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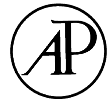

Evaluation of the Percentage of Peripheral T Cells with Two Different T Cell Receptor α-Chains and of their Potential Role in Autoimmunity

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Approximately 25% of mature T cells possess two distinct cytoplasmic T cell receptor (TCR) α-chains, due to productive gene rearrangements of both alleles. Expression of two different α-chains at the cell surface is a potential risk factor for development of autoimmunity. However, it has been difficult to determine the frequency of peripheral T cells with two different α-chains at the surface. Our new approach is based on comparing by flow cytometry the percentage of cells that express a given Vα-chain between wild-type mice and mice that are hemizygous for a disrupted Tcra locus (Tcra+/−) and consequently unable to express two rearranged Tcra genes. We consistently found that ~8% of total peripheral T cells express two surface α-chains. The importance of dual α-T cells in autoimmunity was examined in a mouse model for rheumatoid arthritis, namely collagen-induced arthritis (CIA). No significant difference was observed between Tcra+/− mice and wild-type littermates, considering arthritis incidence, day of disease onset, and maximum arthritic score. We therefore conclude that there is incomplete phenotypic allelic exclusion in TCRα, and that the presence of a significant number of potentially multireactive T cells does not increase the susceptibility to develop autoimmune arthritis.
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Introduction

Allelic exclusion signifies the expression of a gene from only one of the two parental chromosomes bearing that gene in each individual cell. In the immune system, allelic exclusion has been shown to occur for both immunoglobulin heavy and light chain genes [1]. The biological relevance is that individual B cells must produce only one specificity of antibody (the one cell—one specificity rule). This ensures that all the antibodies secreted by an activated B cell are specific for the antigen that originally triggered its proliferation. T cells, like B cells, are characterized by a clonally variable antigen receptor. The T-cell antigen receptor (TCR) is a heterodimeric molecule, which consists, for most T cells in the adult, of an α- and a β-chain. A functional TCR is produced by random somatic rearrangements in TCR α- and β-germline genes. Allelic exclusion has been demonstrated for the TCRβ locus (Tcrb), where acquisition of a productive gene segment rearrangement stops all further rearrangement at the other allele [2]. There is consequently a single productively rearranged Tcrb gene in mature T cells, and the second allele is in a non-productive form due to out-of-frame or incomplete rearrangement. This 'genotypic' form of allelic exclusion ensures that each individual T cell will bear a single TCRβ-chain [2].

In contrast to the β-chain, genotypic allelic exclusion does not apply to the α-chain of the TCR. In almost all mature T cells so far studied, both α-alleles appeared to be fully rearranged, and, in about 25% of the clones, both Tcra genes were found in a productive configuration [2]. It was shown, however, that the presence of two productively rearranged Tcra genes does not necessarily imply that two different α-chains will be expressed at the cell surface. For instance, in a T cell clone containing two different α-chains in the cytoplasm, one of the two chains was shown to be unable to make a pair with the β-chain and was thereby prohibited from surface expression [3]. Preferential association of some αβ chain combinations has also been suggested as a mechanism to prevent one of the two α-chains from reaching the surface [2, 4]. These observations led to the hypothesis that post-translational events could account for a 'phenotypic' type of allelic exclusion ensuring that a given clone will only express one functional TCR α-chain at the surface [2, 5].

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Table 1. *Analysis by flow cytometry double-staining of the frequency of murine peripheral T cells expressing two TCRα-chains at the surface*[a]

Subset[b]	Pair of anti-Vα monoclonal antibodies used for staining						Refs.
	Vα2/Vα3.2	Vα2/Vα8	Vα2/Vα11	Vα3.2/Vα11	Vα3.2/Vα8	Vα8/Vα11	
		14–17	7–21				[7]
		10.8					[8]
Thy1$^+$	0	0	0				[9]
CD4$^+$	51.9–70.9	8.7–15.0	6.8–8.0	>100	>100	16.0	[10]
CD8$^+$	5.5	6.6–24.2	31				[10]
Vα2$^+$		3.7–3.9	2.2–3.1				[11]

[a]All values represent the theoretical percentage of total T cells that express two TCRα-chains. This percentage is calculated by extrapolation based on the % of cells that were stained by two anti-Vα mAb, according to the following formula:

$$X = D \times 100 \times 100 / A \times B$$

where D is the % of cells staining positive for both VαA and VαB, A is the % of cells expressing VαA and B the % of cells expressing VαB.
[b]T cell subset that was analyzed in the respective studies.

The concept of phenotypic allelic exclusion in TCR α was challenged by flow cytometry experiments revealing mature T cells that expressed two different α-chains at the surface. In the first report, staining of human peripheral blood mononuclear cells was performed with monoclonal antibodies (mAb) that recognize TCR Vα2, Vα12 and Vα24 [6]. Cells stained by two Vα mAb were observed at a low frequency (10^{-3} to 10^{-4}) and double-positive T cell clones could be isolated [6]. Gene sequencing in five of the clones confirmed the presence of in-frame mRNAs corresponding to the two expressed α-chains [6]. Similarly, in the mouse, it has been possible to double-stain peripheral T cells with different combinations of the available mAb recognizing specific members of mouse TCR Vα families (Vα2, Vα3.2, Vα8 and Vα11) [7]. T cell clones that had been stained by both anti-Vα2 and anti-Vα11 mAb could be isolated and were shown to contain in-frame Vα2 and Vα11 mRNA transcripts [7].

While the existence of dual α-T cells in the periphery of normal individuals has been demonstrated, the frequency of these cells in the repertoire remains unclear. To date, this frequency has been extrapolated from the observed percentage of cells that can be double-stained with two different anti-Vα mAb [7–11]. This approach, which is limited by the very few anti-Vα reagents that are available, provides inconsistent estimates of the percentage of dual α-T cells, ranging from 0 to over 100% (Table 1). The actual frequency of dual α-T cells in the repertoire is an important issue in T cell biology. If this frequency is low (1% or less of total peripheral T cells), this would mean that there is an efficient phenotypic allelic exclusion taking place in TCRα and T cells could consequently be considered as essentially monospecific cells. In contrast, if the proportion of dual α-T cells is as high as several percent of total T cells, this would imply that a significant number of T cells bear two different antigen receptors at the surface and potentially are multispecific. The existence of large numbers of such potentially multispecific T cells would challenge the current view (the one cell—one specificity rule) on how antigen-specific immune responses are mediated by T cells. Dual α-T cells have also been suggested to play a critical role in autoimmunity: any dual α-T cell may be positively selected using one TCR-αβ pair, but may prove autoreactive via its second αβ combination, particularly if escape is facilitated by the second receptor being expressed at low density on emergent cells [6, 12, 13].

In this report, we present a novel method to measure directly by flow cytometry the frequency of T cells with two different TCRα-chains at the surface. This method is based on comparing the percentage of total cells that express a given Vα-chain, between wild-type mice and mice that are hemizygous for a disrupted Tcra locus (Tcra+/−) and consequently unable to express two rearranged Tcra genes. With this new approach, we consistently found that ~8% of total peripheral T cells express two α-chains. We also examined the importance of dual α-T cells in autoimmunity by using a mouse model for rheumatoid arthritis, namely collagen-induced arthritis (CIA). In this model, no significant difference was observed in disease course and severity between Tcra+/− mice and wild-type littermates. We therefore conclude that there is incomplete phenotypic allelic exclusion in TCRα, and that the presence of significant number of potentially multireactive T cells does not appear to increase the susceptibility to develop autoimmune arthritis.

Materials and Methods

Mice

A targeted mutation of the Tcra locus in 129 strain mice [14] was crossed for 10 or more generations on to the B10.Q strain. Mice carrying the targeted disruption were identified by PCR, using the following primers specific for the inserted Tn5 neomycin

Table 2. *Percentage of peripheral T cells that express a given TCR Vα-chain in Tcra +/− versus wild-type mice and extrapolated dual TCRα T cell frequency*[a]

Subset	Mice age (months)	*n*	Staining	% positive cells (mean±SEM)		Difference[b]	*P* value[c]	Extrapolated dual α-T cell frequency (%)[d]
				Tcra +/−	Wild-type			
CD4	3	4–6	Vα2	10.34±0.06	11.32±0.18	0.98	0.0105	10.6
CD4	6–8	16	Vα2	9.48±0.1	10.18±0.12	0.70	0.0004	8.2
CD8	6–8	12–14	Vα2	4.64±0.09	4.92±0.08	0.28	0.0087	6.3
CD4	3	4–6	Vα8	12.95±0.17	13.71±0.2	0.76	0.033	6.7
CD4	6–8	16	Vα8	12.92±0.14	13.77±0.16	0.85	0.0006	7.6
CD8	6–8	10–14	Vα8	7.26±0.12	7.85±0.14	0.59	0.0071	8.8

[a]Peripheral blood mononuclear cells from male mice hemizygotes for a targeted deletion in the Tcra locus and wild-type littermates were stained with combinations of anti-CD4, -CD8, -TCRVα2 and -TCRVα8 mAb. Flow cytometry analysis was performed with a typical gate for lymphocytes. The % of cells positive for Vα2 or Vα8, in either the CD4 or the CD8 population, was determined. Background staining (isotype-matched control mAb) was subtracted.
[b]Difference between the % of positive cells in Tcra +/− and wild-type mice.
[c]Mann-Whitney *U*-test.
[d]This represents the theoretical % of the total T cell population that express two TCRα-chains. This value is calculated by the following formula:

$$X=\left(\frac{B-A}{(100-A)\times A}\right)\times 100\times 100$$

where A is the % of cells staining positive in Tcra +/− mice and B is the % of cells staining positive in wild-type mice.

resistance gene: Neo2 (CAT CCT GAT CGA CAA GAC C) and Neo3 (CTA TTC GGC TAT GAC TGG). Mice were bred and kept in the animal facility of Medical Inflammation Research at Lund University.

Monoclonal antibodies

Staining for flow cytometry was performed with the following mAb. L3T4-Cy-Chrome™ (anti-CD4) and 53-6.7-Cy-Chrome™ (anti-CD8a) (PharMingen, San Diego, CA, USA). B20.1-FITC (anti-Vα2) and KT65-FITC (anti-Vα8), which were purified from culture supernatant by affinity chromatography on Protein G-Sepharose and conjugated.

Flow cytometry

Blood from the tail was supplemented with ammonium chloride (0.84% (w/v), pH 7.4), in order to lyse the red blood cells. Cells were then washed and resuspended in PBS supplemented with 0.5% (w/v) BSA (Sigma, St Louis, MO, USA) and 0.01% (w/v) NaN_3 (staining buffer). Staining with mAb was performed for 15 min at 4°C. Cells were then washed with staining buffer, and kept at 4°C until analysis. For flow cytometry, a typical forward and side scatter gate for lymphocytes was set to exclude dead cells and aggregates. In total, $0.5–1.5\times10^5$ events in the gate were collected and analysed using a FACSort (Becton Dickinson, San Jose, CA, USA) and Becton Dickinson software.

Induction of arthritis

Rat type II collagen was prepared from the Swarm chondrosarcoma after pepsin digestion [15] and dissolved at a concentration of 2 mg/ml in 0.1 M acetic acid. Arthritis was induced by intradermal injection at the base of the tail with 100 µl of 100 µg rat type II collagen emulsified with an equal volume of complete Freund's adjuvant containing *Mycobacterium butyricum* (Difco, Detroit, MI, USA). Mice were boosted intradermally, 40 days later, at the base of the tail, with 50 µl of 50 µg rat type II collagen emulsified with an equal volume of incomplete Freund's adjuvant (Difco, Detroit, MI, USA).

Clinical evaluation of arthritis

Arthritis was evaluated by a blinded observer using a scoring system based on the number of inflamed joints in each paw, inflammation being defined by swelling and redness. In this scoring system, described in detail elsewhere [16], each inflamed toe or knuckle gives 1 point, whereas an inflamed wrist or ankle gives 5 points, resulting in a score of 0–15 (5 toes+5 knuckles+1 wrist/ankle) for each paw and 0–60 points for each mouse. Healed joints that are deformed or swollen without redness are not considered in this system.

Statistical analysis

Comparison of data between the groups were performed by the Mann–Whitney *U*-test (for the percentages of positive cells in Table 2, the mean day of onset in Table 3, and mean arthritic score in Table 3 and Figure 1) and the Fisher's exact test (for arthritis incidence, Table 3 and Figure 1). $P<0.05$ was considered significant.

Table 3. *CIA is similar in Tcra+/− mice as compared to wild-type littermates*[a]

Mice phenotype	Incidence of arthritis[b]	Day of onset (mean±SD)	Arthritic score[c] (mean±SD)
Tcra+/−	11/18 (61%)	52.1±20.3	8.4±8.2
Tcra+/+	10/16 (63%)	47.9±15.1	9.6±10.1

[a]Mice hemizygotes for a targeted deletion in the Tcra locus and wild-type littermates were immunized with 100 µg of rat type II collagen in complete Freund's adjuvant and boosted 40 days later with 50 µg of rat type II collagen in incomplete Freund's adjuvant. Mice were scored for arthritis until day 96. Statistical analysis showed no significant differences between Tcra+/− mice and wild-types for any measured parameter.
[b]Number of arthritic mice/total number of mice in each group.
[c]Mean maximum arthritic score for arthritic mice only.

Results

Frequency of dual TCR α-T cells

In order to estimate the frequency of T cells with two distinct TCR α-chains at the surface, we used mice that are hemizygous for a targeted mutation at the Tcra locus (Tcra+/−) [14]. These mutant mice are unable to generate two productively rearranged Tcra loci, but are otherwise phenotypically and functionally normal [8, 14]. Peripheral T cells from Tcra+/− mice or from wild-type (Tcra+/−) littermates were stained with mAb anti-TCRVα2 or anti-TCRVα8, and the percentage of positive cells was determined by flow cytometry (Table 2). As we expected, the observed percentage of positive cells was increased in wild-type mice, as compared to Tcra+/− littermates, because of the presence of dual α-T cells expressing the specific Vα we stained for, together with another Vα. For instance, as reported in the first row of Table 2, the percentage of Vα2 positive cells within the $CD4^+$ T cell subset, in 3-month-old mice, was 10.34% in Tcra+/− mice and 11.32% in wild-types. We assume that the difference between the two groups (11.32−10.34=0.98%) represents a direct measurement of the frequency of dual T cells that express Vα2 together with any other Vα at the surface. If all peripheral T cells were carrying two different cell surface α-chains, the frequency of dual T cells that express Vα2 together with another Vα would be in this example (100−10.34)×(10.34/100)=9.27, where '10.34/100' is the probability for a T cell to express Vα2, and '100−10.34' the frequency of T cells that express other Vα's than Vα2 in Tcra+/− mice. By comparing the observed percentage (i.e. 0.98 in this example) with the expected one if all T cells were carrying two α-chains at the surface (i.e. 9.27), it is possible to make an estimate of the overall frequency of dual α-T cells (0.98/9.27=0.106, i.e. 10.6%). It should be noted that $CD4^+$ and $CD8^+$ cells were analysed separately, in order to eliminate inaccuracies resulting from differential skewing of Vα usage toward the CD4 or the CD8 subset [17]. As reported in Table 2, when we made separate calculations based on staining with either anti-Vα2 and anti-Vα8 mAb, using cells from either young adult (3 months old) or older mice (6–8 months old), we found consistent estimates for the frequency of dual α-T cells, ranging from 6.3 to 10.6% (mean ~8%) of total T cells.

Dual α-T cells are not required for development of autoimmune arthritis

We aimed to investigate if dual α-T cells are critical for development of autoimmunity as it has been suggested by others [6, 12, 13]. For this purpose, we chose a mouse model for rheumatoid arthritis, namely CIA, which we have recently shown to be strictly αβ T cell-dependent [18]. In this model, autoimmune arthritis is induced by immunization with type II collagen, a joint cartilage-specific protein, emulsified in adjuvant [19]. A few weeks after the immunization, inflammation occurs in peripheral joints, leading to cartilage and bone destruction [19]. When we compared Tcra+/− mice and wild-type littermates for the development of CIA, we did not observe any statistically significant difference between the groups, considering arthritis incidence, mean day of disease onset, mean maximum arthritic score, and disease course (Figure 1, Table 3). We can thus conclude that dual α-T cells are not required for development of CIA, and most probably do not play a major role in this autoimmune disease.

Discussion

The first objective of the present study was to determine the proportion of peripheral T cells that express two distinct TCRα-chains, and thereby two distinct TCRs, at the surface. Previous attempts to address this issue were based on extrapolations made from the observed frequency of cells that could be stained by two different anti-Vα mAb [7–11]. This approach gave inconsistent results (see Table 1), and this can be explained by some major inherent limitations. First, the very low frequency of double-positive cells (10^{-3} to 10^{-4}) renders it difficult to make accurate measurements by flow cytometry. Second, this approach is limited by the very few available anti-Vα mAb (four in total in the mouse) that only account for a portion of the Vα repertoire. And third, the extrapolated frequency of double-positive cells appears to vary considerably depending on the identity of the α-chains investigated (Table 1). A possible explanation for this last observation is that not all Vα-chains pair equally well with all Vβ-chains [3, 4]. Within each dual α-T cells, the two different TCRα-chains are competing for binding to the unique β-chain. Therefore, one can expect some combinations of Vα-chains with similar binding ability to Vβ-chains (both in terms of affinity and selection for some Vβ family members) to be more commonly found on dual α-T cells than others. This implies that calculations based on staining a limited

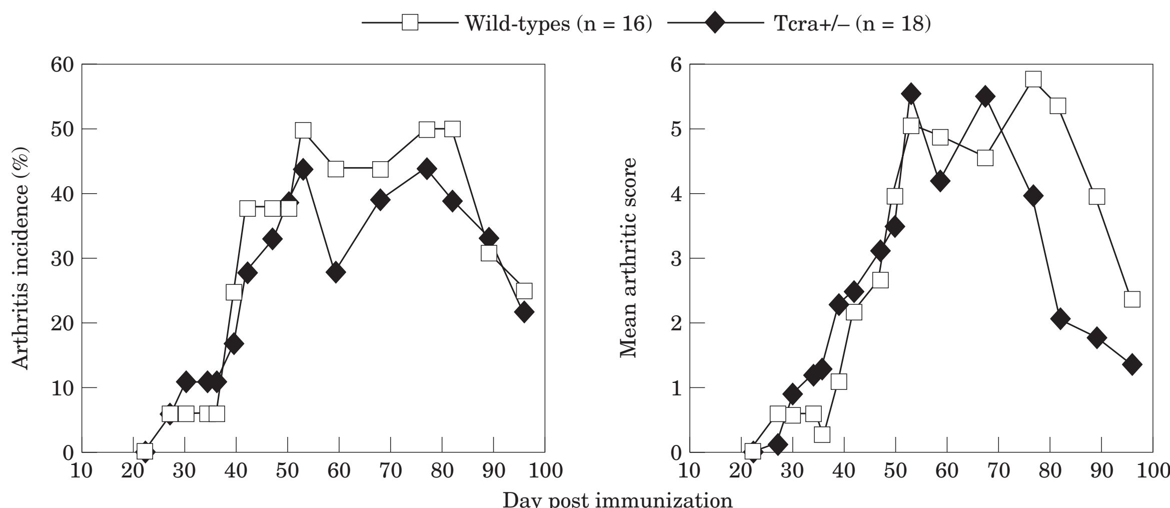

Figure 1. CIA is similar in Tcra + / − mice as compared to wild-type littermates. The disease course is represented by arthritis incidence and mean arthritic score (for all mice in each group). B10.Q male mice were immunized with 100 μg rat type II collagen in complete Freund's adjuvant at day 0 and boosted 40 days later with 50 μg rat type II collagen in incomplete Freund's adjuvant. No statistically significant difference was observed between the two groups at any time point.

number of Vα pairs may be of limited value to estimate the overall frequency of dual α-T cells.

We present here a novel method to estimate by flow cytometry the frequency of dual α-T cells in the periphery. This method is based on comparing the percentage of total T cells that express a given TCR Vα-chain, between wild-type mice and mice that are hemizygous for a disrupted Tcra locus (Tcra + / −). The Tcra + / − mutant mice are unable to generate two productively rearranged Tcra loci, but are otherwise phenotypically and functionally normal [8, 14]. In these hemizygous mice, the targeted mutation on one chromosome results in a strict allelic exclusion at the Tcra locus, occurring in all T cells. We expected that the percentage of cells positive for a given Vα-chain would be higher in wild-type mice, as compared to Tcra + / − littermates, because of the presence of dual α-T cells expressing the specific Vα together with any other Vα. Flow cytometry staining of peripheral T cell with two different mAb (anti-Vα2 and anti-Vα8) confirmed that this was the case. Our assumption is that the observed difference between the percentage positive cells in Tcra + / − versus wild-type mice, is a direct measurement of the frequency of all dual T cells expressing a specific Vα, together with any member of the other Vα families, thereby covering the whole Vα repertoire. Therefore, by comparing this observed frequency with the expected one, if all T cells carried two cell surface α-chains, it is possible to make an estimate of the overall frequency of dual α-T cells.

This novel method circumvents the problems encountered by the double-staining approach and is technically more simple, and therefore more robust, as it is based on single Vα staining. Furthermore, we selected for our measurements two mAb (anti-Vα2 and -8), that each stain over 5% of the T cell repertoire, in order to reduce the influence of non-specific background staining and to assure the accuracy of the data. For the same reason, the two other anti-mouse TCRVα mAb available (anti-Vα-3.2 and -11), which only stain 1–2% of the repertoire were not judged appropriate. The main innovation with our method is that it provides direct measurements of the frequency of dual α-T cells that express Vα2 (or Vα8) together with any other Vα (Vα1, Vα3, Vα4, etc.) at the surface, thereby covering the whole Vα repertoire. Therefore, the new method is circumventing the limitations caused both by the very few anti-Vα reagents available and by studying only a limited number of Vα pairs.

Our new method to estimate the percentage of dual α-T cells is based on the assumptions that Tcra hemizygous mice possess the same TCR repertoire than the wild-type mice. We believe that this is highly likely, as the two groups of mice share all the genes (except of course for the mutated Tcra) expected to influence the TCR repertoire, e.g. MHC and TCR haplotypes. Furthermore, most T cells in wild-type mice and all T cells in Tcra + / − bear a single α-chain at the surface, and it is difficult to conceive why the TCRα repertoire of these single α-T cells should be different between the two groups of mice. In contrast, there is a theoretical possibility that the TCR repertoire might be different for dual α-T cells as compared to single α-T cells. There is presently no report supporting such a variation, but this possibility has not been properly investigated and can therefore not be completely excluded. However, the very consistent results obtained with our method, based on separate measurements with two different anti-Vα mAb, is indirectly supporting the constancy of the TCR repertoire in the two groups of mice and thereby for the validity of the new method.

The frequency of dual α-T cells calculated with our new method, may be somewhat underestimated for two reasons. First, a possible limitation of the method, which is inherent to flow cytometry, is that some dual α-T cells may be missed if the expression of the second

α-chain is so low that it is under detection limits. Second, there is a possibility that Tcra + / − mice may possess some dual α-T cells, due to secondary rearrangements on their unique Tcra locus. Due to such sequential Tcra rearrangements, a given T cell, in a Tcra + / − mouse, may, during a transition period, express two different mRNA coding for TCRα-chains and could therefore express two different TCRα-chains at the surface. This phenomenon has been demonstrated in the thymus [20] but it is yet unclear if and to what extent it occurs in the periphery.

We report here that ~8% of all peripheral T cells bear two α-chains at the surface. This percentage is quite impressive, if one keeps in mind that most T cells possess a single productively rearranged α-chain in the cytoplasm, because of the recombinations in TCR genes, which generate a majority of out-of-frame products. The proportion of T cells with both Tcra genes in a productive configuration has been previously reported to approximate 25% [2]. Our data imply that about one third of these cells (8/25) bear two α-chains at the surface. This is a rather high proportion, as it has been shown that some α-chains are prohibited from surface expression, because they are unable to make a pair with the β-chain [2–4]. The observation that as many as one third of the potentially dual α-T cells express two α-chains at the surface, demonstrate the absence of regulatory mechanisms that would prohibit dual TCR expression on a cell. We can therefore conclude that there is incomplete phenotypic allelic exclusion in TCRα.

In contrast to the α-chain, allelic exclusion seems to be the rule for TCRβ-chains. Interestingly, however, allelic exclusion in TCRβ does not seem to be an absolute process. Peripheral T cells bearing two distinct TCRβ-chains have been detected by several laboratories and the proportion of such dual β-T cells in humans was estimated to 1% of peripheral blood cells [21–23]. These observations indicate that dual TCR expression is compatible with both central and peripheral selection. These data also open the intriguing possibility that a given T cell may bear two distinct α-chains and two distinct β-chains, and thereby as many as four different αβ combinations.

Our data showing that the frequency of dual α-T cells is as high as ~8% suggest that there might be a biological relevance for a T cell to express two different surface α-chains. Current knowledge on TCRα-chain biology suggest that TCRα-chains may have a more important role than β-chains in the TCR–MHC interaction (reviewed in [5]). It also appears that several mechanisms make it possible for a T cell to test several α-chains during its intrathymic development. All T cells rearrange both Tcra loci [2], and sequential rearrangement occur at the Tcra locus, through excision of an existing Vα–Jα exon by rearrangement of an upstream V-region to a downstream J-region (reviewed in [5]). Altogether, this suggests that sequential TCRα-chain rearrangements occur in parallel on both chromosomes, and that this process goes on until production of an α-chain that is suitable for pairing with the β-chain and for positive selection [5]. The generation of T cells with two distinct α-chains at the surface may therefore be a side effect of an effective process, which allows T cells to test a number of different α-chains during its development, in order to produce a functional TCR. Alternatively, possession by a cell of two distinct α-chains, and thereby two distinct TCRs, may also by itself increase the chances of progression through positive selection.

We show in the present report that dual α-T cells are not required for development of autoimmune arthritis, in the CIA model, by comparing disease development in Tcra + / − versus wild-type mice. Our data confirms experiments, which used the same approach to investigate several other models for autoimmune diseases in the mouse. Thus, a critical role for dual α-T cells could be excluded in experimental autoimmune encephalomyelitis (EAE) in the SJL strain, in lupus in the MRL *lpr/lpr* strain, and for diabetes in the NOD mouse [8, 24]. However, investigation of CIA was of special interest, because in this model, in contrast to the above-mentioned experimental diseases, the autoantigen (i.e. type II collagen) is not expressed in the thymus [25]. CIA is therefore a good model of autoimmunity induced by escape from peripheral self-tolerance. The experiments with Tcra + / − hemizygous mice do not rule out that a certain percentage of dual TCR-expressing T cells recognizing a self antigen may escape from tolerance induction mechanisms, but they demonstrate that this is not the only mechanism by which autoimmune disease can be explained.

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References

1. Bergman Y. 1999. Allelic exclusion in B and T lymphpoiesis. *Semin. Immunol.* **11:** 319–328
2. Malissen M., Trucy J., Jouvin Marche E., Cazenave P.A., Scollay R., Malissen B. 1992. Regulation of TCR alpha and beta gene allelic exclusion during T-cell development. *Immunol. Today* **13:** 315–322
3. Kuida K., Furutani-Seiki M., Saito T., Kishimoto H., Sano K., Tada T. 1991. Post-translational attainment of allelic exclusion of the T cell receptor alpha chain in a T cell clone. *Int. Immunol.* **3:** 75–82
4. Couez D., Malissen M., Buferne M., Schmitt-Verhulst A.M., Malissen B. 1991. Each of the two productive T cell receptor alpha-gene rearrangements found in both the A10 and BM 3.3 T cell clones give rise to an alpha chain which can contribute to the constitution of a surface-expressed alpha beta dimer. *Int. Immunol.* **3:** 719–729

5. Gascoigne N.R., Alam S.M. 1999. Allelic exclusion of the T cell receptor alpha-chain: developmental regulation of a post-translational event. *Semin. Immunol.* **11:** 337–347
6. Padovan E., Casorati G., Dellabona P., Meyer S., Brockhaus M., Lanzavecchia A. 1993. Expression of two T cell receptor alpha chains: dual receptor T cells. *Science* **262:** 422–424
7. Heath W.R., Carbone F.R., Bertolino P., Kelly J., Cose S., Miller J.F. 1995. Expression of two T cell receptor alpha chains on the surface of normal murine T cells. *Eur. J. Immunol.* **25:** 1617–1623
8. Elliott J.I., Altmann D.M. 1995. Dual T cell receptor alpha chain T cells in autoimmunity. *J. Exp. Med.* **182:** 953–959
9. Alam S.M., Crispe I.N., Gascoigne N.R. 1995. Allelic exclusion of mouse T cell receptor alpha chain occurs at the time of thymocyte TCR upregulation. *Immunity* **3:** 449–458
10. Elliott J.I. 1998. Selection of dual Valpha T cells. *Eur. J. Immunol.* **28:** 2115–2123
11. Alam S.M., Gascoigne N.R. 1998. Post-translational regulation of TCR Valpha allelic exclusion during T cell differentiation. *J. Immunol.* **160:** 3883–3890
12. Zal T., Weiss S., Mellor A., Stockinger B. 1996. Expression of a second receptor rescues self-specific T cells from thymic deletion and allows activation of autoreactive effector function. *Proc. Natl. Acad. Sci. USA* **93:** 9102–9107
13. Sarukhan A., Garcia C., Lanoue A., von Boehmer H. 1998. Allelic inclusion of T cell receptor alpha genes poses an autoimmune hazard due to low-level expression of autospecific receptors. *Immunity* **8:** 563–570
14. Philpott K.L., Viney J.L., Kay G., Rastan S., Gardiner E.M., Chae S., Hayday A.C., Owen M.J. 1992. Lymphoid development in mice congenitally lacking T cell receptor alpha beta-expressing cells. *Science* **256:** 1448–1452
15. Andersson M., Holmdahl R. 1990. Analysis of type II collagen reactive T cells in the mouse. I. Different regulation of autoreactive versus non-autoreactive anti-type II collagen T cells in the DBA/1 mouse. *Eur. J. Immunol.* **20:** 1061–1066
16. Holmdahl R., Carlsen S., Mikulowska A., Vestberg M., Brunsberg U., Hansson A.-S., Sundvall M., Jansson L., Pettersson U. 1998. Genetic analysis of mouse models for rheumatoid arthritis. In *Human Genome Methods*. K.W. Adolph, ed. CRC Press, New York, pp. 215–238
17. Sim B.C., Lo D., Gascoigne N.R. 1998. Preferential expression of TCR V alpha regions in CD4/CD8 subsets: class discrimination or co-receptor recognition? *Immunol. Today* **19:** 276–282
18. Corthay A., Johansson A., Vestberg M., Holmdahl R. 1999. Collagen-induced arthritis development requires alpha beta T cells but not gamma delta T cells: studies with T cell-deficient (TCR mutant) mice. *Int. Immunol.* **11:** 1065–1073
19. Courtenay J.S., Dallman M.J., Dayan A.D., Martin A., Mosedal B. 1980. Immunization against heterologous type II collagen induces arthritis in mice. *Nature* **283:** 666–668
20. Wang F., Huang C.Y., Kanagawa O. 1998. Rapid deletion of rearranged T cell antigen receptor (TCR) Valpha-Jalpha segment by secondary rearrangement in the thymus: role of continuous rearrangement of TCR alpha chain gene and positive selection in the T cell repertoire formation. *Proc. Natl. Acad. Sci. USA* **95:** 11834–11839
21. Padovan E., Giachino C., Cella M., Valitutti S., Acuto O., Lanzavecchia A. 1995. Normal T lymphocytes can express two different T cell receptor beta chains: implications for the mechanism of allelic exclusion. *J. Exp. Med.* **181:** 1587–1591
22. Davodeau F., Peyrat M.A., Romagne F., Necker A., Hallet M.M., Vie H., Bonneville M. 1995. Dual T cell receptor beta chain expression on human T lymphocytes. *J. Exp. Med.* **181:** 1391–1398
23. Balomenos D., Balderas R.S., Mulvany K.P., Kaye J., Kono D.H., Theofilopoulos A.N. 1995. Incomplete T cell receptor V beta allelic exclusion and dual V beta-expressing cells. *J. Immunol.* **155:** 3308–3312
24. Elliott J.I., Altmann D.M. 1996. Non-obese diabetic mice hemizygous at the T cell receptor alpha locus are susceptible to diabetes and sialitis. *Eur. J. Immunol.* **26:** 953–956
25. Malmstrom V., Michaelsson E., Burkhardt H., Mattsson R., Vuorio E., Holmdahl R. 1996. Systemic versus cartilage-specific expression of a type II collagen-specific T-cell epitope determines the level of tolerance and susceptibility to arthritis. *Proc. Natl. Acad. Sci. USA* **93:** 4480–4485