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Review

Dual Vα T cells

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Abstract. The assumption that T cells can only express a single receptor for antigen has in recent years been shown to be incorrect. However, the finding that a substantial number of T cells express two distinct antigen receptors at the cell surface raises a number of questions. In particular, it has been suggested that cells expressing low levels of a self-reactive T cell receptor may escape self-tolerance mechanisms and in certain situations trigger the onset of autoimmune disease. Such a hypothesis in turn raises questions central to the understanding of the nature of T cell recognition and the process of thymocyte maturation.

Key words. T cell selection; TCR gene rearrangement.

Introduction

The ability of the T lymphocyte arm of the immune system to recognise a diverse array of antigens is dependent on the clonal variability of T cell receptors (TCRs). On most T cells the TCR is a heterodimer comprised of α and β chains. Genes encoding the variable regions of both chains are assembled during intrathymic maturation of T lymphocytes. For example, the gene encoding the variable region of the β chain is assembled by the ordered joining of individual members of libraries of Vβ (variable), Dβ (diversity) and Jβ (joining) gene segments. Consequently, in the absence of control mechanisms an individual T cell could make productive rearrangements of both TCRβ chain and both TCRα chain loci, and hence express four distinct antigen receptors ($\alpha1\beta1$, $\alpha1\beta2$, $\alpha2\beta1$ and $\alpha2\beta2$). That this does not happen is in part due to allelic exclusion at the TCRβ locus, such that in-frame rearrangement of one β chain gene inhibits rearrangement at the second locus. Rearrangement of the TCRα loci is very different and indeed is not turned off until T-cell-positive selection has occurred and expression of RAG 1 and RAG 2 genes has been shut down (fig. 1). Murine and human T cells commonly carry productive rearrangements of both TCRα loci and express two independent TCRs. Such dual Vα T cells are, at least potentially, capable of expressing two distinct cell surface TCRα ($\alpha1\beta$ and $\alpha2\beta$). However, at this point consensus is lost. In particular agreement has not been reached over the questions of (i) what proportion of peripheral T lymphocytes are dual Vα T cells, (ii) what proportion of immature thymocytes are dual Vα cells (iii) whether autoimmunity can result from the inappropriate activation of dual Vα cells carrying one self-reactive TCR.

Dual Vα T cells in the peripheral immune system

It is clear from studies on TCR transgenic mice and T cell clones that allelic exclusion at the TCRα locus is incomplete. Indeed, the existence of multiple Vα and Jα segments potentially allow any TCRα locus to undergo multiple, sequential V-J rearrangements, and several lines of evidence suggest that this does occur [1–17]. Moreover, in over 90% of clones analysed, both α-chain

loci were rearranged. Whilst in the majority of cases only one rearrangement was functional, approximately one in four clones possessed two in-frame rearranged TCRα loci. However, the existence of two rearranged TCRα loci does not necessarily mean that both TCRα chains are expressed at the cell surface. Failure to express both potential TCR $\alpha\beta$ pairs at the cell surface could result either from poor expression of one α chain or from failure of one $\alpha\beta$ combination to pair due to structural constraints. The first indication that cells expressing two cell surface $\alpha\beta$ pairs are present in the periphery in significant numbers was provided by Lanzavecchia and colleagues [18]. Using monoclonal antibodies specific for the human V regions Vα2, Vα12 and Vα24, they showed that a significant proportion of human peripheral T cells stained positive for any two antibodies. Further, when isolated by cell sorting, one-third to one-half of the clones generated stably expressed both Vα chains. As anti-Vα antibodies to either chain could be used to induce cytotoxicity and down-regulate TCR, each Vα chain appeared to be part of a functional receptor. Though the paper is frequently cited as providing an estimate that 30% of the human peripheral T cell repertoire consists of dual Vα T cells, in fact it is difficult to derive this figure from the data. This is because though the paper unambiguously demonstrates the presence of dual Vα T cells in the normal repertoire, it is impossible to distinguish signal from noise in the primary flow cytometric data. Rather, the paper shows that the number of dual Vα cells is likely to be substantial. As the upper limit to the number of dual Vα T cells was taken to be the proportion of T cell clones found by others to possess two in-frame TCRα rearrangements, the authors concluded that an estimate of 30% dual Vα T cells in the peripheral repertoire was reasonable. That thymocytes or peripheral T cells expressing two cell surface Vα chains, or more rarely two cell surface β chains, exist in normal individuals has subsequently been confirmed in several studies, though debate remains about their frequency [19–26].

Thymic selection and TCR Vα chains

One of the notable features about TCRα usage in mice is the degree to which individual Vα chains are expressed preferentially in CD4 or CD8 subsets, the subset preference being largely independent of major histocompatibility complex (MHC) haplotype. For example, in C57BL/10 mice the proportional usage of Vα3.2 in the CD8 subset appears to be over 10 times that amongst CD4+ cells [27]. The phenomenon has a number of implications for the study of dual Vα T cells. First, the fact that Vα usage is skewed between CD4 and CD8 subsets suggests that individual Vα elements interact preferentially with either MHC class I or class II molecules [28]. Skewing of Vα3.2 usage to the CD8 population could result from interactions between Vα3.2 and MHC class II which are either too 'strong', leading to the deletion of CD4+ Vα3.2+ cells, or too 'weak', such that Vα3.2+ cells fail to be positively selected to the CD4+ compartment. In the former case it is easy to see that on dual Vα cells of which one Vα chain is Vα3.2, the higher the level at which Vα3.2 is expressed, the more likely it is that the cell will be deleted from the CD4 subset. Thus CD4+ dual Vα cells expressing Vα3.2 might be expected to be Vα3.2^{lo}. Similar expectations might arise if the interaction between Vα3.2 and MHC class II is too 'weak' to permit efficient thymic selection of CD4+ Vα3.2+ cells. In such a case, on a dual Vα thymocyte committed to the CD4 subset, the higher the proportion of cell surface TCR which is comprised of 'weak' Vα3.2 chains, the less likely it would be that the cell is positively selected. Thus the phenomenon of Vα skewing allows the prediction that on dual Vα T cells the level at which individual Vα chains are expressed will be Vα chain-specific.

The second implication of the phenomenon of Vα chain skewing affects the methodology for analysis of dual Vα cell frequencies. For example, whereas Vα3.2 is predominantly expressed on CD8+ cells, Vα11 usage is strongly skewed to the CD4 subset. Consequently the frequency of Vα3.2+ α11+ cells may be substantially lower than predicted from use of Vα3.2 and Vα11 in

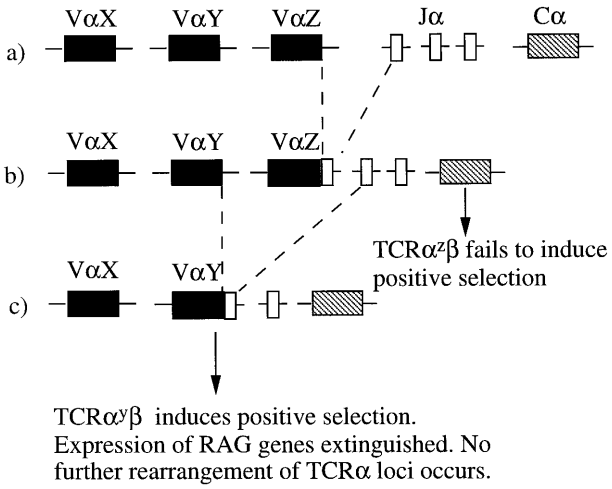

Figure 1. Rearrangement of the TCRα locus. Individual members of libraries of Vα and Jα gene segments of the unrearranged TCRα locus (a) are spliced together. Due to the absence of an intervening D gene segment, multiple sequential rearrangements are permissible (b, c). Cell surface expression of a TCR$\alpha\beta$ pair which induces positive selection results in the shutdown of expression of RAG genes, and rearrangement of the TCRα locus ceases.

total T cell preparations. Frequencies of dual Vα cells should therefore be derived from analysis of either CD4+ or CD8+ populations.

Secondary TCRs as passengers in thymic selection

Lanzavecchia and colleagues in their original description of dual Vα T cells suggested that such lymphocytes may play an important role in the development of autoimmune disease [18]. The hypothesis was that during thymic selection positive selection might be mediated by one of the two TCRs on a dual Vα thymocyte, allowing potentially autoreactive TCRs to escape as passengers into the periphery. Subsequent activation of the T cell in response to exogenous antigen via the positively selected TCR would also trigger the autoreactive potential of the covertly selected TCR. As further developed by Mason [29], the notion of a covertly selected TCR is largely dependent on the assumption that random rearrangement of TCRα and β loci and subsequent association of TCRα and β chains is highly likely to result in the production of an αβ TCR pair which fails to interact with self-MHC. That is, most thymocytes will die of neglect. Whilst data addressing the question of what proportion of thymocytes die through neglect and through active deletion are mixed, there are, I believe, good reasons for favouring those which suggest that very many thymocytes are actively deleted. Surh and Sprent, for example, assessed the comparative frequencies of apoptotic thymocytes in wild-type mice and those lacking MHC molecules (which consequently cannot negatively select) [30]. As these workers found little difference in the frequency of apoptotic cells in the two groups of mice, they argued that the great majority of thymocyte apoptosis occurs due to neglect. However, as pointed out by others [31], because thymocytes do not mature in MHC-deficient mice, the level of apoptosis will necessarily be very high, and the comparison with normal mice is not a legitimate one. Several groups have analysed the T cell repertoire selected by self-MHC class II apparently complexed with a single peptide [32–35]. Such mice possess around a third of the normal numbers of CD4 T cells. Significantly, 70% of T cell hybrids from such mice react with wild-type antigen-presenting cells expressing self-MHC/peptide. However, others have recently suggested that selection in such systems is dependent on the presence of low-abundance, 'contaminating' peptides in such systems [36]. A more direct approach was taken by Merkenschlager et al., who showed that around one in five thymocytes generated in the absence of MHC will react with MHC-expressing thymic stroma in short-term reaggregate cultures [31]. Similar data has also been reported by others [37]. It might be expected that as such work measures a snapshot of self-reactivity measured by upregulation of activation markers at 24 h and will not, for example, accurately measure the number of cells which may apoptose rapidly in response to MHC, it provides a minimal estimate of the propensity of TCRs to interact with self, and favours the concept that randomly expressed TCRs have an innate propensity to interact with MHC. In an alternative approach, van Meerwijk

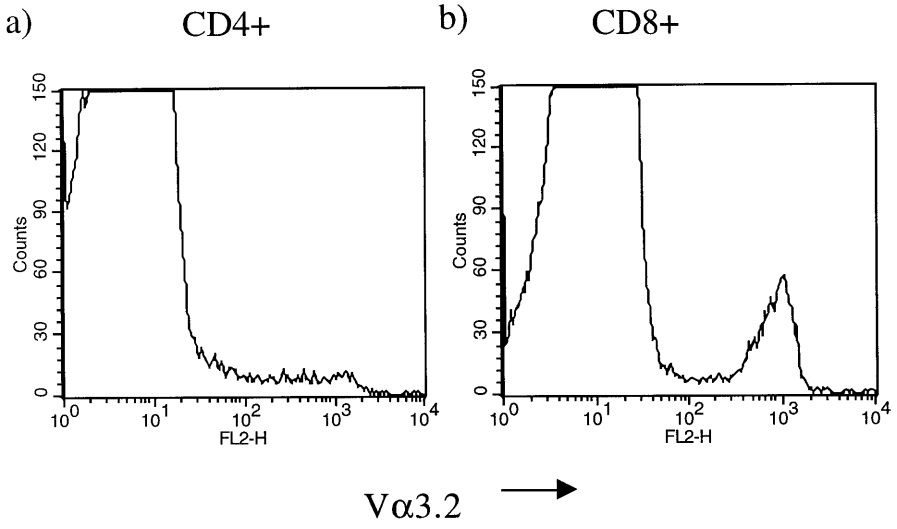

Figure 2. Vα3.2+ populations in CD4 and CD8 subsets in C57BL/10 mice. Mesenteric lymph node cells were stained with anti-CD4, -CD8 and -Vα3.2 antibodies. Vα3.2 use is heavily skewed toward the CD8 subset (*b*), and is often negligible amongst CD4+ cells (*a*).

and colleagues constructed radiation bone marrow chimeras in which irradiated mice were repopulated with bone marrow cells from MHC class II-positive or -negative mice [38]. From the excess production of CD4+ cells in mice lacking MHC class II on negatively selecting bone marrow-derived cells, they argue that half to two-thirds of thymocytes able to undergo positive selection die due to negative selection. As I have suggested elsewhere, there are several other reasons to believe that the innate self-reactivity of the unselected thymocyte repertoire is very high [39].

Concepts of the propensity of randomly generated TCRs to interact with self are intimately associated with the study of dual Vα T cells. Thus the hypotheses that (i) most T cells die of neglect and (ii) the level of cell surface TCR is fixed are difficult to reconcile with the production of large numbers of functional dual Vα T cells because any reduction in the level of a critical positively selecting TCR through competition with an irrelevant receptor would be likely to severely compromise thymic selection. Similarly, if only one of a pair of cell surface TCRs is relevant to selection then (in contrast to the argument put forward in the section above) one would expect to see little or no evidence of Vα subset skewing in the secondary 'irrelevant' TCR. One of the aims of the study of dual Vα T cells is therefore to shed light on the dynamics of TCR/MHC interactions in the thymus.

Selection of Vα3.2+ dual Vα cells

Two groups have used a comparison of lymphocytes from wild-type mice and littermates hemizygous for a mutation at the TCRα locus [40] and consequently unable to express two rearranged TCRα genes to study dual Vα T cells. As described above, use of Vα3.2 in C57BL/10 mice is strongly skewed toward the CD8 subset (fig. 2). The analysis of Vα3.2 expression also indicates the lessons that can be learned from the study of dual Vα cells and the limitations of such work. As shown in table 1, the most striking finding in estimation of dual Vα T cell frequencies in C57BL/10 mice is the disproportionate number of CD4+Vα3.2+ cells which express a second receptor. Indeed, from frequencies of CD4+Vα8+α3.2+ and CD4+Vα11+α3.2+ cells, the extrapolated frequency is over 100%. That estimated frequencies of dual Vα cells can exceed 100% is due to the fact that to minimise background noise the overall usage of Vα3.2 in the CD4+ population is defined by the proportion of CD4+Vα3.2^{hi} cells. Indeed, the same approximation is made for all Vα populations. However, in the case of CD4+Vα3.2-expressing cells it appears that the majority are Vα3.2^{lo} (i.e. probably express a second Vα chain). Indeed, often no discrete population of CD4+Vα3.2+ cells could be detected (fig. 2).

The second, potentially related, feature of CD4+ Vα3.2+ dual Vα T cells is that when coexpressed with a second Vα chain there is a strong tendency for Vα3.2 to be expressed at very low levels (fig. 3a). Of the Vα chains analysed, this phenomenon is specific to Vα3.2, such that whilst CD4+Vα2^{hi}α8^{hi} cells are common (fig. 4), Vα2^{hi}3.2^{hi} cells are not. At least two explanations for the low expression level of Vα3.2 could be given. First, when coexpressed with a second Vα chain, due to structural constraints Vα3.2 might compete poorly for binding to Vβ. Second, as discussed above, high-level Vα3.2 expression might predispose against positive selection on MHC class II. To distinguish between the two hypotheses, the pattern of dual Vα2+α3.2+ expression was compared between CD4+ and CD8+ cells. Thus, if Vα3.2 competes poorly with other Vα chains for binding to Vβ, this should be apparent in both CD4 and CD8 subsets. As shown in figure 3b, this was not the case. Relative levels of Vα3.2 in CD4 and CD8 subsets differ, and no predominant Vα2^{hi}α3.2^{lo} population is apparent in the CD8 subset. As is shown in table 1, the proportion of Vα3.2+ dual Vα cells is also much lower in the CD8+ than the CD4+ subset.

Thus the low efficiency with which Vα3.2+ cells are positively selected by MHC class II is associated both with a very high relative frequency of Vα3.2+ dual Vα T cells and with low expression of Vα3.2 on dual Vα cells.

Table 1. Frequency of dual Vα T cells.

Vα pair	Subset	Frequency of dual Vα T cells (%)
2/8	CD4	13.6
2/8	CD8	6.5
2/11	CD4	7.5
2/11	CD8	31
8/11	CD4	16
2/3.2	CD4	60
2/3.2	CD8	5.5
8/3.2	CD4	>100
11/3.2	CD4	>100

Mesenteric lymph node cells from mice carrying a targeted mutation of the TCRα locus and from wild-type littermates were stained with combinations of anti-CD4, -CD8 and Vα antibodies. The mean number of cells within a gate for dual Vα T cells in wild-type mice had subtracted from it the number of events within an equivalently gated population from TCRα+/− littermates. The extrapolated percentage of dual Vα T cells is derived from the observed frequencies of cells staining high for each Vα population. For example, a mean of 43.9 Vα2+α8Vα3.2+ T cells was found per 10^5 CD4+ cells. As the total percentages of CD4+Vα2^{hi} and Vα8^{hi} cells were 11.8 and 2.73, respectively, if all T cells carried two cell surface Vα chains, the proportion of Vα2+ cells coexpressing Vα8 would be 0.118×0.0273, that is 322 of 10^5 CD4+ cells. Therefore, the extrapolated percentage of dual Vα T cells is $43.9/322 = 13.6$. This calculation is relatively accurate when dual Vα T cells are a small fraction of the total, but can give percentages of >100 when the proportion of dual Vα cells is high.

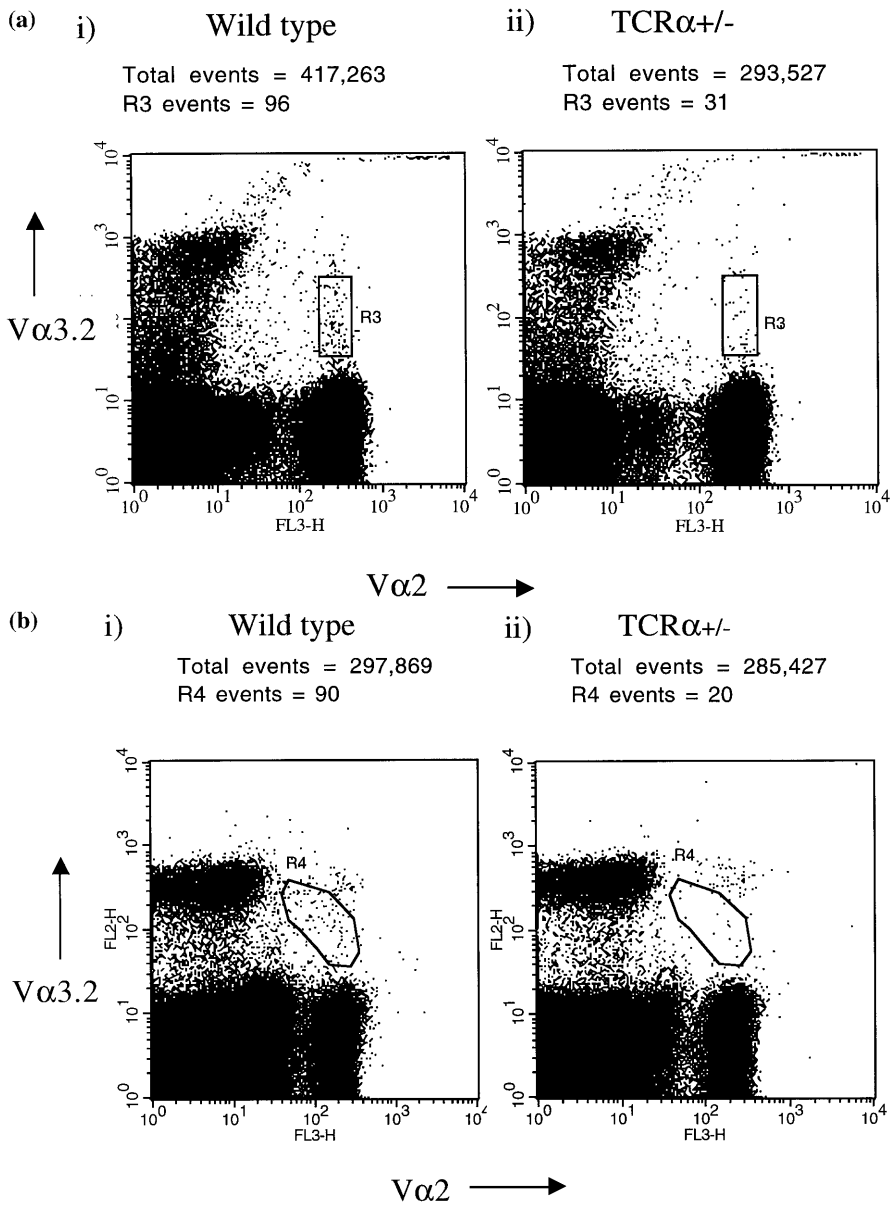

Figure 3. Relative use of Vα2 and Vα3.2 on dual Vα T cells in CD4 and CD8 lineages in C57BL/10 mice. Mesenteric lymph node cells from TCRα+/− mice and wild-type littermates were triple-stained with appropriate combinations of (*a*) anti-CD4, (*b*) -CD8, and anti-Vα antibodies. Gates show the major populations of dual Vα cells in wild-type mice. Whilst Vα3.2 expression on CD4+ dual Vα cells is predominantly very low, this is not the case of Vα3.2 expression on CD8+ cells.

Vα11+ dual Vα T cells

In C57BL/10 mice Vα2 expression is skewed toward the CD4 subset; Vα3.2 expression is strongly skewed toward the CD8 subset. Thus in analysing Vα2+α3.2+ cells, one is addressing a cell population carrying Vα chains with opposing positive selection preferences. Vα2+α11+ cells constitute a population in which both TCRα chains shared an MHC class selection preference. Surprisingly, some CD8+Vα2+α11+ but not CD4+Vα2+α11+ cells appear to express levels of Vα11 somewhat higher than that on the majority of Vα11+ lymphocytes (fig. 5). However, such Vα11^{hi} cells coexpress Vα2 at a high level, strongly suggesting that such cells express abnormally high levels of cell surface TCR, perhaps more than twice the average level (that is, normal levels of Vα2 plus elevated levels of Vα11). The most appealing explanation for the high level of TCR on CD8+Vα2+α11+ cells is that it is due to the relative inefficiency with which both Vα chains are selected by MHC class I. Thus many thymo-

cytes expressing either Vα11 or Vα2 may recognise MHC class I ligands below a threshold required for positive selection. As those thymocytes coexpressing Vα2 and Vα11 at high levels increase the number of selecting ligands available, a population of cells will exist which will be dependent on signals derived from both receptors to cross the threshold between death by neglect and positive selection. A variation on this derives from analysis of Vα/Vβ pairing in thymic selection. For example, the presence of H2-E favours the selection of both Vα11- and Vβ6-bearing cells into the CD4 subset. However, the high frequency of Vα11 + Vβ6 + cells in H2-E + mice is consistent with the suggestion that the cumulative result of two independent positive selection effects is synergistic [27]. Consequently, by analogy two weak positive selection events mediated by interaction of Vα2 and Vα11 with MHC class I may interact synergistically to result in strong positive selection. This differs from the former model in that neither independent Vα interaction with MHC class I need be below the threshold for positive selection. Alternative explanations are of course possible.

Selection of dual Vα T cells

The main conclusion derived from the analysis described above and of other combinations of TCRVα pairs is that the frequency of dual Vα T cells and the relative expression levels of different Vα chains is closely related to the efficiency with which the two Vα chains mediate thymic selection into a given T cell subset. First, inefficient positive selection correlates with high frequencies of dual Vα T cells. This is likely to be related to two interrelated factors:

1) As originally hypothesised by others [41], the sooner a TCRαβ pair which can mediate differentiation to the single positive state (positive selection) can be expressed at the cell surface, the sooner expression of RAG genes is extinguished, inhibiting further TCRα rearrangement, reducing the chance that a second TCRα chain will be coexpressed. Hence, weak positive selection signals, insufficient to efficiently drive terminal maturation, would be predicted to be associated with increased frequencies of dual Vα T cells. This appears to be the case. In certain circumstances (e.g. CD8 + Vα2 + α11 + cells) expression of two Vα chains also appears to be associated with increased levels of cell surface TCR.

2) If, for example, high-level expression of Vα3.2 is strongly associated with the deletion of CD4 + T cells, then Vα3.2 + -monospecific cells will be eliminated more readily than Vα3.2^{lo} (dual Vα) cells, resulting in a high relative frequency of the latter. Monospecific cells would be similarly affected by the presence of a Vα chain which mediates excessively 'weak' MHC interactions.

T cell receptor repertoire selection in TCRα + / − mice

Given the hypothesis that thymic selection of CD4 + Vα3.2 + cells is inefficient but can be enhanced through

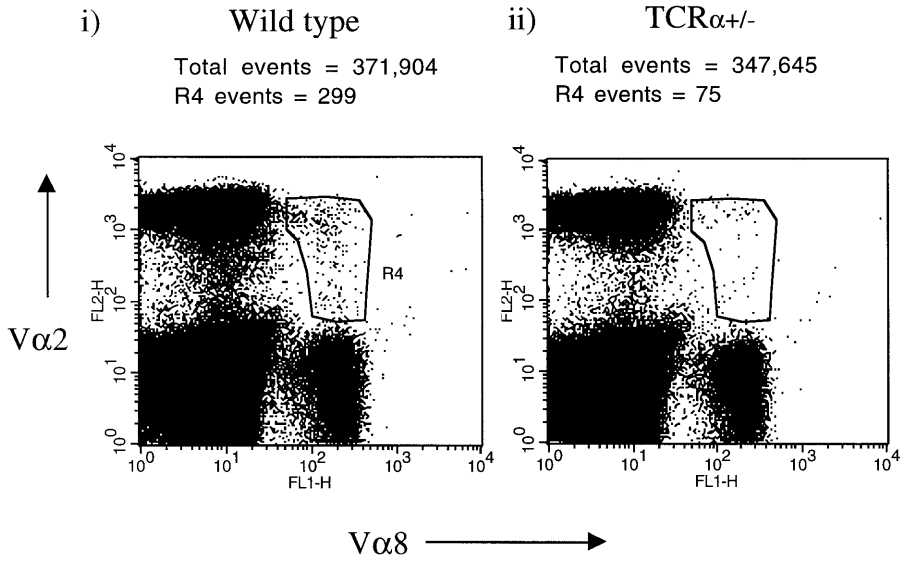

Figure 4. Relative use of Vα2 and Vα8 on CD4 + dual Vα T cells in C57BL/10 mice. Mesenteric lymph node cells from TCRα + / − mice and wild-type littermates were triple-stained with appropriate combinations of anti-CD4 and anti-Vα antibodies. Gates show the major populations of dual Vα cells in wild-type mice.

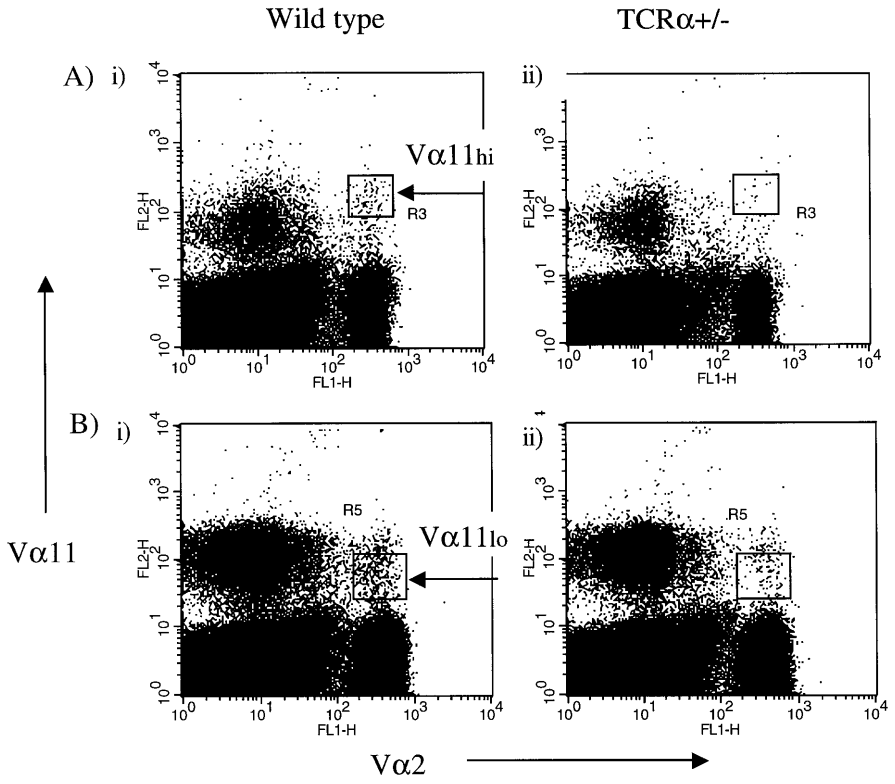

Figure 5. Relative use of Vα2 and Vα11 on dual Vα T cells in C57BL/10 mice. Mesenteric lymph node cells from TCRα+/− mice and wild-type littermates were triple-stained with appropriate combinations of anti-Vα antibodies and (*a*) anti-CD8 and (*b*) anti-CD4. A significant number [shown in gates in (*a*)] of CD8 + Vα2 + α11 + dual Vα T lymphocytes appear to express Vα11 chains at levels higher than those found on the bulk of monospecific cells.

expression of a second Vα chain, one might predict that TCRα+/− mice possess fewer CD4 + Vα3.2 + T lymphocytes than wild-type littermates, as the former cannot rescue selection through the generation of dual Vα cells. The corollary of the prediction is that as a proportion of the CD4 + repertoire, wild-type mice should possess fewer CD4 + T cells bearing efficiently selected TCRα chains (e.g. Vα2, Vα11) than do TCRα+/− littermates. In fact, the percentage of both Vα2 + and Vα11 + cells appear to be higher in wild-type than TCRα+/− mice. For example, in one experiment in a group of 13 mice the percentages of (CD4 +) Vα11 + cells were 4.49 and 4.11 in wild-type and TCRα+/− mice, respectively, and of CD4 + Vα2 + cells were 12.4 and 10.6, respectively (unpublished). At least two possible explanations for this finding can be given. First, wild-type mice may possess more T cells than do TCR+/− littermates. Though there is no evidence that this is the case, it perhaps has not been assessed with sufficient accuracy. In this case the number of both CD4 + Vα3.2 + and CD4 + Vα2 + cells could rise in wild-type mice even if only the latter increases as a proportion of the CD4 + repertoire. Second, the prediction of increased numbers of CD4 + Vα3.2 + cells in wild-type mice assumes that thymocytes and peripheral T cells do not compete for survival, that is essentially that all T cells that can be selected will be selected. However, if the maximal number of peripheral T cells is fixed and the number of potentially selectable thymocytes increases, then thymoctes and/or T cells must compete for survival. There are a number of reasons why one might envisage that the total number of 'selectable' thymocytes is higher in wild-type mice than TCRα+/− littermates. For example, as above, expression of a second Vα chain may rescue thymocytes expressing poorly selected chains. Second, many TCR+/− thymocytes may die before functionally rearranging the TCRα locus. This number would presumably be lower in wild-type mice able to attempt rearrangement of two loci. In addition, considering an efficiently selected TCR, in TCRα+/− mice the frequency of in-frame rearrangements of the Vα2 locus may limit the frequency of CD4 + Vα2 + cells. This constraint on the frequency of Vα2 + cells will be lifted in wild-type mice. Thus in a system in which thymocytes and T cells compete with each other, for

example for limiting quantities of essential cytokines, though expression of a second Vα chain may rescue inefficiently selected thymocytes, this may not be reflected in an increase in the percentage of CD4+ Vα3.2+ cells if the enhanced selection of Vα2+ cells gives the greater selective advantage for survival.

The relative frequencies of CD4+Vα3.2+ cells in TCR+/− and wild-type mice has proved difficult to assess. The main problem is that the number of CD4+ Vα3.2+ cells is very low in both groups. Differences in the frequency of such cells may therefore be masked be masked by minor intermouse variability. More important, apparently CD4+Vα3.2^{lo} cells can be found even in TCRα+/− mice. It is unclear whether such cells reflect (i) weak cross-reactivity of the anti-Vα3.2 antibody with other Vα chains, (ii) Vα3.2+ cells coexpressing a second Vα chain at the cell surface as a relic of a prior recombination event at the intact TCRα locus, (iii) memory T cells—as these express comparatively low levels of TCR—or (iv) Vα3.2+ cells which have generally downregulated TCR as a means of escaping deletion. Consequently, whilst there appears no significant difference in the number of CD4+Vα3.2^{hi} cells in TCRα+/− and wild-type mice, it is not clear whether the result is accurate. Given the considerations outlined above and that data are recorded as percentages of CD4+ cells, not absolute cell numbers, little can be deduced from the apparent equivalence of frequencies of CD4+Vα3.2+ cells in TCRα and wild-type mice.

Intracellular expression of TCRα chains

Not all studies have shown dual Vα T cells to constitute a substantial proportion of the T cell repertoire. Notably, Alam et al. initially found the periphery to be devoid of dual Vα T cells [24], though the same group has subsequently revised this figure to around 3% [25]. This frequency nevertheless remains substantially below the findings outlined above. Alam et al. also find, however, that though frequencies of peripheral T cells expressing two cell surface TCR Vα chains is very low, around 25% express two intracellular TCR Vα chains [25]. It is perhaps easiest to resolve the differences in published data in terms of sensitivity of detection. For example, cells expressing very low levels of cell surface Vα3.2 may express relatively high levels of cytoplasmic Vα3.2 and hence be more readily detected as permeabilised cells. If so, when two Vα chains are expressed at the cell surface at differing densities (e.g. on CD4+ Vα2^{hi}α3.2^{lo} cells), the low expression of Vα3.2 may in part be due to a relative inability to pair with Vβ. However, as indicated from the relative intensities of Vα2 and Vα3.2 on CD8+ cells, there is probably no intrinsic difference in the ability of these two chains to pair with Vβ. Therefore, whereas the high numbers of CD4+Vα2^{hi}α3.2^{lo} cells indicates a strong bias against the selection of Vα3.2^{hi} cells, the control of Vα3.2 expression levels appears to occur at the level of competition for Vβ and is not transcriptional.

Intrathymic dual Vα cells

There are conflicting reports as to whether immature thymocytes are enriched for dual Vα T cells [24, 26, 42]. Whilst it is not clear why the two sets of data differ, the groups have performed different experiments. Thus, whilst wild-type sorted and restained immature Vα2^{lo} thymocytes appeared to possess very large numbers of cells expressing a second Vα chain [24], no dual Vα thymocytes could be detected amongst freshly isolated wild-type cells when analysed in parallel with TCRα+/− control cells, suggesting either that very few immature thymocytes are dual Vα cells or, more probably, that the relatively low level of TCR on the surface of immature thymocytes prevents their detection [42]. It may be difficult to resolve this question. Certain dual Vα populations, for example Vα2^{hi}α3.2^{hi} cells, are very likely to be specifically deleted late in thymic maturation. In other cases expression of a second receptor is likely to inhibit deletion of autoreactive cells [43, 44]. Thus the relative intensities and frequencies with which cells stain with specific pairs of anti-Vα antibodies will probably differ between immature and mature thymocytes. Ultimately the ability to assess the frequency of dual Vα cells is highly dependent on the intensity with which anti-Vα antibodies stain lymphocytes, and this is especially true of immature thymocytes on which the level of cell surface TCR is 10% of that on cells following positive selection [45, 46]. With regard to the specific disagreement between groups as to the frequency of immature dual Vα thymocytes, it remains possible that in time away from the thymic stroma, for example during cell sorting, levels of TCR increase, which favours the hypothesis that frequencies of dual Vα thymocytes are high and are detected through increased staining sensitivity, and/or that mechanisms affecting the control of TCRα rearrangement or cell surface retention are affected, favouring the hypothesis that thymocytes are not enriched for dual Vα cells.

Dual Vα cells and autoimmunity

A substantial amount of effort has been spent on attempts to determine whether dual Vα T cells have an important role in the development of autoimmune disease. Two distinct approaches have been taken. In the first, a mutation of the TCRα locus was backcrossed for

several generations onto non-obese diabetic (NOD), MRL lpr/lpr and SJL backgrounds [19]. This allows a comparison to be made between the susceptibility to autoimmune disease of wild-type mice and those possessing only one functional TCRα locus and consequently unable to generate dual Vα T cells. The initial data suggested that no difference in susceptibility to spontaneous lupus (MRL lpr/lpr) or induced experimental autoimmune encephalomyelitis (SJL) could be found between wild-type and TCR$\alpha+/-$ mice, but that NOD.TCR$\alpha+/-$ mice appeared to be protected from development of spontaneous IDDM. However, further backcrossing of the TCRα mutation onto the NOD background indicated that inhibition of IDDM on the NOD background was due not to any lack of dual Vα cells, but to an IDDM susceptibility gene cosegregating with the TCRα locus on chromosome 14 [47]. Thus the presence or absence of dual Vα cells appears to make no difference to autoimmune susceptibility in the models tested to date. The second approach is to investigate TCR transgenic mice. If one assumes that susceptibility to autoimmune disease is related to the frequency of autoreactive T cells, then it is not surprising that TCR transgenic mice often yield dramatic results. However, it is not always obvious what general interpretations should be drawn. Nevertheless, what such data indicate is that autoreactive TCR transgenic T cells can escape to the periphery in the presence of endogenous coexpressed TCR chains, presumably due to a concomitant reduction in the level of cell surface TCR^{tg} [43]. Further, such transgenic T cells which escape tolerance induction can retain the capacity to induce autoimmune disease [44, 48]. However, it must be remembered that TCR transgenes encoding autoreactive TCRs on an RAG-deficient background (consequently unable to express endogenous TCRs) have also been found to be susceptible to autoimmune disease and indeed can develop disease at a higher rate than those on a wild-type [49, 50]. Thus the simplest explanation for the TCR transgenic data implicating dual Vα T cells in autoimmune disease pathogenesis is that, as suggested by others, T cells positively selected close to the border with negative selection are relatively autoreactive [51]. Thus TCR transgenes which when expressed at high level induce deletion can escape tolerance induction if expressed at reduced density. In such situations there is a bias toward positive selection at the border with deletion and consequent selection toward autoreactivity.

Expression of a second TCRα chain is very likely to be a means by which T cells can escape deletion. This is supported by studies of TCR transgenic mice and is also the simplest interpretation of the low level at which Vα3.2 tends to be expressed on CD4+ dual Vα cells. However, it is also clear that autoreactive cells can escape into the periphery in the absence of a second T cell receptor. It is worth considering two factors in the development of autoimmune disease. First is the hypothesis that T cells selected close to the border with negative selection are relatively autoimmune [51]. Second, not only must T cells for any key autoantigen escape to the periphery, they must do so in sufficient numbers to induce disease. As illustrated (fig. 6), if a requirement for an autoreactive T cell is to be positively selected close to the border with negative selection, this can be achieved by the expression of a second TCRα chain (b) which reduces the overall avidity for self of a thymocyte which would otherwise be deleted (a). This T cell, specific for autoantigen 'y', has been given a notional avidity for self of 'x'. However, a crucial determinant of whether an individual develops autoimmune disease is likely to be the number of autoreactive T cells and, importantly, the expression of a second Vα chain is only one way in which the critical autoreactive avidity can be achieved. The most obvious other means is that T cells unrelated to the autoreactive dual Vα T cell, and which do not rely on the expression of a second Vα chain to escape deletion, can be weakly autoreactive (fig. 6c,d). Such T cell/MHC interactions may represent those studied by Wraith et al. [51]. Thus the importance of dual Vα T cells in autoimmunity may depend on whether they are a minor or a major fraction of those cells which bind autoantigen 'y' with avidity 'x'. As the absence of dual Vα T cells does not appear to increase spontaneous autoimmune disease susceptibility, the probability is that dual Vα T cells are not a major factor in pathogenesis. Indeed, as outlined earlier, both TCRs on dual Vα T cells appear to contribute toward T cell selection, and consequently dual and mono Vα T cells will be subject to the same selection criteria.

Concluding remarks

The existence of dual Vα T cells is now accepted by all working in the area. Their relative frequency is still contentious with estimates varying from 3–30%. It is likely that the disagreement is not fundamental. Detection of low-level cell surface TCR is inevitably limited by the efficiency of Vα-specific antibodies, and those supporting estimates of dual Vα T cell frequencies at the lower end of the range nevertheless find that considerably more T cells express two intracytoplasmic Vα chains. As it is unlikely that retention of one of two Vα chains in the cytoplasm is absolute, many dual Vα T cells presumably express higher levels of the second Vα chain in the cytoplasm than they do at the cell surface. Why the immune system has evolved to allow the production of dual Vα cells is not clear. In my opinion, because dual Vα T cells do not appear to be a significant autoimmune hazard, there is no reason for the

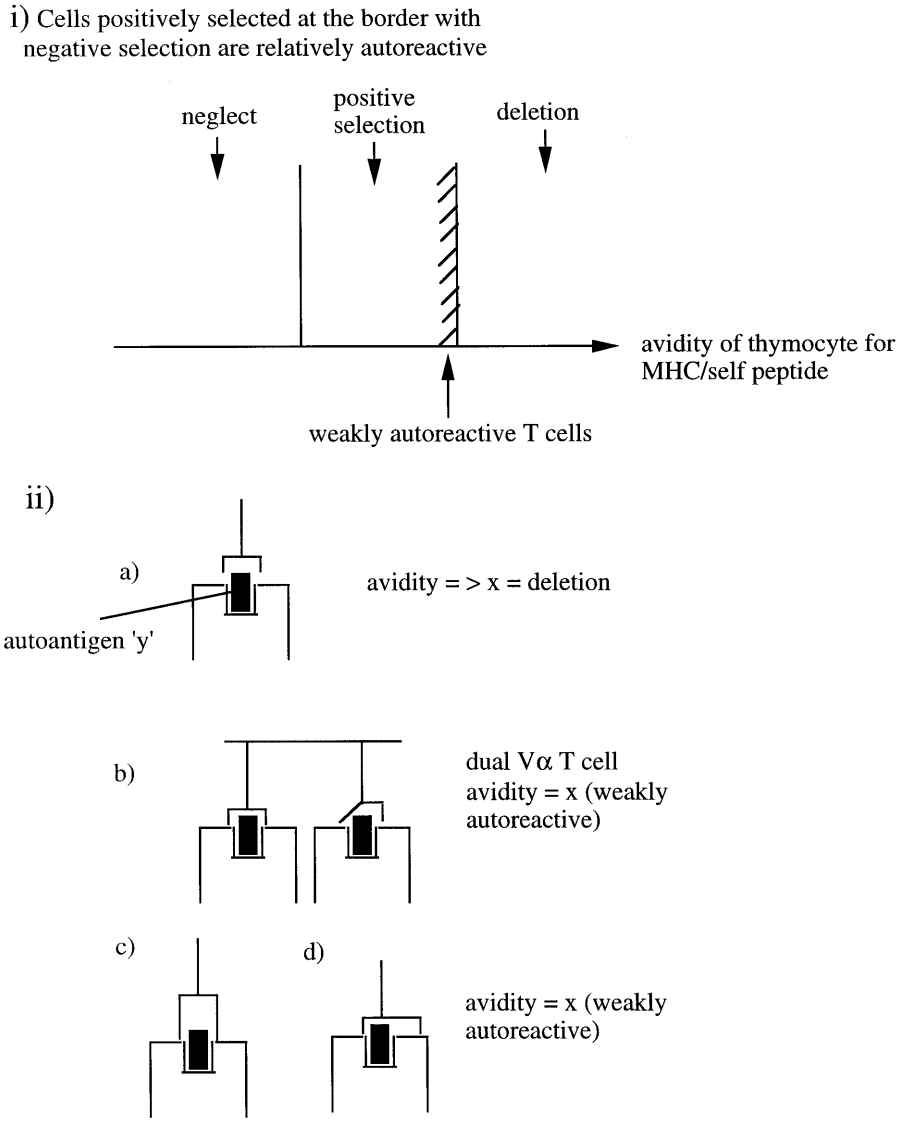

Figure 6. Dual Vα and monospecific T cells in autoimmune disease. (*i*) It has been suggested that T cells positively selected close to the border with negative selection are relatively self-reactive. (*ii*) If so, T cells carrying high-affinity self-reactive specific for autoantigen are deleted *(a)*, but can be rescued *(b)* by means of expression of a second Vα chain which competes for binding to Vβ, decreasing the overall avidity of the T cell/MHC/self-peptide interaction. In normal animals T cells specific for (or cross-reactive with) the same autoantigen ('y') binding with the same low avidity ('x') as possessed by the dual Vα T cell may carry unrelated TCRs *(c, d)*. Whether dual Vα T cells play a significant role in autoimmune disease depends on the relative frequencies of weakly autoreactive cells carrying a second Vα chain *(a)*, or single TCRs of similar overall avidity *(c, d)*.

immune system to exclude them. Both TCR specificities appear to participate in thymic selection and dual Vα cells mature and survive in an environment in which they compete for space with monospecific cells. Presumably, therefore, dual Vα cells exist simply because they increase diversity and outcompete other T cells following the same rules which govern the selection of monospecific lymphocytes.

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