Dual T Cell Receptor β Chain Expression on Human T Lymphocytes

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Summary

Allelic exclusion of lymphocyte antigen receptor chains has been hypothesized as a mechanism developed by the immune system to ensure efficient lymphocyte repertoire selection and tight control of lymphocyte specificity. It was effectively shown to be operative for both the immunoglobulin (Ig) and the T cell receptor (TCR) β chain genes. Our present observations suggest that close to 1% of human T lymphocytes escape this allelic control, and express two surface TCR β chains with distinct superantigenic reactivities. Since this high frequency of dual β chain expressers did not result in any dramatic immune dysregulations, these results question the need for a mechanism ensuring clonal monospecificity through allelic exclusion.

Lymphocyte antigen receptors are composed of two variable glycoprotein subunits, the Ig heavy and light chains on B cells and the TCR α and β or γ and δ chains on T cells. The genes coding for Ig and TCR chains are formed through somatic rearrangement of V, D, and J elements (for reviews see references 1 and 2). Given its diploid nature, any lymphocyte clone could theoretically express up to four distinct combinations of antigen receptor chains on its surface. Since clonal plurispecificity, possibly resulting from expression of multiple antigen receptors on the same cell, is expected to lead to immune dysregulations (that is, autoimmunity), control processes ensuring allelic exclusion of antigen receptor chains were postulated more than a decade ago, and their existence demonstrated through elegant Ig and TCR transgenic mouse models (3–5).

More recently it has become clear that the stringency of allelic exclusion greatly differs from one antigen receptor chain gene to another. In the case of TCR β chain genes, expression of any productively rearranged gene prevents further rearrangement in the β locus, a process referred to as “genotypic” exclusion (6). Such a genotypic control is illustrated by the arrest of endogenous TCR β gene rearrangements in mice carrying a functional or truncated β transgene (5, 7). However, similar transgenic studies have failed to demonstrate a dramatic influence of functional α transgenes on the occurrence of rearrangements within endogenous TCR α loci (8–12). Moreover, studies performed on normal T cells have revealed the expression of distinct productive α transcripts (13) or even distinct surface α chains (14) on a fraction of murine and human T cell clones.

Although lack of allelic exclusion of α chains can result in a violation of the “one cell, one receptor” rule, current ontogenic models suggest that dual α chain expression would have limited physiological consequences. Experiments with normal and transgenic T cells suggest that a developing lymphocyte will keep on rearranging its α loci until it produces a TCR α chain able to pair with the available TCR β chain and form a TCR showing sufficient affinity for self MHC-peptide complexes (12, 13, 15). The corollary to this hypothesis, which proposes a close coupling between arrest of TCR α gene rearrangements within the thymocyte and its positive selection, is that any lymphocyte carrying two distinct α chains should systematically express one α chain unable to form a selectable TCR, that is, with a physiologically relevant specificity. Therefore lack of genotypic exclusion of TCR α chain genes, which would merely be the consequence of a low frequency of selectable α chains within the immature repertoire, should remain compatible with maintenance of clonal monospecificity. This might not necessarily be true in the case of dual TCR β chain expression. First, since rearrangements within TCR β loci are completed before production of TCR α chains and subsequent α/β TCR selection (for reviews see references 16 and 17), the two α/β TCR present in dual β expressers could not in theory be sequentially tested for affinity to a given MHC–peptide complex. Second, because β chains, unlike α chains, display superantigenic reactivity (for a review see reference 18), dual TCR β chain expression should systematically lead to bispecificity, possibly hampering regulation of immune responses directed against exogenous superantigens. Hence, this may justify the need for a tight control of TCR β gene rearrangement, whose primary aim would be to ensure clonal monospecificity through maintenance of
TCR β chain clonal distribution. This interpretation is put into question by the present data, which suggest that a normal immune function can accommodate itself to a relatively high frequency of dual β chain expressors.

Materials and Methods

**Antibodies.** The following mAbs were used for flow cytometry and sorting experiments: BMA031 (pan β), 360 (anti-Vγ9), LE89 (anti-Vγ3), IMMU157 (anti-Vβ5.1), 36213 (anti-Vβ5.2), 3D11 (anti-Vβ5.3), OT145 (anti-Vβ6.7), S6C5.2 (anti-Vβ8), SS11 (anti-Vβ12.2), JU74 (anti-Vβ13.3), 417.53 (anti-Vβ19), IG125 (anti-Vβ21.3), and IMMU546 (anti-Vβ22) (19-25). The VB nomenclature used is from reference 26.

**Generation of T Cell Lines and Clones.** PBL from healthy donors were sorted, cultured, and cloned as previously described (27, 28). In brief, cells were incubated with TCR V-specific mAbs for 45 min, washed once, and rotated for 4 h at 4°C with magnetic beads coated with sheep anti-mouse Ig (Dynal, Oslo, Norway). After eight washes, bead-adherent cells were cultured in medium (RPMI 1640, 10% human serum, 1 mM L-glutamine, rIL-2, and leukaglutinin) and irradiated PBL and B lymphoblastoid cells. Beads were removed between day 5 and 8, and cells were cultured further, and at day 14, they were subjected to a second immunomagnetic separation. After two more weeks of culture, cells were cloned by limiting dilution in culture medium and irradiated feeder cells. Growing colonies with a probability of monoclonality above 95% were kept for further analysis.

**Flow Cytometry Analysis.** Cells were phenotyped by indirect immunofluorescence (IF)1 as follows. Cells were incubated first with the unconjugated mAbs (single or pooled VB-specific mAbs, 1:1,000 dilutions) at 4°C for 30 min; second with FITC-conjugated rabbit anti-mouse Ig for 30 min at 4°C; third with normal mouse serum (1:10 dilution) for 10 min at room temperature; fourth with biotinylated Vβ-specific mAbs for 30 min at 4°C; and fifth with PE-conjugated streptavidin for 30 min at 4°C. Between each incubation, cells were washed twice with PBS, 1% BSA. Cells were analyzed by flow cytometry on a FACScan® (Becton Dickinson & Co., Mountain View, CA) using LYSYS II software. To have an accurate estimate of infrequent subsets (representing <0.2% of the total population), 0.4 × 10⁶ events were accumulated during the analysis. Staining background was reduced by gating out dead cells using propidium iodide (read on the FL3 channel). To reduce the proportion of doublets, which could represent up to 2% of the total population (see Materials and Methods). Under these conditions, double positive cells recognized by both a Vβ-specific mAb and a mAb specific for another Vγ-region (green fluorescence) was performed on cells sorted with the Vβ8-specific mAb for 45 min at 4°C with magnetic beads coated with sheep anti-mouse Ig (Dynal, Oslo, Norway). After eight washes, bead-adherent cells were cultured in medium (RPMI 1640, 10% human serum, 1 mM L-glutamine, rIL-2, and leukaglutinin) and irradiated PBL and B lymphoblastoid cells. Beads were removed between day 5 and 8, and cells were cultured further, and at day 14, they were subjected to a second immunomagnetic separation. After two more weeks of culture, cells were cloned by limiting dilution in culture medium and irradiated feeder cells. Growing colonies with a probability of monoclonality above 95% were kept for further analysis.

**Amplification and Sequencing of TCR Transcripts.** Molecular analysis of TCR β transcripts was performed as previously described (33) on several T cell clones stained by two distinct Vβ-specific mAbs (in all cases, 100% of the cells were recognized by the two antibodies). Reverse transcription of T cell clone RNA, amplification, and sequencing were performed using the following primers: VB3 (5'TAG AAT TCA TGG GAA GAA GTC TCA CAT GCC), VB5 (5'TAG AAT TCA TGG GCT CCA GCA GCC TGC TCT GTT), VB6 (5'TCG AAT TCA TCA TGG GCA CCA GCC TGC TCT G), Vβ8 (5'ATG AT CGG GGA CTG GAG TTG CTC), Vβ19 (5'TAG AAT TCA TGA GCC ACC AGG TGC TCT G), Vβ21 (5'AAA GGA GTA GAC TCT AAC AAA GCC TCT GCC), VB8 (5'AGA AAA GGA GAA GTC TAT GCC), VB19 (5'TAG AAT TCA TGA GCC ACC AGG TGC TCT G), Vβ21 (5'AAA GGA GTA GAC TCC ACT CTC), VB22 (5'ATG TCT CAG TTG AAA GCC CTG), and VB8 (5'GGG AGA GTC CCT CTG ATG GCT). V, NDN, and J assignments were deduced from a comparative analysis of cDNA sequences with those of germline elements. The Vβ and Jβ nomenclatures used were from Wilson et al. (26) and from Toyonaga et al. (34).

Results

**Detection by Flow Cytometry of Peripheral T Lymphocytes Recognized by Distinct Vβ-specific mAbs.** We recently described T cell clones expressing two distinct TCR γ chains on their surface, and demonstrated by 2C-IF that 1-7% of PBL were recognized by mAbs directed against distinct Vγ regions (33). In the course of this study, PBL-derived α/β T cell lines were analyzed in parallel by 2C-IF using mAbs directed against distinct Vβ regions, to compare the stringency of TCR γ vs. TCR β chain exclusion. In light of previous studies suggesting a tight genotypic exclusion of TCR β chain genes (5), the proportion of cells recognized by two distinct Vβ-specific mAbs was expected to be low, if not negligible. Therefore, we set up technical conditions that allowed detection and accurate quantification of cell subsets representing <0.2% of the total population (see Materials and Methods). Under these conditions, double positive cells recognized by both a Vβ8-specific mAb and a mAb specific for another Vβ-region were reproducibly detected in all the cell lines studied (Fig. 1 B and Table 1).

Three possible artifacts could explain the above results: (a) a binding of the FITC-conjugated antiserum to the PE-conjugated mAb; (b) a doublet discrimination problem (that is, due to lack of discrimination by the flow cytometer soft-

1 Abbreviations used in this paper: IF, immunofluorescence; MFI, relative mean fluorescence intensity; SEB, staphylococcal enterotoxin B; SEE, staphylococcal enterotoxin E; SAgs, superantigens; 2C-IF, two-color immunofluorescence.
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ware between a doublet of single positive events and a double positive event); and (c) a cross-reactivity of some VB-specific mAbs to other regions. The first possibility was ruled out by 2C-IF experiments using irrelevant isotype-matched mAbs (Fig. 1 A and data not shown). Moreover, the fact that no double positive events were detected when analyzing mixed samples stained by 1C-IF using either the PE- or FITC-conjugated mAbs allowed us to rule out a doublet discrimination problem (Fig. 1 A). Finally, the staining profiles of the double positive population was not compatible with cross-reactivity of some of the VB-specific mAbs tested. Instead, the inverse correlation noted between staining intensities obtained with the VB8-specific mAb vs the pool of VB-specific mAbs was strongly indicative of the presence of two distinct TCR β chains on the same cell, competing for pairing with limiting amounts of other TCR or CD3 components (Fig. 1 B).

All T Cell Clones Recognized by Distinct VB-specific mAbs Express Two Distinct Productively Rearranged TCR β Genes. To formally prove the existence of dual β expressors, we generated T cell lines after sequential sorting of PBL using one VB-specific mAb first and then a pool of mAbs specific for other distinct VB regions (Fig. 2), and cloned them by limiting dilution. More than 50 clones recognized by two distinct VB-specific mAbs were generated this way (see for example, Fig. 2 D). It is noteworthy that the relative mean fluorescence intensities (rMFI) obtained with each VB-specific mAb, which were calculated by dividing the MFI obtained with the VB mAb by the MFI obtained with a pan β mAb, were inversely correlated (Fig. 3). Again these results, which were reminiscent of those obtained with dual TCR χ- and dual TCR α chain–expressing clones (14, 33), strongly suggested the presence of distinct β chains on the above cells.

To formally prove this point, we performed a molecular analysis of TCR β transcripts derived from T cell clone RNA, after reverse transcription and polymerase chain amplification using Cβ- and VB-specific primers followed by sequencing of the VDβ junctions. In all cases, surface phenotype of clones recognized by two distinct VB-specific mAbs matched the expression of the corresponding productive TCR β chain gene transcripts (Fig. 4).
Figure 2. Flow cytometry analysis of dual TCR β chain–expressing cells. (A) 2C-IF analysis of a T cell line (FlaBx-1) generated after sequential sorting of PBL using a Vβ8-specific mAb and a pool of mAbs specific to other Vβ regions. The pool of Vβ-specific mAbs used for sorting is the same as that in Fig. 1. Note the presence of almost all the possible Vβ8/VβX combinations in this cell line (see also Table 3). (B) CD8 coreceptor expression by dual β chain expressors. Cells from line FlaBx-1 were stained with PE-conjugated CD8 mAb (ordinate) and various Vβ-specific mAbs (abscissa). All cells were recognized by the Vβ8-specific mAb (see A). Reciprocal results were obtained with a PE-conjugated CD4 mAb (data not shown). Note that the majority of the subsets recognized by a given pair of Vβ–specific mAbs comprised CD4+ and CD8+ cells. (C) 1C-IF analysis of Vα2+ cells derived from line FlaBx-1. Cells were sorted using a Vα2.1–specific mAb, cultured and analyzed by IF. All cells were recognized by the Vα2- and Vβ8–specific mAb, some were also recognized by the Vβ22-, Vβ21-, Vβ12-, and Vβ6–specific mAb, which indicated that Vα2+ cells from line FlaBx-1 were still highly polyclonal. (D) Flow cytometry analysis of two T cell clones derived from line FlaBx-1. Shown are the overlayed fluorescence histograms obtained after IF staining of two T cell clones (#10, upper histograms and #5, lower histograms) using Vβ21-, Vβ6- and Vβ8–specific mAbs. The presence of distinct β chains in clones #10 and #5 was also confirmed by molecular analysis (see Fig. 4).

Figure 3. Surface expression levels of Vβ8+ and Vβ19+ chains on dual Vβ8/Vβ19 expressors. The relative mean fluorescence intensities (rMFI) obtained with a Vβ8 (ordinate) and a Vβ19–specific mAb (abscissa), calculated by dividing the MFI obtained with the Vβ mAb by the MFI obtained with a pan β mAb BMA031 (19), were estimated on two Vβ8+ T cell clones (filled circles) and 6 Vβ8+ Vβ19+ T cell clones (unfilled circles). Note the inverse correlation between the Vβ8 and Vβ19 rMFI within the dual β-expressing cell population.

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binant SEB, whereas reciprocal results were obtained with Vβ3*Vβ19* clones (Table 2 and data not shown). It is noteworthy that Vβ8*Vβ19* cells responded to both SEE and SEB, which indicated that their two TCR β chains were functional (Table 2).

**Table 2. Reactivity of Dual TCR β Chain-expressing T Cell Clones towards Staphylococcal SAgs**

| Clone | Phenotype | Vβ | NDN | γp | Vp | NDN | Jp |
|-------|-----------|----|-----|----|----|-----|----|
| 3β3   | Vβ18Vβ8  | 10 |     | 1.5 | 8 |     | 2.7 |
| 3β4   | Vβ8*     | 10 |     | 4.5 | 8 |     | 2.5 |
| 3β5   | Vβ3*Vβ19* | 10 |     | 1.1 | 8 |     | 2.1 |
| 3β6   | Vβ3*Vβ19* | 10 |     | 7.5 | 8 |     | 2.3 |
| 3β7   | Vβ8*Vβ19* | 10 |     | 2.2 | 8 |     | 2.2 |
| 3β8   | Vβ8*Vβ19* | 10 |     | 3.7 | 8 |     | 2.7 |
| 3β21  | Vβ3*Vβ19* | 10 |     | 1.0 | 8 |     | 2.0 |

Indeed, most of the expected Vβ8/Vβx combinations were detected in the majority of the cell lines (Fig. 2 A and Table 3). Moreover, within a given line, most subsets recognized by a pair of Vβ-specific mAbs comprised CD4+CD8- and CD4-CD8* cells (Fig. 2 B and data not shown). Finally, IF analysis of Vα2* cells derived from the above lines still demonstrated the presence of subsets expressing distinct Vβ combinations (Fig. 2 C).

**Table 3. TCR Vβ Surface Expression of PBL-derived Lines Generated after Sequential Sorting Using a Vβ8-specific mAb First and then a Pool of mAbs Specific to Vβ3, 5, 6, 8, 12, 13, 19, 21, and 22**

| Clone | Vβ line | Vβ3 | Vβ5 | Vβ6 | Vβ8 | Vβ12 | Vβ13 | Vβ19 | Vβ21 | Vβ22 |
|-------|---------|-----|-----|-----|-----|------|------|------|------|------|
| 3β4   | Vβ8*    | 0.2 | 3.8 | 14.4 | 98.0 | 34.7 | 0.5  | 31.3 | 7.2  | 2.5  |
| 3β5   | Vβ3*Vβ19* | 4.5 | 0.2 | 7.6 | 99.1 | 58.1 | 0.2  | 7.5  | 9.4  | 4.4  |
| 3β7   | Vβ3*Vβ19* | 2.4 | 1.1 | 39.9 | 99.9 | 1.9  | 0.3  | 11.0 | 14.0 | 23.8 |
| 3β10  | Vβ8*Vβ19* | 7.9 | 2.6 | 10.5 | 99.9 | 14.5 | 0.1  | 29.4 | 8.5  | 20.6 |

**Figure 4. Deduced amino acid sequences of TCR β chain gene transcripts expressed in PBL-derived T cell clones.** The T cell clone phenotype was deduced from IF analysis (100% of the cells were recognized by the Vβ-specific mAb mentioned in the figure). These sequence data are available from EMBL/EBI data bank under accession numbers X84265 to X84283.
the relatively high frequency of dual β expressors estimated here (that is, close to 1%) raises questions regarding its physiological consequences and the way cells with such an unusual phenotype were generated.

Rearrangements of TCR β genes are activated within early CD3-CD4-CD8+ thymocytes. At that stage, expression of any productively rearranged β chain gene will lead to a transient arrest of the thymocyte recombination machinery, presumably upon pairing between the β chain gene product and a surrogate α chain (35). TCR α gene rearrangements are activated at a later stage of development (that is, within CD4+CD8+ thymocytes), when the β loci are no longer accessible to the recombinases. According to this scheme, three major hypotheses might be put forward to account for the generation of dual TCR β chain expressors. First, escape from β genotypic exclusion might be due to an intrinsic inability of the first β chain produced to generate a recombinase modulating signal. However, this possibility seems rather unlikely because such a signal can even be provided by truncated β chains (7) and because both the β chains on dual expressors were shown to be functional (Table 2). Second, escape from β chain exclusion might happen if for some unknown reasons, rearrangements occur synchronously on the two β alleles or if signaling through the first β chain produced is delayed. Third, an accessibility of the β loci to the recombinases at an abnormally late stage of development (that is, at the CD4+CD8+ stage) might result in secondary TCR β rearrangements.

As mentioned, one might expect the frequency of cells with two receptors with distinct physiologically relevant specificities to be higher among dual β than dual α expressors. However, it is likely that for several reasons exposed below, a relatively high incidence of dual β expressors (even in the order of 1%) will have limited consequences on a normal immune function. First, as mentioned previously, current models suggest that a thymocyte will stop its recombination machinery once it has produced a selectable α chain, able to form a receptor with sufficient affinity for self MHC–peptides. Consequently, the fact that T cells have almost systematically rearranged their two α alleles (6) would indicate that in most cases, the first α chain produced was unable to generate a recombinase modulating signal, which would mean that the proportion of selectable α/β TCR within the immature (unselected) repertoire is extremely low. Therefore, the probability that both TCR on dual β expressors will be self MHC restricted (that is, physiologically relevant) should be almost negligible. Second, even if a dual expressor turned out to be plurispecific, it would likely be controlled by the powerful negative selection processes operating in the thymus and in the periphery. Third, although the in vivo significance of superantigenic responses is yet unclear, the fact that the immune system tolerates a relatively high frequency of clones truly bispecific towards distinct SAgS might indicate that clonal plurispecificity has limited consequences in terms of immune response specificity.

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References

1. Tonogawa, S. 1983. Somati generation of antibody diversity. Nature (Lond.). 302:575–583.
2. Davis, M.M., and P.J. Bjorkman. 1988. T cell antigen receptor genes and T cell recognition. Nature (Lond.). 334:395–400.
3. Ritchie, K.A., R.L. Brinster, and U. Storb. 1984. Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in k transgenic mice. Nature (Lond.). 312:517–520.
4. Weaver, D., F. Constantini, T. Imanishi-Kari, and D. Baltimore. 1985. A transgenic immunoglobulin mu gene prevents rearrangement of endogenous genes. Cell. 42:117–127.
5. Uematsu, Y., S. Ryser, S. Dembic, P. Borgulya, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. In transgenic mice the introduced functional T cell receptor β gene prevents expression of endogenous β genes. Cell. 52:831–841.
6. Malissen, M., J. Trucy, E. Jouvin-Marche, P.A. Cazenave, R. Scollay, and B. Malissen. 1992. Regulation of TCR α and β gene allelic exclusion during T-cell development. Immunol. Today. 13:315–322.
7. Krimpenfort, P., F. Ossendorp, J. Borst, C. Melief, and A. Berns. 1989. T-cell depletion in transgenic mice carrying a mu gene allic exclusion during T-cell development. Immunol. Today. 13:315–322.
8. Teh, H.S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Bluthmann, and H. von Boehmer. 1988. Thymic major histocompatibility complex antigens and the specificity of the αβ T cell receptor determine the CD4/CD8 phenotype of T cells. Nature (Lond.). 335:232–235.
9. Sha, W.C., C.A. Nelson, R.D. Newberry, D. Kranz, J.H. Russell, and D.Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature (Lond.). 336:73–76.
10. Berg, L.J., A.M. Pullen, B. Faezakas de St. Groth, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell. 58:1035–1046.
11. Pincher, H., K. Burki, R. Lang, H. Hengartner, and R.M. Zinkernagel. 1989. Tolerance induction in doubly specific T cell receptor transgenic mice with antigen. Nature (Lond.). 342:559–562.
12. Borgulya, P., H. Kishi, Y. Uematsu, and H. Von Boehmer. 1992. Exclusion and inclusion of α and β T cell receptor alleles. Cell. 69:529–537.
13. Malissen, M., J. Trucy, F. Letourneur, N. Rebai, D.E. Dunn, F.W. Hitch, L. Hood, and B. Malissen. 1988. A T cell clone expresses two T cell receptor α genes but uses one α/β heterodimer for allorecognition and self MHC-restricted antigen recognition. Cell. 55:49–59.
14. Padovan, E., G. Casorati, P. Dellabona, S. Meyer, M. Brockhaus, and A. Lanzavecchia. 1993. Expression of two T cell receptor α chains: dual receptor T cells. Science (Wash. DC). 262:422–424.
15. Marolleau, J.P., J.D. Fondell, M. Malissen, J. Trucy, E. Barbier, K.B. Marcu, P.A. Cazenave, and D. Priml. 1988. The joining of germ-line Vα to Jα genes replaces the preexisting Vα-Jα complexes in a T cell receptor α,β positive T cell line. Cell. 55:291–300.
16. von Boehmer, H. 1990. Developmental biology of T cells in T cell receptor transgenic mice. Annu. Rev. Immunol. 8:531–547.
17. Wilson, A., A. D'Amico, T. Ewing, R. Scollay, and K. Shortman. 1989. Subpopulations of early thymocytes. J. Immunol. 140:1461–1469.
18. Scherer, M.T., L. Ignatowicz, G.M. Winslow, J.J. Kappler, and P. Marrack. 1993. Superantigens: bacterial and viral proteins that manipulate the immune system. Annu. Rev. Cell Biol. 9:101–126.
19. Borst, J., J.J.M. van Dongen, E. de Vries, W.M. Cowans-Bitter, M.J.D. van Tol, J.M. Vossen, and R. Kurle. 1990. BMA031, a monoclonal antibody suited to identify the T cell receptor αβ/CD3 complex on viable human T lymphocytes in normal and disease states. Hum. Immunol. 29:175–181.
20. Davodeau, F., I. Houde, G. Boulot, P. Romagné, A. Necker, N. Canavo, M.A. Peyrat, M.M. Hallet, H. Vié, Y. Jacques, R. Mariuzza, and M. Bonneville. 1993. Secretion of disulfide-linked human T-cell receptor γδ heterodimers. J. Biol. Chem. 268:15455–15555.
21. Diu, A., F. Romagné, C. Genevèe, C. Rocher, J.M. Bruneau, A. David, F. Praz, and T. Hercend. 1992. Fine specificity of the OtB/CD3 complex on viable human T lymphocytes in normal and HPB-ALL. Eur. J. Immunol. 22:649–652.
22. Posnett, D.N., C.Y. Wang, and S.M. Friedman. 1986. Inherited idiotype-like molecules on cells of a human T cell leukemia. Proc. Natl. Acad. Sci. USA. 83:7888–7892.
23. Bigler, R.D., D.E. Fisher, C.Y. Wang, E.A. Rinnov Kan, and H.G. Kunkel. 1983. Idiotype-like molecules on cells of a human T cell leukemia. J. Exp. Med. 158:1000–1005.
24. Romagné, F., L. Besnardeau, and B. Malissen. 1992. A versatile method to produce antibodies to human T cell receptor V beta segments: frequency determination of human V beta 2+ cells that react with toxic shock syndrome toxin-1. Eur. J. Immunol. 22:2749–2752.
25. Wilson, R.K., E. Lai, P. Concannon, R.K. Barth, and L.E. Hood. 1988. Structure, organisation and polymorphism of murine and human T cell receptor α and β chain gene families. Immunol. Rev. 101:149–169.

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27. Davodeau, F., M.A. Peyrat, I. Houde, M.M. Hallet, H. Vié, and M. Bonneville. 1993. Peripheral selection of antigen receptor junctional features in a major human γδ T cell subset. Eur. J. Immunol. 23:804–810.

28. Vié, H., S. Chevalier, R. Garand, J.P. Moisan, V. Praloran, M.C. Dewilder, J.F. Moreau, and J.P. Soullilou. 1989. Clonal expansion of lymphocytes bearing the gamma/delta receptor in a patient with a large granular lymphocyte disorder. Blood. 74:285–293.

29. Moreau, J.F., D.D. Donaldson, F. Bennett, J. Witek-Giannotti, S.C. Clark, and G.G. Wong. 1988. Leukaemia-inhibitory factor is identical to the myeloid growth factor, human interleukin for DA cells. Nature (Lond.). 336:690–692.

30. Moreau, J.F., M. Bonneville, M.A. Peyrat, A. Godard, Y. Jacques, C. Desgranges, and J.P. Soullilou. 1986. T lymphocyte cloning from rejected human kidney allografts: growth frequency and functional/phenotypic analysis. J. Clin. Invest. 78:874–879.

31. Kappler, J., B. Kotzin, L. Herron, E.W. Gelfand, R.D. Bigler, A. Boylston, S. Carrel, D.N. Posnett, Y. Choi, and P. Marrack. 1989. V beta-specific stimulation of human T cells by staphylococcal toxins. Science (Wash. DC). 244:811–813.

32. Choi, Y.W., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler. 1989. Interaction of staphylococcus aureus toxin “superantigens” with human T cells. Proc. Natl. Acad. Sci. USA. 86:8941–8954.

33. Davodeau, F., M.A. Peyrat, I. Houde, M.M. Hallet, G. De Libero, H. Vié, and M. Bonneville. 1993. Surface expression of two distinct functional antigen receptors on human γδ T cells. Science (Wash. DC). 260:1800–1802.

34. Toyonaga, B., Y. Yoshikai, V. Vadassz, B. Chin, and T.W. Mak. 1985. Organization and sequences of the diversity, joining and constant region genes of the human T cell receptor β chain. Proc. Natl. Acad. Sci. USA. 82:8624–8629.

35. Groettrup, M., K. Ungewiss, O. Azogui, R. Palacios, M.J. Owen, A.C. Hayday, and H. Von Boehmer. 1993. A novel disulfide-linked heterodimer on pre-T cells consists of the T cell receptor β chain and a 33 kDa glycoprotein. Cell. 75:283–293.

36. Lozano, F., C. Roda, J.M. Jarvis, and C. Milstein. 1993. Affinity maturation leads to differential expression of multiple copies of a κ light chain transgene. Nature (Lond.). 363:271–274.

37. Schittek, B., E. Unkelbach, and K. Rajewsky. 1989. Violation of allelic exclusion of the T cell receptor beta genes in a helper T cell clone. Int. Immunol. 1:273–280.

38. Jameson, S.C., K.A. Hogquist, and M.J. Bevan. 1994. Specificity and flexibility in thymic selection. Nature (Lond.). 369:750–752.