EXPRESSION OF T-CELL RECEPTOR Vβ REGIONS IN GRANULAR LYMPHOCYTE-PROLIFERATIVE DISORDERS

To the Editor:

In a recent issue of *Blood,* de Totero et al[1] reported the preferential T-cell receptor (TCR) V region usage in the expansion of $CD8^+$ granular lymphocytes (GL). They found that both monoclonal and polyclonal $CD8^+TCR\alpha\beta^+$ GL shared the Vβ5.3 region, as shown by their positivity for LC4 monoclonal antibody (MoAb), and suggested a preferential Vβ gene segment usage in the rearrangement process of $CD8^+$ GL.

We also studied the usage of TCR Vβ regions of peripheral blood lymphocytes (PBL) in patients with GL-proliferative disorders (GLPD). GLPD are characterized by the proliferation of GL in the peripheral blood.[2,3] Based on the surface phenotypes of the proliferating GL, GLPD are divided into $CD3^+$ T-cell–lineage GLPD (T-GLPD) and $CD3^-$ natural killer cell-lineage GLPD. The GL of most T-GLPD patients are monoclonally rearranged, and these GL are thought to be a proliferation of cytotoxic T lymphocytes of unknown antigen specificity.[4,5] Investigation of the usage of TCR variable regions in T-GLPD is thought to be important in the study of the pathogenesis of the disorders.

Murine MoAbs reactive with human TCR variable regions are now commercially available to study the repertoire of TCR expressed under many conditions. In this study, we used MoAb Diversi-T αβ TCR Screening Panels 1F (lot 2800; T Cell Diagnostics, Cambridge, MA) and those from Immunotech S.A. (Marseille, France). Data analysis was performed with EPICS Profile II (Coulter Co, Hialeah, FL) by appropriately gating lymphoid populations.

Seventeen cases of T-GLPD were studied (Table 1). Patients no. 1 through 15 had $CD4^-CD8^+TCR\alpha\beta^+$ GL and patients no. 16 and 17 had $CD4^+CD8^-TCR\alpha\beta^+$ GL. The details of their clinical features have been described elsewhere.[2,3] Interestingly, $CD8^+$ T-GLPD cells from all the patients and $CD4^+$ T-GLPD cells from one patient were LC4 positive. This finding suggests that these GL shared the usage of Vβ5.3 gene, as de Totero et al[1] had reported. However, in our experiments, 4 patients (nos. 7, 11, 14, and 15) were strongly positive for other MoAbs that react with different TCR Vβ regions, and the sum of the number of positive cells in each patient exceeded 100%. Figure 1 shows the representative pattern of TCR Vβ analysis. LC4 seemed to be positive in almost all the lymphocytes in the patients, as shown by the right shift of the whole cells. Other MoAbs, OT145 and 16G8, stained the positive cells much more strongly, and some populations remained negative. We then examined the $CD4^-CD8^-TCR\alpha\beta^+$ lymphocytes of a patient with lymphoma (patient no. 18) and the cells were found to be negative for LC4, thus denying the possibility that the staining pattern with the MoAb LC4 is nonspecific. When examined with PBL in five normal donors, the percentage of $LC4^+$ cells was similar to that of $CD8^+$ cells (25% ± 5% and 23% ± 7%, respectively), and the number of $LC4^+$ cells was more than that stated in the manufacturer's instruction, ie, 1% to 5% of PBL. Next, $CD8^+$ cells were purified from PBL of three normal donors with magnetic beads (Dynabeads; Dynal Co, Oslo, Norway) and tested for LC4 MoAb reactivity. Almost all $CD8^+$ cells were found to be $LC4^+$.

These findings indicate that the MoAb LC4 reacts with antigen expressed mainly on $CD8^+$ lymphocytes and different from or including Vβ 5.3 region of TCR chain, and that T-GLPD cells do not use common TCR Vβ regions.

Table 1. Percentage of Positive Cells in PBL

Patient No.	CD3	CD4	CD8	TCRβ Gene	E22* Vβ2	LE89 Vβ3	1C1 Vβ5a	W112 Vβ5b	LC4 Vβ5c	OT145 Vβ6	16G8 Vβ8	S511 Vβ12	JU74 Vβ13	BA62 Vβ17	E17.5 Vβ19	F1 Vα2
1	88	26	71	M	5	0	5	6	19	6	6	5	0	0	6	5
2	96	8	89	M	3	0	4	4	45	5	4	3	0	0	2	3
3	92	9	91	M	1	0	3	4	66	4	5	14	0	0	2	2
4	97	1	95	M	0	0	5	4	52	3	4	4	0	0	3	3
5	95	13	80	M	1	0	5	4	45	4	6	5	0	0	2	3
6	79	17	78	M	2	0	3	2	54	3	3	3	0	0	2	2
7	98	7	91	M	1	0	3	2	80	2	48	3	0	0	2	2
8	64	15	61	M	1	0	3	1	30	2	2	2	0	0	1	1
9	91	30	58	P	17	0	4	2	59	3	6	4	0	0	4	3
10	95	6	90	M	1	0	4	2	80	2	3	2	0	0	2	1
11	99	7	91	M	86	0	3	3	66	3	4	3	0	0	2	2
12	84	26	62	M	6	0	6	3	62	3	7	4	0	0	4	3
13	98	4	95	M	2	0	3	2	25	2	2	2	0	0	6	2
14	98	8	73	M	1	0	5	1	45	87	2	2	0	0	2	1
15	99	4	96	M	2	0	3	3	67	3	57	3	0	0	3	3
16	98	96	6	M	1	0	2	1	27	2	2	1	0	0	1	1
17	95	84	12	M	1	0	2	2	13	2	2	4	0	0	2	2
18	98	9	17	M	0	0	4	3	6	4	4	3	0	0	3	3
Normal donors (n = 5)	66 ± 6	38 ± 7	23 ± 7		6 ± 1	0 ± 0	11 ± 3	12 ± 5	25 ± 5	11 ± 5	13 ± 4	17 ± 9	0 ± 0	0 ± 0	9 ± 2	12 ± 5

Patients no. 1 through 15 had GLPD of $CD3^+CD4^-CD8^+$ phenotypes, patients no. 16 and 17 had GLPD of $CD3^+CD4^+CD8^-$ phenotype, and patient no. 18 had leukemic phase of non-Hodgkin's lymphoma and $CD3^+CD4^-CD8^-TCR\alpha\beta^+$ phenotype.

Abbreviations: M, monoclonally rearranged; P, polyclonally rearranged.

* The name of MoAb-producing clones.

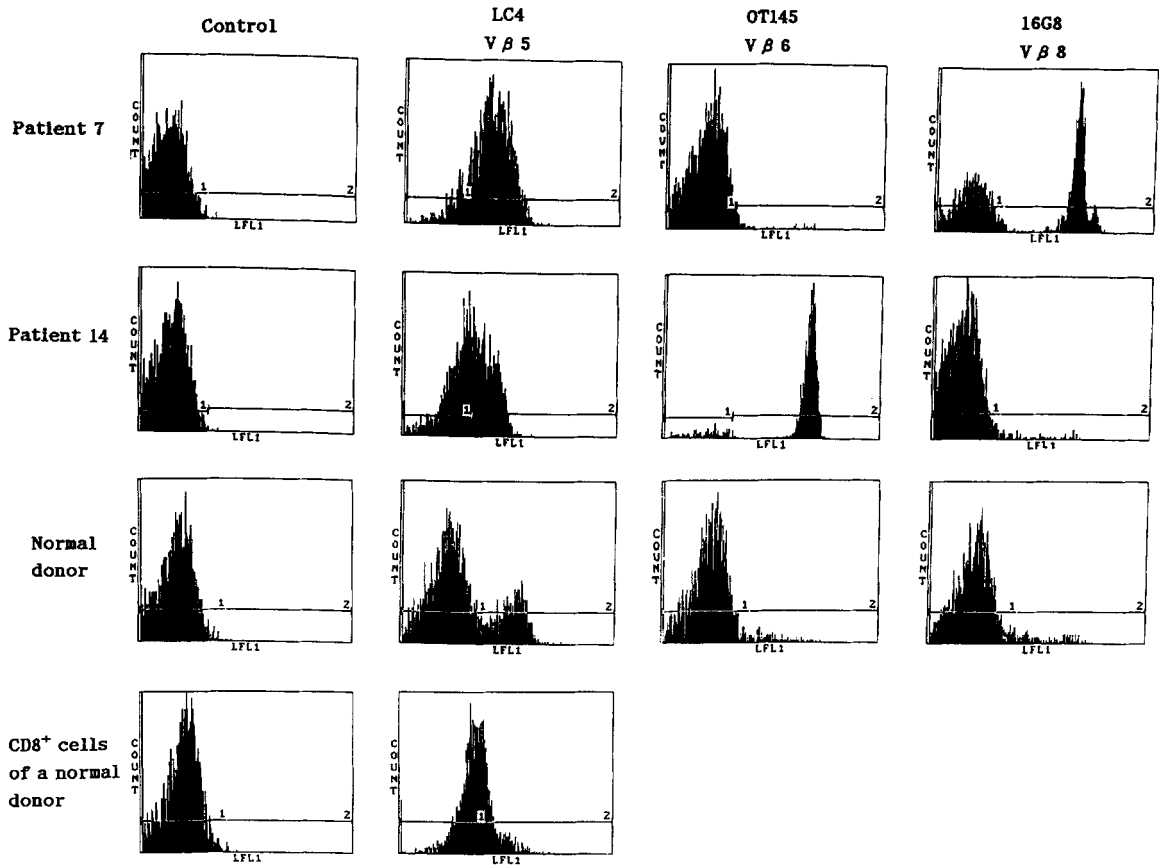

Fig 1. Cytofluorometric analysis of PBL with MoAb against TCR Vβ. The data from representative cases, normal donors, and purified normal CD8$^+$ lymphocytes are presented.

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