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Brief Definitive Report

Expression of Two α Chains on the Surface of T Cells in T Cell Receptor Transgenic Mice

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Summary

$CD8^+$ T cells taken directly from mice expressing a K^b-specific T cell receptor (TCR) transgene expressed the transgenic TCR in a bimodal profile as detected by flow cytometric analysis using a clonotype-specific monoclonal antibody. Those cells expressing the lower density of the transgenic TCR expressed the transgenic β chain and two different α chains on their surface. One α chain was the product of the α transgene, whereas the other was derived by endogenous rearrangement. This report provides the first demonstration that T cells isolated directly from mice may express two different TCR clonotypes on their surface. The potential consequences of this finding for studies using TCR transgenic mice and for the induction of autoimmunity are discussed.

T cell development involves several important processes including rearrangement of the TCR genes, positive selection for MHC restriction, and negative selection against autoreactive clones (1). Rearrangement of the α and β chains of the TCR is critical in the generation of a diverse T cell repertoire (2). Current evidence suggests that the β chain alleles are the first to rearrange and do so sequentially (3). Once a productive rearrangement is achieved, further rearrangement is allelically excluded (4, 5). By contrast, α chain rearrangement, which is less well understood, may occur simultaneously (6) and continue to do so until a positive selection signal is received (7). The less stringent allelic exclusion of the α chain may account for the finding that some T cell clones express two different α chains (1, 6).

In this report, we raise the issue of multiple α chain expression by TCR transgenic T cells. Evidence is provided for the in vivo presence of T cells expressing two different α chains on their surface. This finding has important implications for experimental systems that utilize TCR transgenic mice.

Materials and Methods

Mice. B10.BR and transgenic mice were bred at the Walter and Eliza Hall Institute for Medical Research. RIP-K^b mice were derived as previously described (8) and were maintained by mating with H-2^k CBA/CaHWehi mice. Des-TCR transgenic mice were the kind gift of Drs. B. Arnold, G. Schönrich and G. J. Hämmerling (Deutsches Krebsforschungszentrum, Heidelberg, Germany), and were derived as previously described (9). The Des-TCR line was maintained by mating with H-2^k B10.BR mice.

Antibodies. The Désiré mAb was the kind gift of Dr. A.-M. Schmitt-Verhulst (Centre d'Immunologie de Marseille-Luminy, Marseille, France) (10). PE- and FITC-conjugated CD8 mAbs were purchased from Becton Dickinson and Co., Mountain View, CA. V region–specific mAbs B20.1 (anti-$V_\alpha 2$) and B20.6 (anti-$V_\beta 2$) were the kind gift of Dr. B. Malissen (Centre d'Immunologie de Marseille-Luminy) (11). RR3-15 (anti-$V_\beta 11$) (12) and KT50 (anti-$V_\alpha 8$) (13) mAbs were used as biotinylated mAbs. The GK1.5 mAb was used for staining $CD4^+$ cells (14). FITC-conjugated sheep anti–mouse Ig was purchased from Silenus Laboratories Pty. Ltd., Hawthorn, Australia.

Flow Cytometry. Two different methods of staining were used. All incubations were performed on ice in the dark. When cells were stained with only two different mAbs, 5×10^5 cells were stained with CD8-FITC and the appropriate biotinylated mAb for 30 min. After two washes, cells were then stained with streptavidin-PE (Caltag Labs., S. San Francisco, CA) for a further 30 min, washed once, and analyzed.

When cells were stained with three different mAbs, 5×10^5 cells were stained with the appropriate mAb (B20.1, B20.6, or GK1.5) for 30 min. After two washes, cells were stained with sheep anti–mouse Ig for 30 min. Cells were then washed twice and exposed to 20 μl of rat serum for 15 min to block any vacant mouse Ig binding sites on sheep antibodies. This was followed by staining with the appropriate biotinylated TCR-specific mAb (B20.1 or Désiré) and CD8-PE for 30 min, two washes, and staining with streptavidin conjugated to Tri-color (Caltag Labs.) for 30 min. After one further wash, cells were analyzed by flow cytometry.

Analysis was performed on a FACScan® (Becton Dickinson and Co.). Live gates were set on lymphocytes by forward and side scatter profiles. Live gates were also set on $CD8^+$ cells, and 10,000 cells were analyzed.

Results and Discussion

Identification of Bimodal Expression of a Transgenic TCR. We have been interested in addressing the fate of T cells that are specific for antigens expressed outside the thymus. For these studies, we have used the RIP-K^b model in which transgenic

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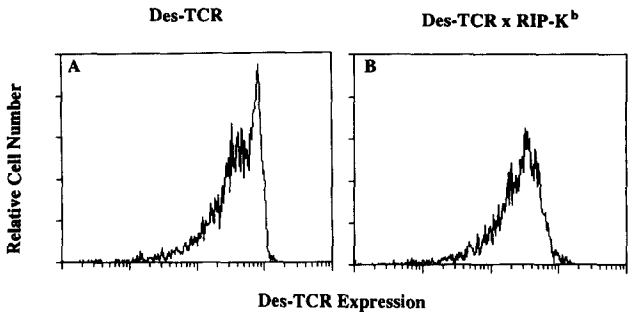

Figure 1. Expression of the Des-TCR by CD8$^+$ lymph node cells from Des-TCR and Des-TCR × RIP-K^b transgenic mice. Lymph node cells from (*A*) Des-TCR and (*B*) Des-TCR × RIP-K^b transgenic mice were stained for CD8 and Des-TCR expression and analyzed by flow cytometry.

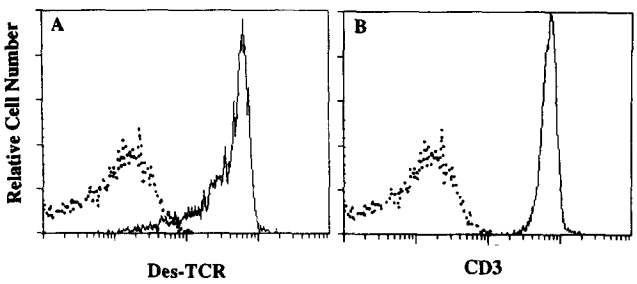

Figure 3. CD3 is expressed as a single peak by CD8$^+$ lymph node cells from Des-TCR transgenic mice. Lymph node cells from Des-TCR transgenic mice were stained for the expression of CD8 and either (*A*) the Des-TCR or (*B*) CD3, and analyzed by flow cytometry. Histograms show Des-TCR or CD3 expression by CD8$^+$ cells from Des-TCR transgenic mice (*solid lines*). As a negative control, Vβ11 expression by CD8$^+$ cells is shown (*dotted lines*).

mice express the class I molecule K^b in the β cells of the pancreas, under the control of the rat insulin promoter (8). To examine the fate of K^b-specific T cells in RIP-K^b mice, these mice were crossed with TCR transgenic mice (Des-TCR mice) that expressed a transgene encoding a K^b-specific TCR. This receptor could be detected by the clonotype-specific mAb Désiré (10). Flow cytometric analysis of lymph node CD8$^+$ T cells revealed that both single Des-TCR transgenic mice and double Des-TCR × RIP-K^b transgenic mice had K^b-specific T cells in their periphery (Fig. 1 and reference 15). Of note, however, was the significant reduction in the number of cells expressing the highest density of the Des-TCR in the double transgenic mice. These analyses highlighted the fact that the CD8$^+$ T cells in the periphery of Des-TCR mice expressed variable levels of the clonotypic Des-TCR characterized by a bimodal profile of low and high TCR densities. Des-TCR mice were analyzed on numerous occasions and the bimodal expression was highly reproducible, although the proportion of CD8$^+$ cells that comprised each peak was variable.

CD8$^+$ T Cells Expressing the Lower Density of Des-TCR Express the Transgenic α Chain. One explanation for the bimodal expression of the Des-TCR in single transgenic mice was that some cells expressed an endogenous α chain, rather than the transgenic α chain, paired with the transgenic β chain. Together these chains may form a new TCR detected with lower affinity by the Désiré mAb. To test this, lymph node cells from a Des-TCR mouse were double stained for CD8 and V$_\alpha$2 expression, the latter being the V$_\alpha$ expressed by the transgene (Fig. 2). Virtually all CD8$^+$ cells expressed the V$_\alpha$2 transgene, and this was expressed with a similar bimodal profile as seen for the Des-TCR. This suggested that those transgenic CD8$^+$ cells expressing the lower density of the Des-TCR also expressed a low density of the transgenic α chain.

CD8$^+$ T Cells from Des-TCR Mice Do Not Show Bimodal Expression of CD3 and the Transgenic β Chain. An alternative possibility was that cells expressing the lower density of the Des-TCR might express two different receptors, only one of which could be identified by the Désiré mAb. To examine this possibility, Des-TCR transgenic lymph node cells were double stained for CD3 and CD8 (Fig. 3). This revealed that in contrast to Des-TCR expression, CD3 was expressed as a single peak by lymph node CD8$^+$ T cells. To determine whether the transgenic CD8$^+$ T cells expressed the transgenic β chain as a single or double peak, Des-TCR lymph node cells were stained for CD8 and for the transgenic β chain Vβ2 (Fig. 4). In this case, a biotinylated Vβ2-specific mAb was not available, requiring an alternative staining ap-

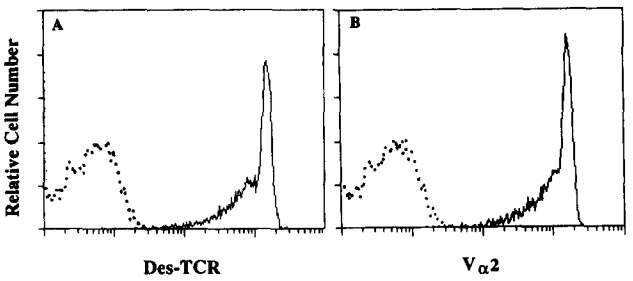

Figure 2. V$_\alpha$2 is expressed in a bimodal manner by CD8$^+$ lymph node cells from Des-TCR transgenic mice. Lymph node cells from Des-TCR transgenic mice were double stained for the expression of CD8 and either (*A*) the Des-TCR or (*B*) V$_\alpha$2, and analyzed by flow cytometry (*solid lines*). As a negative control, Vβ11 expression by CD8$^+$ cells is shown (*dotted lines*).

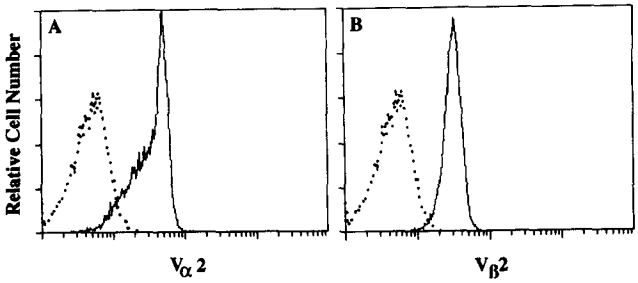

Figure 4. Vβ2 is expressed as a single peak by CD8$^+$ lymph node cells from Des-TCR transgenic mice. Lymph node cells from Des-TCR transgenic mice were stained for CD8 and either (*A*) V$_\alpha$2 or (*B*) Vβ2, and analyzed by flow cytometry (*solid lines*). As a negative control, cells stained with all antibodies except the primary V region–specific mAb are shown (*dotted lines*).

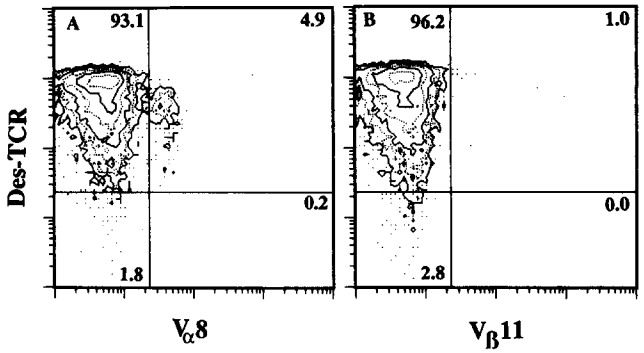

Figure 5. $V_\alpha 8$ is expressed by a subpopulation of Des-TCR$^+$CD8$^+$ lymph node cells from Des-TCR transgenic mice. Lymph node cells from Des-TCR transgenic mice were triple stained for CD8, Des-TCR, and either (*A*) $V_\alpha 8$ or (*B*) $V_\beta 11$, and analyzed by flow cytometry. Contour plots show expression of Des-TCR and either $V_\alpha 8$ or $V_\beta 11$ by CD8$^+$ cells.

proach. Thus, while control staining for $V_\alpha 2$ showed a bimodal peak, it was not as clearly revealed as in Fig. 2. It is, however, evident that the profile of expression of $V_\beta 2$ is a single peak, as seen for CD3. Thus, CD3 and the $V_\beta 2$ transgene were expressed as a single peak, whereas the clonotypic receptor and the $V_\alpha 2$ transgene were expressed in a bimodal manner.

Des-TCR Density Variation Is Due to the Expression of Two α Chains by Some CD8$^+$ T Cells. These results suggested that those cells expressing the lower density of the Des-TCR in the Des-TCR transgenic mice may represent cells expressing the transgenic β chain and two α chains: one being the transgenic α chain and the other endogenously rearranged. To test this possibility, Des-TCR transgenic lymph node cells were triple stained for CD8, the Des-TCR, and $V_\alpha 8$ (Fig. 5). Staining for $V_\alpha 8$ was of low intensity, but in control B10.BR mice this method identified a percentage of cells

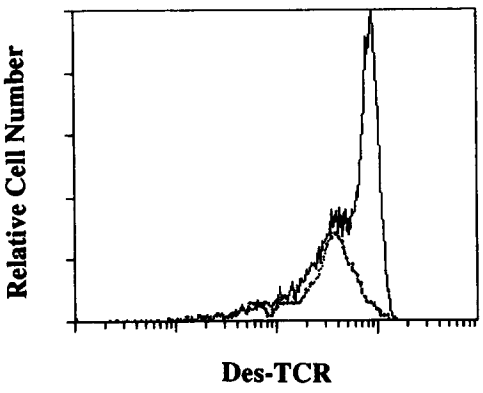

Figure 6. CD8$^+$V$_\alpha$8$^+$ T cells from Des-TCR transgenic mice express the lower density of the Des-TCR. Lymph node cells from Des-TCR transgenic mice were stained as in Fig. 5 and CD8$^+$ cells (*solid line*) or CD8$^+$V$_\alpha$8$^+$ cells (*dotted line*) were examined for their expression of the Des-TCR. For convenience of comparison, the profile of V$_\alpha$8$^+$ cells has been magnified relative to the unseparated population. V$_\alpha$8$^+$ cells represent 4.9% of the unseparated population.

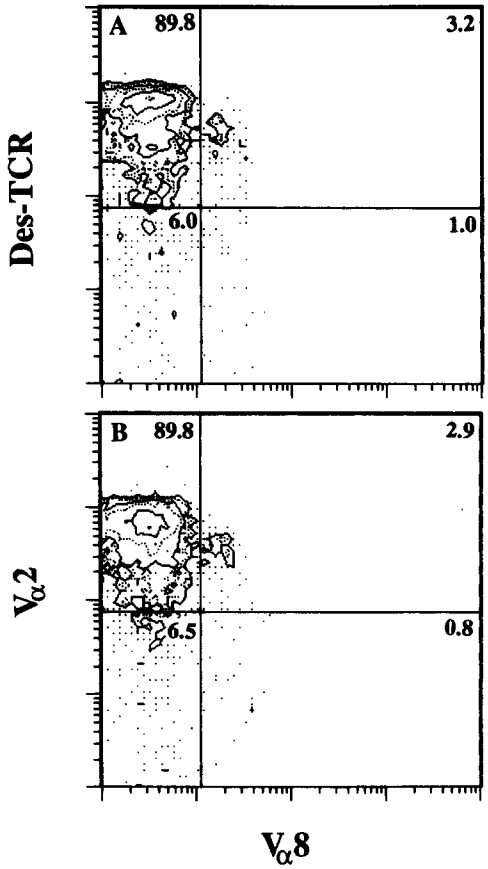

Figure 7. Both $V_\beta 8$ and $V_\alpha 2$ are expressed by some CD8$^+$ T cells from Des-TCR transgenic mice. Lymph node cells from Des-TCR transgenic mice were triple stained for CD8, $V_\alpha 8$, and either (*A*) the Des-TCR or (*B*) $V_\alpha 2$, and analyzed by flow cytometry. Contour plots show expression of $V_\alpha 8$ and either Des-TCR or $V_\alpha 2$ by CD8$^+$ cells.

similar to that previously published (13) (data not shown). Importantly, Des-TCR mice showed a small percentage of CD8$^+$Des-TCR$^+$ cells that expressed $V_\alpha 8$. In five experiments, an average of 4.1 ± 0.8% of CD8$^+$Des-TCR$^+$ cells expressed $V_\alpha 8$. When the CD8$^+$V$_\alpha$8$^+$ cells were examined for Des-TCR expression, these cells were found to be included in the population of cells expressing the lower density of the Des-TCR (Fig. 6). Furthermore, the mean intensity of $V_\alpha 8$ on Des-TCR$^+$ cells was found to be half that of normal B10.BR cells (mean fluorescence intensity of 41.5 vs. 84.2). Triple staining for CD8, $V_\alpha 2$ and $V_\alpha 8$ revealed that some cells expressed both $V_\alpha 2$ and $V_\alpha 8$ (Fig. 7). Together these data showed that Des-TCR transgenic mice possess CD8$^+$ T cells that express different densities of the Des-TCR, of which at least some of those expressing the lower density also express an endogenous α chain on their surface.

Potential Consequences of the Expression of Two α Chains. To our knowledge, this finding represents the first demonstration that in vivo cells can express two different types of TCR on the surface of the same cell. Whether such cells are also present in normal T cell repertoires must await further analysis. These findings have extremely important implications

for at least two areas that utilize TCR transgenic mice. First, when addressing the various haplotypes that positively select a given TCR transgene, the endogenous expression of an α chain together with the transgenic α chain could lead to false conclusions. In situations where the transgenic receptor cannot receive a positive selection signal, T cells may be positively selected by their nontransgenic receptor. T cells bearing the transgenic receptor would enter the periphery of these mice, leading to the false conclusion that positive selection had occurred via this receptor.

The second, and more major consequence of expressing two α chains is that such T cells may have two completely different antigen specificities. This is particularly relevant to autoimmunity studies. We recently reported the induction of autoimmune diabetes in transgenic mice coexpressing three different transgenes, the Des-TCR, RIP-K^b, and RIP-IL-2 (IL-2 expressed by the pancreatic β cells) (15). In some mice, diabetes was induced as early as 7 d after birth. Since the $CD8^+$ T cells in these triple transgenic mice expressed the lower density of the Des-TCR, due to the thymic deletion of those cells expressing the higher density (15), it is likely that they also expressed a second TCR comprised of the transgenic β chain and an endogenous α chain. Since naive T cells do not express IL-2 receptors, and do not usually infiltrate tissues (16), it was unclear how these K^b-specific cells were initially activated so that they could respond to IL-2 and destroy islet tissue. How they were attracted to the islets without expressing IL-2 receptors is still unresolved. While it is possible that these cells were attracted by secondary lymphokines induced as a consequence of IL-2 synthesis, the likely expression of two receptors raises an alternative possibility. These cells may have been activated via recognition of an environmental antigen by their endogenously derived TCR and, after activation, responded to IL-2 produced by the islet β cells and caused K^b-specific destruction via their transgenic TCR.

This scenario may be applicable to several other models. Recently, $\alpha\beta$ transgenic nonobese diabetic mice were shown to be susceptible to autoimmune diabetes despite their expression of a transgenic TCR with no specificity for islet antigens (17). In this study, the expression of endogenously rearranged α chains was not examined. Such α chains may have combined with the transgenic β chain to produce a broad repertoire still capable of causing specific autoimmunity.

It has also been reported that TCR transgenic mice specific for myelin basic protein developed spontaneous autoimmunity, but that this varied depending on the animal housing facility (18). This might be explained if two receptors were expressed by the transgenic T cells. Once these cells responded to an environmental antigen via their endogenously rearranged TCR, they may have caused autoimmunity via their transgenic TCR. This would explain why animal facilities possessing a greater number of pathogens were more likely to be associated with autoimmunity.

Relevance to Autoimmunity. These findings question the view that stimulation by a cross-reactive environmental antigen is required for the induction of autoimmunity. Analysis of T cell clones has revealed that T cells from normal repertoires can express two productively rearranged α chains (3). In these examples, however, only one α chain was detected on the cell surface. Due to the low frequency of any one α chain in a normal repertoire, and the limited range of α chain–specific mAbs available, demonstration of cells expressing two α chains on their surface is technically difficult. If a certain proportion of T cells do express two rearranged α chains on their surface, it is possible that T cells bearing autoreactive TCR may be activated to cause autoimmunity via recognition of an environmental antigen by their second, nonautoreactive TCR. In this case, no molecular mimicry would be required. The frequency of such cells among T cells bearing two different TCR is, however, likely to be very low. Since, of the two TCR, the autoreactive receptor would, by definition, be MHC restricted, there would be no requirement for MHC restriction of the second receptor; positive selection in the thymus could occur via the autoreactive receptor. The likelihood that the second receptor would recognize an environmental antigen in an MHC-restricted manner would, therefore, depend on the innate frequency of MHC-restriction of TCRs. This might be increased somewhat by the presence of a β chain that is able to form an MHC-restricted receptor.

While it remains unclear whether the presence of T cells expressing two TCR clonotypes is applicable to a normal T cell repertoire, we have provided direct evidence that transgenic T cells may possess this phenotype in vivo. Future experiments may clarify the importance of this finding to autoimmunity.

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