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Patterns of membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ chain expression by normal blood $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^-CD8^{dim+}$ and $CD4^-CD8^-$ lymphocytes

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SUMMARY

Enriched $CD4^+CD8^-/CD4^-CD8^-$, $CD4^-CD8^+/CD4^-CD8^-$ and $CD4^-CD8^-$ cell suspensions were prepared from normal peripheral blood by selective immunomagnetic depletion of monoclonal antibody-defined lymphocyte populations. Subsequent examination of these modified cell fractions by two-colour flow cytometry provided a means of determining the expression of membrane T-cell receptor (TcR)$\alpha\beta$ and TcR$\gamma\delta$ chains by both major ($CD4^+$ and $CD8^+$) and minor ($CD3^+CD4^-CD8^{dim+}$ and $CD3^+CD4^-CD8^-$) lymphocyte subpopulations. Normal $CD4^+CD8^-$ lymphocytes were almost invariably (>99%) TcR$\alpha\beta^+$, whereas lymphocytes expressing membrane CD8, which could be further subdivided according to differences in fluorescent staining intensity into $CD3^+CD4^-CD8^+$, $CD3^+CD4^-CD8^{dim+}$ and $CD3^-CD4^-CD8^{dim+}$ components, were characterized by distinct differences in patterns of TcR chain expression. In contrast to $CD3^+CD4^-CD8^+$ cells, which were predominantly (99%) TcR$\alpha\beta^+$, $CD3^+CD4^-CD8^{dim+}$ lymphocytes showed a significant proportion (33%) of TcR$\gamma\delta^+$ cells (natural killer-associated $CD3^-CD4^-CD8^{dim+}$ cells were uniformly TcR^-). The highest proportion (62%) of TcR$\gamma\delta^+$ cells was associated with the $CD3^+CD4^-CD8^-$ fraction, but these studies also revealed that a significant minority of this population was TcR$\alpha\beta^+$. Despite some evidence for normal inter-individual variation, further analysis of membrane CD8 fluorescent intensities confirmed clear differential relationships for TcR$\alpha\beta$ and TcR$\gamma\delta$ chain expression.

INTRODUCTION

The majority of normal human peripheral blood T lymphocytes express, in association with the CD3 complex, a disulphide-linked heterodimeric T-cell antigen receptor (TcR$\alpha\beta$) defined by monoclonal antibodies (mAb) βF1 and WT31 (Kung, 1987). There is, in addition, a small proportion of $CD3^+$ lymphocytes that lacks TcR$\alpha\beta$ chains but expresses a putative second T-cell receptor heterodimer (TcR$\gamma\delta$), defined by reactivity with mAb TcRδ1 (Brenner *et al.*, 1986; Kung, 1987). Whilst the expression of TcR$\alpha\beta$ and TcR$\gamma\delta$ chains by major lymphocyte fractions, such as $CD4^+CD8^-$ helper/inducer cells and $CD4^-CD8^+$ suppressor/cytotoxic cells, is well documented, few studies have investigated minor populations. Indeed, although a number of reports suggest that $CD3^+CD4^-CD8^-$ cells are predominantly TcR$\gamma\delta^+$ (Lanier & Weiss, 1986; Van de Griend *et al.*, 1988), there has been no systematic evaluation of the relative TcR$\alpha\beta$/TcR$\gamma\delta$ expression by this T-cell subset and little is known about the differential expression of TcR chains in relation to $CD8^+$ subpopulations. These deficiencies are largely due to the paucity of such cells in normal peripheral blood and a technical inability to accurately determine the immunophenotypic characteristics of cell populations which may represent <5% of the lymphocyte fraction being studied. However, by selectively removing various lymphocyte populations by immunomagnetic depletion, a number of modified cell suspensions have been successfully prepared which were subsequently analysed by two-colour flow cytometry. Reported here are the results for these investigations which have provided a means of determining the expression of TcR$\alpha\beta$ and TcR$\gamma\delta$ chains by both major and minor lymphocyte populations.

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MATERIALS AND METHODS

Immunological reagents used in this study

The following FITC/phycoerythrin (PE) two-colour combinations were employed for the analysis of membrane determinant expression in this investigation; CD4 (Leu-3a, PE conjugate; Becton-Dickinson, Mountain View, CA) with WT31 (TcR$\alpha\beta$, FITC conjugate; Becton-Dickinson) or TcRδ1 (TcR$\gamma\delta$, FITC conjugate; Laboratory Impex, Twickenham, Middlesex); CD8 (Leu-2a, PE conjugate; Becton-Dickinson) with FITC-conjugated WT31 or TcRδ1.

Unconjugated mAb used in the immunomagnetic depletion procedures included T4 (CD4; American Type Culture Collec-

tion, Rockville, MD), RFT8 (CD8; Royal Free Hospital, London), 63D3 (CD14; American Type Culture Collection) and HD37 (CD19; Dako, High Wycombe, Bucks).

Preliminary mononuclear cell separation

Normal peripheral blood samples, obtained from healthy adult volunteers, were evaluated in this study. Initially, mononuclear cells were fractionated from 50 ml of EDTA-anti-coagulated blood by density sedimentation with Lymphoprep (Nyegaard, Oslo, Norway). After washing in phosphate-buffered saline (PBS)-azide (0·01%), the cells were pelletted and mixed with saturating concentrations of various mAb combinations, as described below.

Preparation of $CD4^+CD8^-$ enriched cell fractions

$CD4^+CD8^-$ enriched peripheral blood fractions were prepared from mononuclear cell suspensions by selective depletion of $CD8^+$ T-suppressor/cytotoxic cell, $CD14^+$ monocyte and $CD19^+$ B-cell components using mAb RFT8, 63D3 and HD37, respectively. To achieve this, predetermined amounts of all three mAb were mixed with the cell pellets and, after 30 min incubation at 4°, the cells were washed free of excess antibodies and resuspended in 0·5 ml Hanks' balanced salt solution (HBSS) (pH 7·2) supplemented with 1% bovine serum albumin (BSA/HBSS). To this were added 50 μl of washed sheep anti-mouse immunoglobulin-coated M450 immunomagnetic particles (Dynabeads; Dynal, Wirral, Merseyside) in 1% BSA/HBSS. Following incubation at room temperature for 30 min with regular gentle mixing, the leucocyte–Dynabead mixture was resuspended to 1·0 ml in 1% BSA/HBSS and placed on a magnetic separator. The supernatant was removed and tested by indirect immunorosetting (MacKarill *et al.*, 1987) for the presence of residual mAb-coated cells and the procedure repeated with 50 μl Dynabead aliquots until these comprised $<2\%$ of the modified lymphocyte fraction. In practice, this rarely required more than two depletion steps. Following removal of $CD8^+$, $CD14^+$ and $CD19^+$ populations, the resulting enriched cell suspension, comprising a mixture of $CD4^+CD8^-$ and $CD4^-CD8^-$ lymphocytes, was washed and resuspended in PBS-azide prior to immunophenotyping.

Preparation of normal $CD4^-CD8^+$ enriched cell fractions

For the preparation of $CD4^-CD8^+$ enriched lymphocyte fractions, $CD4^+$ T-helper/inducer cell, $CD14^+$ and $CD19^+$ components were removed by immunomagnetic depletion with mAb T4, 63D3, and HD37. Depletion was again considered complete when residual mAb-coated cells were $<2\%$ and when re-analysis of the cell suspension with a CD4/CD14/CD19 antibody cocktail gave $<2\%$ positive cells. This modified lymphocyte fraction, comprising a mixture of $CD4^-CD8^+$ and $CD4^-CD8^-$ cells, was resuspended in PBS-azide for subsequent immunophenotypic analyses.

Preparation of normal $CD4^-CD8^-$ enriched cell fractions

For the preparation of a $CD4^-CD8^-$ enriched cell suspension, $CD4^+$, $CD8^+$, $CD14^+$ and $CD19^+$ components were removed by immunomagnetic depletion using mAb T4, RFT8, 63D3 and HD37. Again, satisfactory depletion was considered complete when re-analysis of the cell suspension with a CD4/CD8/CD14/CD19 antibody cocktail gave $<2\%$ positive cells. These $CD4^-CD8^-$ cells were resuspended in PBS-azide for subsequent immunophenotypic analyses.

Immunological procedures for two-colour immunofluorescence

Analysis of membrane determinant co-expression, using the two-colour combination of fluorescein isothiocyanate (FITC) and phycoerythrin (PE), was carried out as follows: 10 μl of each primary reagent were pipetted into microtitre plate wells containing $1{\cdot}0 \times 10^6$ cells (in a maximum volume of 10μl). The cell–antibody mixture was mixed, incubated at 4° for 5 min and the cells then pelletted by centrifugation. After removal of excess antibody and washing twice in 150 μl volumes of PBS-azide, the cells were resuspended in the same prior to FACS analysis. Control studies comprised of either unstained cells or cells incubated with IgG1-FITC and IgG2-PE irrelevant mAb.

Acquisition and analysis was carried out with a FACSCAN flow cytometer (Becton-Dickinson) using Consort 30 software. Data were stored in list mode files in the order: FSC (forward scatter), SSC (side scatter), FL1 (FITC) and FL2 (PE). An electronic gate was set around the lymphocyte population, as defined by FSC and SSC characteristics, and single histogram plots examined to determine the percentage positive cells for a single antigen. Contour (quadrant) graphs of FL1 versus FL2 were assessed for single and dual antigen-positive components.

RESULTS

Phenotypic characteristics of modified cell fractions produced by immunomagnetic depletion

A total of 11 different blood mononuclear fractions were depleted of $CD8^+$, $CD14^+$ and $CD19^+$ components. The resulting cell suspensions comprised a mixture of $CD4^+CD8^-$ and $CD4^-CD8^-$ lymphocytes with mean relative frequencies of 84% and 16% (Fig. 1). Similarly, 19 individual mononuclear fractions were depleted of $CD4^+$, $CD14^+$ and $CD19^+$ components to produce an enriched mixture of $CD4^-CD8^+$ and $CD4^-CD8^-$ cells (mean frequencies 71% and 29%). FACS histogram analysis of the latter cell suspensions indicated that the $CD8^+$ fraction could be further divided, according to differences in membrane staining intensity, into $CD8^{dim+}$ and $CD8^+$ subpopulations with mean relative frequencies ($n=19$) of 30% and 70%, respectively. Supplementary studies, detailed in a separate communication (Richards & Scott, 1990), of CD3 expression by these cell fractions revealed that virtually all normal $CD4^+CD8^-$ and $CD4^-CD8^+$ lymphocytes were $CD3^+$, whereas most $CD4^-CD8^{dim+}$ cells lacked membrane CD3 (Fig. 1). The third group ($n=23$) of modified cell suspensions prepared in this study comprised $>98\%$ $CD4^-$ $CD8^-$ lymphocytes, which could be further subdivided, according to CD3 expression, into $CD3^+$ (mean, 19%) and $CD3^-$ components.

Membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ expression by $CD4^+CD8^-$, $CD4^-CD8^+$ and $CD4^-CD8^{dim+}$ lymphocytes

Normal blood mononuclear fractions, modified by immunomagnetic depletion procedures, were further studied by two-colour immunocytometry. For the first of these suspensions, comprising a mixture of $CD4^+CD8^-$ and $CD4^-CD8^-$ cells, the proportions of $CD4^+$ lymphocytes co-expressing either TcR$\alpha\beta$ or TCR$\gamma\delta$ chains, as well as the distribution of $CD4^+TcR^-$ cells,

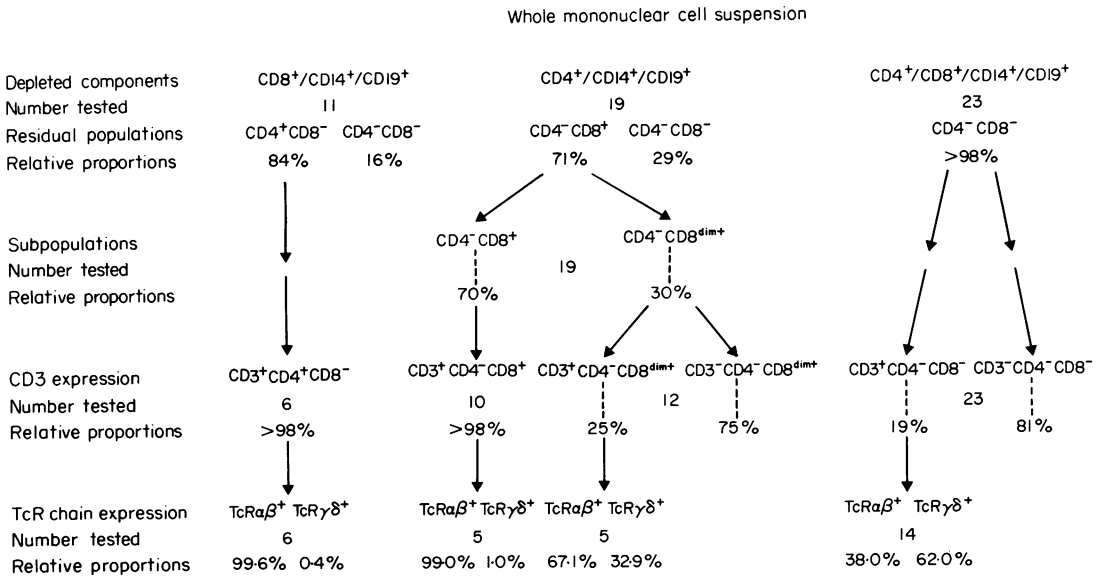

Figure 1. Immunophenotypic analysis of normal $CD4^+CD8^-/CD4^-CD8^-$, $CD4^-CD8^+/CD4^-CD8^-$ and $CD4^-CD8^-$ lymphocyte fractions, prepared by immunomagnetic depletion of selected mAb-defined populations, for the expression of TcR$\alpha\beta$, and TcR$\gamma\delta$ determinants. Schematic representation of the methodological and analytical procedures incorporated in this study, and the relative immunological composition of cell fractions at each stage of the investigation. Whole mononuclear cell suspensions, from which various lymphocyte populations were depleted, were prepared by conventional density sedimentation of peripheral blood.

were determined. The results (Table 1 and Fig. 1) for the six fractions examined showed that virtually all (mean 98%) $CD4^+$ cells were membrane TcR$\alpha\beta^+$ and that $CD4^+$TcR$\gamma\delta^+$ and $CD4^+$TcR$^-$ components were insignificant.

In the same way, relationships between CD8 and TcR chain expression were determined by immunocytometric analysis of the enriched $CD4^-$ $CD8^+$ and $CD4^-$ $CD8^-$ cell mixtures. It was possible by FACS analysis to further subdivide $CD8^+$ cells into $CD8^+$ and $CD8^{dim+}$ subpopulations and therefore membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ chain expression by each of these CD8 fractions was additionally examined. As with lymphocytes expressing CD4, most $CD8^+$ cells were TcR$\alpha\beta^+$ and only a small minority were TcR$\alpha\beta^+$ (Table 1). However, the proportion of $CD8^+$TcR$^-$ cells appeared greater than that observed for the $CD4^+$ fractions although, with the exception of one case (no. 5, detailed separately below), this did not exceed 6·5%. For comparison, membrane TcR expression by $CD8^{dim+}$ cells was characterized by a predominance of TcR$^-$ cells, with the TcR$^+$ fraction showing considerable individual variation with respect to differential TcR$\alpha\beta$ and TcR$\gamma\delta$ expression (Table 1). It has previously been demonstrated that most normal $CD8^{dim+}$ cells are $CD3^-$ and express a number of natural killer cell-associated (NKa) markers, most notably CD11b, CD16, CD56 and CD57 (Richards & Scott, 1990). These current studies extended these findings by demonstrating that the $CD3^+CD8^{dim+}$ component, which shows little evidence of NKa phenotypic differentiation, has a much higher proportion (mean 32·9%) of TcR$^+$ cells expressing $\gamma\delta$ chains than $CD3^+CD8^+$ cells (Fig. 1).

Membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ expression by normal blood $CD4^-CD8^-$ lymphocytes

Fourteen $CD4^-CD8^-$ lymphocyte-enriched fractions were phenotypically analysed for the distributions of TcR$\alpha\beta^+$, TcR$\gamma\delta^+$ and TcR$^-$ populations. As expected from analyses of CD3 expression by these cell fractions, the majority (mean 82·1%) of $CD4^-CD8^-$ lymphocytes were TcR$^-$ (Table 2), with the remainder expressing either TcR$\alpha\beta$ or TcR$\gamma\delta$ chains. The individual variation for relative expression of TcR$\alpha\beta^+$ and TcR$\gamma\delta^+$ components in the series of fractions studied was considerable, although, in most instances the predominant TcR$^+$ population expressed $\gamma\delta$ chains (mean TcR$\alpha\beta^+$, 38%; TcR$\gamma\delta^+$, 62%; Fig. 1).

Relationships between CD8 and TcR chain expression

The above studies showed that $CD8^-$ and $CD8^{dim+}$ fractions both comprised mixtures of TcR$\alpha\beta^+$ and TcR$\gamma\delta^+$ cells whereas, in contrast, $CD8^+$ lymphocytes were predominantly TcR$\alpha\beta^+$. However, one of the six normal peripheral bloods analysed (no. 5, Table 1) showed a pattern of membrane TcR expression that, because of the high proportion (35·9%) of $CD8^+$TcR$^-$ cells, clearly differed from the other five. To investigate this further, and in order to assess relationships between CD8 and TcR chain expression, the $CD8^{dim+}$ and $CD8^+$ fluorescent histograms were subdivided in the six cases studied according to differences in CD8 membrane staining intensity. For this analysis, the $CD8^{dim+}$ and $CD8^+$ components, defined by membrane PE fluorescent intensities of 65–145 and >145 PE units, respectively, were further subdivided into three subgroups (Table 3). Examination of case nos 1–4 and no. 6 showed that, in the $CD8^{dim+}$ range of 65–126 PE units, the relative proportions of TcR$\alpha\beta^+$ and TcR$\gamma\delta^+$ cells were remarkably similar, with only a slight predominance (mean 1·47:1) of TcR$\alpha\beta^+$ cells. In contrast, $CD8^+$ cells with staining intensities exceeding 167 PE units showed a marked predominance (>100:1) of TcR$\alpha\beta^+$ cells. The two intermediate groups ($CD8^{dim+}$, 127–145 PE units; and $CD8^+$ 146–166 PE units), however, showed TcR$\alpha\beta^+$:

Table 1. Membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ chain expression by normal CD4$^+$CD8$^-$, CD4$^-$CD8$^+$, and CD4$^-$CD8^{dim+} lymphocyte subpopulations: an analysis of six normal peripheral blood fractions*

	% TcR $\alpha\beta^+$	% TcR $\gamma\delta^+$	% TcR $^-$
CD4$^+$ CD8$^-$ fraction			
Case no.			
1	98·6	0·4	1·0
2	98·8	0·1	1·1
3	99·2	0·4	0·4
4	99·6	0·1	0·3
5	96·5	0·9	2·6
6	93·7	0·4	5·9
Mean	97·7	0·4	1·9
CD4$^-$CD8$^+$ fraction			
Case no.			
1	96·3	1·0	2·7
2	90·5	3·0	6·5
3	97·8	0·2	2·0
4	98·3	0·2	1·5
5	59·2	4·9	35·9
6	94·9	0·6	4·5
Mean†	89·5	1·6	8·9
CD4$^-$CD8^{dim+} fraction			
Case no.			
1	16·0	2·0	82·0
2	7·0	1·5	91·5
3	13·0	13·1	73·9
4	4·8	2·5	92·7
5	4·3	22·4	73·3
6	12·4	6·8	80·8
Mean‡	9·6	8·1	82·3

* TcR$\alpha\beta$ expression defined by mAb WT31; TcR$\gamma\delta$ expression defined by mAb TcRδ1. Results are shown as the percentages of TcR$\alpha\beta^+$, TcR$\gamma\delta^+$ or TcR$^-$ (TcR$\alpha\beta^-$TcR$\gamma\delta^-$) fractions in each lymphocyte subpopulation.

† Mean percentages, excluding case no. 5: TcR$\alpha\beta^+$, 95·6%; TcR$\gamma\delta^+$, 1·0%; TcR$^-$, 3·4%.

‡ Mean percentages, excluding case no. 5: TcR$\alpha\beta^+$, 10·6%; TcR$\gamma\delta^+$, 5·2%. TcR$^-$, 84·2%.

Table 2. Analysis of 14 different normal CD4$^-$ CD8$^-$ lymphocyte fractions for membrane TcR$\alpha\beta$ and TcR$\gamma\delta$ chain expression*

	% TcR$\alpha\beta^+$	% TcR $\gamma\delta^+$	% TcR$^-$
Fraction			
(a)	8	12	80
(b)	2	31	67
(c)	35	5	60
(d)	12	8	80
(e)	3	2	95
(f)	5	8	87
(g)	4	5	91
(h)	7	11	82
(i)	1	27	72
(j)	2	2	96
(k)	3	4	93
(l)	5	4	91
(m)	6	11	83
(n)	4	29	67
Mean	6·5	11·4	82·1

* TcR$\alpha\beta$ expression defined by mAb WT31; TcR$\gamma\delta$ expression defined by mAb TcRδ1. Results are shown as the percentages of TcR$\alpha\beta^+$, TcR$\gamma\delta^+$ or TcR$^-$ (TcR$\alpha\beta^-$TcR$\gamma\delta^-$) fractions in each lymphocyte subpopulation.

TcR$\gamma\delta^+$ ratios of 3·63 and 55·7:1, respectively. When analysed in the same way, case no. 5 clearly showed a different pattern of membrane TcR chain expression. Whilst consistent TcR$\alpha\beta^+$:TcR$\gamma\delta^+$ ratios were again observed throughout the CD8^{dim+} range of 65–126 PE units, with a predominance (mean ratio 0·15:1) of TcR$\gamma\delta^+$ cells, the expected 'transition' at higher staining intensities to a predominance of TcR$\alpha\beta^+$ cells was not seen until > 180 PE units. Relationships between CD8 and TcR expression in case no. 5 are further illustrated in Fig. 2 where clear correlations between (a) CD8^{dim+} and TcR$\alpha\beta^-$ and (b) CD8$^+$ and TcR$\alpha\beta^+$ populations are evident.

DISCUSSION

This investigation has characterized the expression of WT31 (TcR$\alpha\beta$) and TcRδ1 (TcR$\gamma\delta$) membrane determinants by normal peripheral blood lymphocyte subpopulations. The authors believe that the methodological approach to this analysis is the first time that mononuclear cell suspensions have been manipulated by mAb and immunomagnetic depletion to prepare modified lymphocyte fractions, specifically designed to enrich minor lymphocyte populations by negative selection, for subsequent two-colour studies of membrane TcR expression. It is proposed that the subsequent immunophenotypic analysis of such modified lymphocyte fractions is likely to be considerably more accurate and reliable than previous studies of either whole mononuclear cell fractions or cell populations obtained by positive selection.

In contrast to βF1, which recognizes the Cβ domain of the T-cell antigen receptor, the exact specificity of mAb WT31 has not yet been established (Kung, 1987), although it has been shown to react with a common epitope of the C$\alpha\beta$ region (Spits *et al.*, 1985; Brenner *et al.*, 1986; Kung, 1987) and, consequently, recognizes all but a minor proportion of human CD3$^+$ cells. The function of membrane TcR$\alpha\beta$ has not been fully elucidated, but it is considered that it may be associated with antigen recognition and MHC restriction (Brenner *et al.*, 1986). This antigen receptor is a disulphide-linked heterodimer (Ti) (Bank *et al.*, 1986; Lanier *et al.*, 1987) that exists in non-covalent association with three invariant proteins, referred to as the CD3 complex (Kung, 1987). The minor proportion (approximately 3%) of normal lymphocytes that is unreactive with WT31 alternatively appears to express a heterodimer of γ and δ chains (Raulet, 1989). Delineation of this cell population is achieved with mAb TcRδ1, which identifies a framework determinant on the TcRδ chain. The function(s) of TcR$\gamma\delta^+$ cells is still unresolved,

Table 3. Relationships between membrane CD8 and TcR chain expression by normal peripheral blood lymphocytes*

CD8 fraction fluorescent intensity†	$CD4^-CD8^-$	$CD4^-CD8^{dim+}$					$CD4^-CD8^+$		
	<65	65–80	81–95	96–110	111–126	127–145	146–166	167–180	>180
Mean normal values‡									
Total % TcR^+ cells§	17·9¶	10·0	9·0	10·2	13·2	31·5	85·0	98·1	99·0
% $TcR\alpha\beta^+$ cells	6·5	6·4	4·8	6·4	7·4	24·7	83·5	97·3	98·4
% $TcR\gamma\delta^+$ cells	11·4	3·6	4·2	3·8	5·8	6·8	1·5	0·8	0·6
$TcR\alpha\beta$:$TcR\gamma\delta$ ratio	0·57:1	1·78:1	1·14:1	1·68:1	1·28:1	3·63:1	55·7:1	>100:1	>100:1
Case no. 5									
Total % TcR^+ cells§		31·5	29·5	25·7	28·1	21·4	24·3	66·8	95·4
% $TcR\alpha\beta^+$ cells		3·5	2·5	3·3	5·2	3·9	15·1	63·4	94·7
% $TcR\gamma\delta^+$ cells		28·0	27·0	22·4	22·9	17·5	9·2	3·4	0·7
TcR $\alpha\beta$:$TcR\gamma\delta$ ratio		0·13:1	0·09:	0·15:1	0·23:1	0·22:1	1·64:1	18·6:1	>100:1

* For this analysis, the $CD4^-CD8^{dim+}$ and $CD4^-CD8^+$ populations were further subdivided according to level of CD8 membrane staining and re-examined for the proportions of lymphocytes within each fraction that expressed $TcR\alpha\beta$ or $TcR\gamma\delta$.

† $CD4^-CD8^-$ fraction defined as <65 PE units; $CD4^-$ $CD8^{dim+}$ fraction (range 65–145 PE units) subdivided into five subgroups with stated fluorescent values; $CD4^-CD8^+$ fraction (>145 PE units) subdivided into three subgroups with stated fluorescent levels.

‡ Data for $CD4^-CD8^{dim+}$ and $CD4^-$ $CD8^+$ fractions represent mean values obtained from five normal peripheral blood samples nos 1–4 and 6 (Table 1).

§ Total % TcR^+ cells represents sum of $TcR\alpha\beta^+$ and $TcR\gamma\delta^+$ lymphocytes.

¶ Data for $CD4^-CD8^-$ fractions derived from analysis of 14 different normal peripheral bloods.

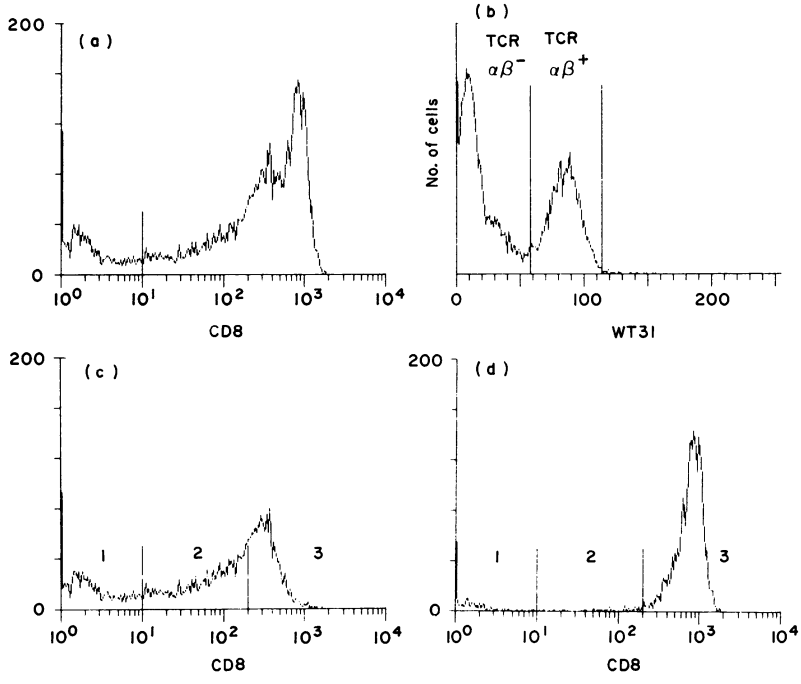

Figure 2. Relationships between membrane CD8 and $TcR\alpha\beta$ expression in case no. 5 as defined by two-colour immunocytometry. $CD4^+$, $CD14^+$ and $CD19^+$ cells were removed by immunomagnetic depletion and the resulting enriched $CD4^-CD8^+$ and $CD4^-CD8^-$ cell mixture examined with PE-conjugated Leu-2a (CD8) and FITC-conjugated WT31 ($TcR\alpha\beta$) mAb. Histogram (a) shows the spectrum of CD8 staining intensities for the 'whole' cell fraction and reveals considerable staining heterogeneity, with evidence of more than one lymphocyte population expressing CD8. Similarly, histogram (b) shows the pattern of $TcR\alpha\beta$ reactivity for the 'whole' cell fraction and clearly shows both $TcR\alpha\beta^-$ and $TcR\alpha\beta^+$ subpopulations. When these different TcR fractions are 'gated' and separately analysed for CD8 expression, it is apparent that the $TcR\alpha\beta^-$ component comprises (c) lymphocytes with CD8 staining intensities ranging from negative (segment 1) through $CD8^{dim+}$ (segment 2) to the lower end of $CD8^+$ (segment 3). In marked contrast, the $TcR\alpha\beta^+$ component essentially comprises (d) a single population of cells with strong CD8 expression (segment 3) with only a minor $CD8^-$ fraction. In this analysis, the pattern of CD8 expression shown in histogram (a) is clearly a composite of histograms (c) and (d).

although several $CD4^-CD8^-Tcr\gamma\delta^+$ T-cell lines derived from synovial tissue of patients with rheumatoid arthritis have been reported (Porcelli *et al.*, 1989) to show specific cytotoxicity of CD1c determinants, which are functionally analogous to MHC class I and II molecules, and there is also some speculation that $Tcr\gamma\delta^+$ cells may be specialized for mycobacterial immunity or destruction of 'stressed' autologous cells (Raulet, 1989).

Whilst a number of studies have established that normal $CD3^+CD4^+CD8^-$ and $CD3^+$ $CD4^-$ $CD8^+$ lymphocyte populations are predominantly $Tcr\alpha\beta^+$, and that $Tcr\gamma\delta^+$ cells are associated in particular with the $CD3^+$ $CD4^-$ $CD8^-$ subset (Lanier & Weiss, 1986; Van de Griend *et al.*, 1988), there are a number of areas which remain less clear. One of these is a lack of information regarding the expression of membrane TcR chains by 'suppressor' T-cells with different membrane CD8 staining intensities (dim+ and +). This is particularly relevant as it is known that $CD8^{dim+}$ cells are phenotypically heterogenous, comprising both $CD3^+NKa^-$ and $CD3^-NKa^+$ subpopulations (Richards & Scott, 1990), and also because some 'suppressor' cells show a deletion of or fail to rearrange $Tcr\beta$ genes (Brenner *et al.*, 1986). The investigations of $CD8^{dim+}$ and $CD8^+$ enriched populations, prepared by selective removal of $CD4^+$, $CD14^+$ and $CD19^+$ components, indicate that the $CD3^+CD8^{dim+}$ fraction comprises a high proportion (mean 32·9%) of $Tcr\gamma\delta^+$ cells, intermediate between that of $CD3^+CD8^+$ (<1·0%) and $CD3^+CD4^-CD8^-$ (62%) lymphocytes. In addition, the results indicate that, for any given individual, the differential composition of $Tcr\alpha\beta^+$ and $Tcr\gamma\delta^+$ cells for specific levels of CD8 expression is constant.

A further point specifically addressed by this current study was the determination of the relative frequencies of $Tcr\alpha\beta^+$ and $Tcr\gamma\delta^+$ cells within the $CD3^+$ fraction of $CD4^-$ $CD8^-$ cells. In contrast to the conclusions of Van de Griend *et al.* (1988), suggesting that $CD3^+CD4^-CD8^-$ cells are almost exclusively $Tcr\gamma\delta^+$, this present study conclusively demonstrated that this lymphocyte fraction comprises both $Tcr\alpha\beta^+$ and $Tcr\gamma\delta^+$ components. This is supported by the recent observations of Shivakumar *et al.* (1989), who additionally reported that an expansion of $CD4^-CD8^-Tcr\alpha\beta^+$ cells was clinically associated with active systemic lupus erythematosus.

The ability to manipulate cell fractions in a predictable way is clearly advantageous in that depletion of 'unwanted' components concomitantly enriches those populations of interest. By adapting such procedures, and incorporating two-colour flow cytometry for the subsequent study of each modified cell suspension, this study has for the first time been able to detail the expression of membrane TcR chains by normal blood lymphocytes and has systematically analysed the distributions of $Tcr\alpha\beta^+$ and $Tcr\gamma\delta^+$ cells within individual lymphocyte subpopulations. That there are distinct patterns between the differential expression of the two TcR heterodimers and various CD4/CD8 composite phenotypes is clear. However, what is also apparent is that there is significant normal inter-individual variation. Whether these patterns of expression are stable or constantly vary according to type and severity of immunological challenge (e.g. common viral infections) is as yet unknown but may be resolved by further studies currently in progress.

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