least a limited number of receptors (*1*). Experimental observations up to now indicate that most T and B lymphocytes carry a single receptor type; this has been referred to as the "one cell, one receptor" rule. In thymocytes developing along the αβ lineage, the presence of a productively rearranged $V_\beta$ chain prevents further β rearrangements (*2*). This is not the case for the TCRα locus, where both alleles rearrange simultaneously and rearrangements continue until a surface TCRαβ heterodimeric molecule is expressed that can be positively selected by thymic major histocompatibility complex (MHC) molecules (*3*). Thus, approximately one-third of mature αβ T cells carry two productive α rearrangements and, as a consequence, have the potential to express two messages and two proteins (*4–7*). However, when mouse T cell clones that carry two in-frame $V_\alpha$ rearrangements were analyzed by immunoprecipitation and two-dimensional gel electrophoresis, only one α chain was found on the cell surface of each of the three clones analyzed (*4, 5*). These results suggested that the "one cell, one receptor" rule might still be valid because of preferential expression (*8*) or preferential pairing of one of the two α chains with the β chain (*9*).

Using three mouse monoclonal antibodies that recognize $V_\alpha 2$, $V_\alpha 12$, or $V_\alpha 24$, we examined surface expression of TCRα. Peripheral blood mononuclear cells (PBMCs) from normal donors were stained with a combination of two antibodies to $V_\alpha$ to detect T cells that expressed the two corresponding $V_\alpha$ chains (*10*). Cells stained by two antibodies to $V_\alpha$ were detected at a low frequency ($10^{-3}$ to $10^{-4}$) (Fig. 1A and Table 1). When the double positive cells were isolated by cell sorting and cloned, one-third to one-half of the clones stably expressed both $V_\alpha$ chains (Fig. 1D and Table 1). We also sorted and expanded T cells expressing $V_\alpha 2$, $V_\alpha 12$, or $V_\alpha 24$ in bulk culture. Almost all cells expressed the $V_\alpha$ for which they had been originally selected, and a portion also expressed a second $V_\alpha$ (Fig. 1, B and C, and Table 1).

A total of 124 clones that stained positive for two antibodies to $V_\alpha$ were isolated. Fifteen of these clones, chosen at random, were analyzed for the presence of a $V_\alpha$-specific message by the polymerase chain reaction (PCR) with specific primers (*11*), and in all cases messages corresponding to the two $V_\alpha$ chains expected from the staining data were found. From five of these clones, the two α alleles were sequenced. All the sequences were in-frame, and the length of the N region was comparable in both alleles, which possibly reflects the activity of terminal transferase at the time of rearrangement (Table 2). These results are in agreement with the antibody staining data.

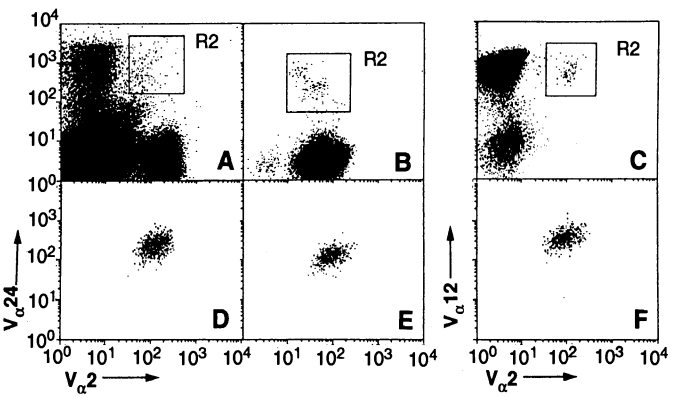


**Fig. 1.** Identification and isolation of T cells expressing two $V_\alpha$ chains (*10*). (**A**) PBMCs stained with antibodies to $V_\alpha 2$ and $V_\alpha 24$. (**B**) A polyclonal $V_\alpha 2^+$ cell line stained with antibodies to $V_\alpha 2$ and $V_\alpha 24$. (**C**) A polyclonal $V_\alpha 12^+$ cell line stained with antibodies to $V_\alpha 2$ and $V_\alpha 12$. The squares show the gates of the sorting. (**D** through **F**) Three representative clones isolated from sorted cells from (A), (B), and (C), respectively.

**Table 1.** Frequency of T cells expressing two $V_\alpha$ chains. The T cells from peripheral blood of healthy donors were analyzed as described in Fig. 1. The frequency of T cells stained by one or two $V_\alpha$ antibodies is indicated. The number of doubly positive clones out of the total number of clones isolated is shown in parentheses. ND, not done.

| Cell source | Frequency of T cells expressing: | | | | |
|---|---|---|---|---|---|
| | $V_\alpha 2$ | $V_\alpha 24$ | $V_\alpha 12$ | $V_\alpha 2, V_\alpha 12$ | $V_\alpha 2, V_\alpha 24$ |
| | | | *Experiment 1* | | |
| PBMC EPA | 1/33 | 1/100 | – | – | 1/3000 (3/7) |
| PBMC SVA | 1/27 | 1/131 | – | – | 1/10,000 (5/18) |
| PBMC ALA | 1/21 | – | 1/32 | 1/900 (5/10) | – |
| PBMC PDB | 1/25 | – | 1/20 | 1/1058 (ND) | – |
| | | | *Experiment 2* | | |
| α2 ALA | 1/1 | – | – | 1/191 (24/24) | 1/425 (ND) |
| α2 ATR | 1/1 | – | – | 1/375 (12/12) | 1/355 (37/42) |
| α2 DSH | 1/1 | – | – | 1/531 (20/20) | 1/331 (ND) |
| | | | *Experiment 3* | | |
| α24 ALA | – | 1/1.1 | – | – | 1/4600 (5/12) |
| α24 ATR | – | 1/1.2 | – | – | 1/1620 (7/12) |
| α24 DSH | – | 1/1.1 | – | – | 1/213 (6/12) |
| | | | *Experiment 4* | | |
| α12 ALA | – | – | 1/1 | 1/1804 (ND) | – |
| α12 ATR | – | – | 1/1 | 1/1511 (ND) | – |

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To investigate whether T cells that express two $V_\alpha$ chains have two functional and independent TCRs, we used antibodies to $V_\alpha$ to induce cytotoxicity and TCR down-regulation. The killing of target cells that express Fc receptors by a T cell clone expressing two $V_\alpha$ chains could be induced by either of the cognate antibodies to $V_\alpha$ (Fig. 2). In addition, incubation of a $V_\alpha2^+V_\alpha24^+$ clone with antibody to $V_\alpha24$ resulted in a selective down-regulation of the cognate $V_\alpha$ (Table 3). Taken together, these results suggest that each $V_\alpha$ chain is part of an independent and functional TCR.

What is the frequency of T cells that express two $V_\alpha$ chains? If all the in-frame rearrangements result in unbiased surface expression of the corresponding α chain, approximately one-third of all T cells should express two $V_\alpha$ chains (*12*). The observed frequencies (Table 1) are close to this expected value. A separate estimate of this frequency can be obtained by analysis of the ratio of TCR $V_\alpha$ type to CD3. The stoichiometry of total TCR to CD3 is fixed (*13*); thus, the $V_\alpha24$:CD3 ratio will be higher in cells that express $V_\alpha24$ only, as compared to cells that express $V_\alpha24$ plus a second $V_\alpha$ (*14*). This was tested by three-color staining of PBMCs with antibodies to $V_\alpha24$, $V_\alpha2$, and CD3. A typical distribution pattern (Fig. 3A) of the $V_\alpha24$:CD3 fluorescence shows that a portion of the $V_\alpha24^+$ cells is near a diagonal with a high $V_\alpha24$:CD3 ratio (compatible with the expression of $V_\alpha24$ only); a sizable fraction of the cells that express $V_\alpha24$ have a lower ratio, compatible with the expression of $V_\alpha24$ plus an additional, unknown $V_\alpha$. $V_\alpha2$, which is not expressed by cells with the high $V_\alpha24$:CD3 ratio, is found among cells with a lower $V_\alpha24$:CD3 ratio at a frequency that is comparable to that in whole blood (Fig. 3, B through D). Similar results have been obtained in three donors with various $V_\alpha$ combinations. These results show that (i) the $V_\alpha$:CD3 ratio can be used to quantify cells that express two $V_\alpha$ chains and (ii) $V_\alpha2$ and $V_\alpha24$ are not overrepresented among cells carrying two $V_\alpha$ chains. These plots also clearly show that the expression of two $V_\alpha$ chains, which can be easily detected by the staining method, is uneven in the sense that the two α chains are expressed in different proportions, possibly because of differential transcription or differential pairing with the β chain (*9, 12, 15*).

The expression of dual receptors by αβ T cells has been directly demonstrated only

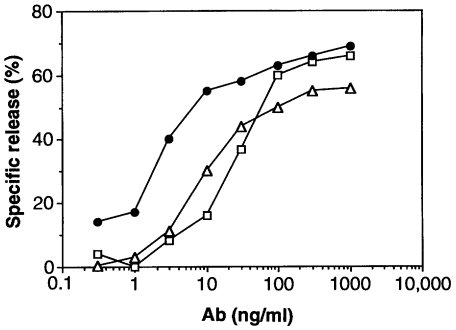


**Fig. 2.** Functional assessment of TCRs in a T cell clone expressing two $V_\alpha$ chains. A $V_\alpha2^+,V_\alpha24^+$ clone was tested for its capacity to kill $^{51}Cr$-labeled P815 target cells in the presence of different concentrations of antibodies to $V_\alpha2$ (□), $V_\alpha24$ (△), or CD3 (●) in a standard 5-hour assay with an effector-to-target ratio of 10 (*24*). Ab, antibody.

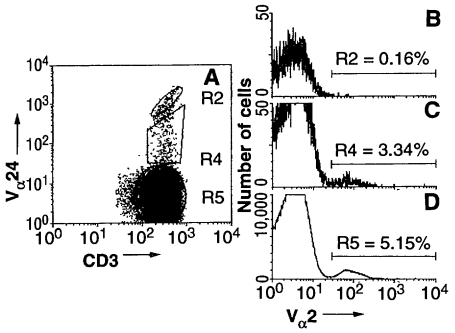


**Fig. 3.** Identification of peripheral blood T cells expressing $V_\alpha24$ only or $V_\alpha24$ plus another $V_\alpha$ chain. The PBMCs were stained in three-color fluorescence with anti-$V_\alpha2$ (IgG2a), biotin-labeled antibody to $V_\alpha24$ (IgG1), and FITC-labeled antibody to CD3 (clone TR66, IgG1) (*24*), followed by goat antibody to mouse IgG2a-PE and streptavidin-allophycocyanin. (**A**) Distribution of $V_\alpha24^+$ versus $CD3^+$ cells. Gate R2 contains cells with a high $V_\alpha24$:CD3 ratio; gate R4 contains cells with a lower ratio. (**B** through **D**) Frequency of $V_\alpha2^+$ cells in gates R2, R4, and R5, respectively.

**Table 3.** Independent down-regulation of the two TCRs in T cells expressing two $V_\alpha$ chains. Two T cell clones expressing $V_\alpha2$ and $V_\alpha24$ were incubated at 37°C with antibody to $V_\alpha24$ (5 μg/ml), with phorbol myristate acetate (PMA) (10 ng/ml) or with medium alone (control). After 8 hours, the cells were stained with an excess of antibody to $V_\alpha2$, $V_\alpha24$, and CD3, followed by the appropriate second-step reagents. The data represent the mean fluorescence intensity from which the background (<5) was subtracted. Numbers in parentheses indicate the percent of specific fluorescence left after down-regulation as compared to the untreated sample.

| Clone | Control | Anti-$V_\alpha24$ | PMA |
|---|---|---|---|
| | *Experiment 1* | | |
| $V_\alpha2$ | 128 | 127 (99) | 41 (32) |
| $V_\alpha24$ | 303 | 38 (12) | 46 (15) |
| CD3 | 430 | 280 (65) | 123 (28) |
| | *Experiment 2* | | |
| $V_\alpha2$ | 39 | 37 (95) | |
| $V_\alpha24$ | 75 | 27 (36) | |
| CD3 | 172 | 138 (80) | |

**Table 2.** Sequence of TCRα from five T cell clones that express two surface $V_\alpha$ products.

| | $V_\alpha$ | N | $J_\alpha$ | |
|---|---|---|---|---|
| | | *EPA 25* | | |
| $V_\alpha2$ | TAC CTC TGT GTG GTG | CA | G ATT TAT AAC CAG | $J_\alpha$H.1 |
| $V_\alpha24$ | TAC ATC TGT GTG GTG AG | AC | AC AGA GGC TCA AAC CCT | $J_\alpha$Q |
| | | *DSH 11* | | |
| $V_\alpha2$ | TAC CTC TGT GTG GTG A | | CG TAC AAT AAC AAT | $J_\alpha$10 |
| $V_\alpha24$ | TAC ATC TGT GTG GTG AG | | G AAT TCA GGA AAC ACA | $J_\alpha$F.1 |
| | | *ATR 6.2* | | |
| $V_\alpha2$ | TAC CTC TGT GTG GTG AAC | CGA GCG CA | C AAC TTC AAC AAA | $J_\alpha$AC 24 |
| $V_\alpha24$ | TAC ATC TGT GTG GTG AG | A GCG GCC TAT GG | T AAT GCA GGC AAC ATG | $J_\alpha$AC-17 |
| | | *DSH 102* | | |
| $V_\alpha2$ | TAC CTC TGT GTG GTG AAC | ATG GGG AAG GGA | ACC TCC TAC | $J_\alpha$F 86 |
| $V_\alpha12$ | TAC TTC TGT GCT CT | G AGT GAG GCG CTA TTG | AAC TTC AAC AAA | $J_\alpha$AC 24 |
| | | *ALA 100* | | |
| $V_\alpha2$ | TAC CTC TGT GTG GTG A | GA TTT TA | C ACC GGT AAC CAG | $J_\alpha$U |
| $V_\alpha12$ | TAC TTC TGT GCT CT | T TTA ATG C | CT GGG GCT GGG AGT TAC | $J_\alpha$V |

for the three $V_\alpha$ products that can be identified with the available antibodies, but it is likely to reflect a general property of αβ T cells (*16*). Thus, our findings, together with the recent report that two TCRγ alleles can be expressed on the same γδ T cell (*17*), show that the "one cell, one receptor" rule is not valid for a portion of both αβ and γδ T cells.

What are the implications of the dual αβ TCR subset? First, it may be more frequently involved in alloreactivity because (i) it has two TCRs rather than one (although each at a lower concentration) and (ii) the non–positively selected receptor may be more prone to see peptides bound to allogeneic MHC molecules (although admittedly self restricted antigen recognition and alloreactivity can be mediated by the same receptor) (*4*, *18*). Second, although the presence of two TCRs on T cells is compatible with central (thymic) tolerance, it could pose a problem for peripheral tolerance when this is based on the inability of tissue cells to stimulate naïve T cells (*19*). Thus, once a dual receptor T cell is activated by a foreign antigen recognized by the first TCR, it may become competent to utilize the second (non–positively selected) TCR to attack the self antigen. This type of cross-reactivity would not be based on structural similarities between foreign and self antigens, but rather on the particular somatic combination of two distinct TCRs.

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10. The mouse immunoglobulin G2a (IgG2a) antibody to human $V_\alpha2$ (clone V2a) was purchased from T Cell Science (Cambridge, MA). The mouse IgG1 antibody to human $V_\alpha12$ (clone 6D6) was provided by M. Brenner (*20*). We produced a mouse IgG1 antibody to human $V_\alpha24$ (*21*). Cells were stained with either antibodies to $V_\alpha2$ plus $V_\alpha24$ or antibodies to $V_\alpha2$ plus $V_\alpha12$, followed by phycoerythrin-conjugated goat antibody to mouse IgG1 plus fluorescein isothiocyanate (FITC)–conjugated goat antibody to mouse IgG2a. In some experiments, T cells that expressed a given $V_\alpha$ were first enriched by sorting, expanded with phytohemagglutinin (PHA), interleukin-2 (IL-2), and irradiated feeder cells, and reanalyzed. Cells stained with two antibodies were sorted on a FACStar Plus (Becton Dickinson) and cloned as described (*22*) by limiting dilution with PHA, IL-2, and irradiated allogeneic PBMCs.
11. RNA extraction and complementary DNA (cDNA) synthesis were performed as described (*23*). We amplified cDNA in 30 μl using 80 ng of one of the TCR $V_\alpha$-specific primers ($V_\alpha1$, 5′-GGCATTAAGGGTTTTGAGGCTGGA-3′; $V_\alpha2$, 5′-CAGTGTTCCAGAGGGAGCCATTGT-3′; $V_\alpha12$, 5′-TCGTCGGAACTCTTTTGATGAGCA-3′; or $V_\alpha24$ 5′-CTGGATGCAGACACAAAGCAGAGC-3′) [C. Geneveè *et al.*, *Eur. J. Immunol.* **22**, 1261 (1992)] plus 80 ng of the TCR $C_\alpha$ primer (5′-TGCTCTTGAATTCCATAGACCTCATGTC-3′) with the following profile: 15 s at 94°C, 20 s at 60°C, and 20 s at 72°C for 30 cycles in a Perkin-Elmer 480 thermal cycler. The PCR products were fractionated on a 2% agarose gel and isolated with low melting point agarose. The single PCR fragments were directly sequenced with a U.S. Biochemicals Sequenase kit as described [J.-L. Casanova *et al.*, *Nucleic Acids Res.* **18**, 4028 (1990)] with 0.25 pmol of template and 10 pmol of the TCR $C_\alpha$ oligonucleotide 5′-CAGACAGACTTGTCACT-3′.
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14. This ratio can be measured by two-color staining with antibodies to $V_\alpha$ and to CD3. All $V_\alpha24^+$ clones tested show a homogeneous staining with a clear correlation between the level of $V_\alpha24$ and CD3 and a constant ratio. Within the same experiment, this ratio has a fixed high value for the majority of the clones and a lower value for those that are known to express a second $V_\alpha$ chain.
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16. The possibility has to be considered that there could be a mechanism that favored $V_\alpha2$, $V_\alpha12$, and $V_\alpha24$ for dual expression. However, this is unlikely for two reasons. First, rearrangements of both $V_\alpha$ loci occur simultaneously and independently of thymic selection (*12*); rearrangement is therefore expected to be random. Thus, it is unlikely that, upon positive selection of one of the two $V_\alpha$ chains, a bias is introduced for the other unselected $V_\alpha$ chain. Second, the results shown in Fig. 3 indicate that $V_\alpha2$ and $V_\alpha24$ are not overrepresented among co-expressed $V_\alpha$ chains.
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21. A monoclonal antibody (C15, IgG1) was produced from mice immunized with a T cell clone that expressed a $V_\alpha24,J_\alpha Q,V_\beta11$ TCR (*23*). T cells stained by C15 were sorted from peripheral blood and cloned, and the structure of their TCRs was determined by sequencing. All the clones recognized by the C15 antibody express $V_\alpha24$, which can be associated with different $J_\alpha$ segments as well as with different $V_\beta$ chains. Antibody titration experiments indicate that the affinity of C15 is not influenced by $J_\alpha$ or $V_\beta$.
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# Altered Fluid Transport Across Airway Epithelium in Cystic Fibrosis


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**In cystic fibrosis (CF), absence or dysfunction of a phosphorylation-regulated chloride channel [CF transmembrane conductance regulator (CFTR)] leads to the loss or reduction of chloride secretion into the airways. Active sodium absorption is also increased in CF, and both of these ion transport changes could alter fluid transport across the airways. Under baseline conditions, cultured human airway epithelia from normal individuals absorbed fluid, and this absorption was increased in epithelia from patients with CF. In normal and CF epithelial cultures fluid absorption was inhibited by amiloride. Adenosine 3′,5′-monophosphate stimulated fluid secretion in normal epithelial cultures but not in cultures from individuals with CF. In contrast, fluid secretion induced by nucleotide triphosphates (uridine triphosphate or adenosine triphosphate) was unaltered in cultures of epithelia from patients with CF, suggesting an approach to the treatment of CF.**


The surface of the airway epithelium is covered by a 5-μm layer of fluid (sol) and a blanket of mucus (gel) that are produced by various surface and submucosal gland cells. Cilia on the cells' apical membranes beat within the sol and contact the underside of the mucus blanket, thereby clearing it together with entrapped particulate matter from the lungs. It is believed that in CF this mucociliary clearance is impaired because alterations in CFTR lead to $Cl^-$ impermeability of the luminal membrane, thereby reducing fluid secretion and dehydrating the sol and gel layers (*1*, *2*). In human airways, $Na^+$ absorption is increased in patients with CF (*3*–*5*), and this may also dehydrate the airway surface and contribute to the pathology.

To directly test whether airway fluid transport is altered in CF, we have made continuous measurements of active ion–linked fluid movement across cultured human airway epithelia. We used primary cultures of nasal (HNE) and tracheal (HTE) epithelia, which have ion transport properties that closely resemble those of