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STUDIES OF HUMAN Tγ CELLS: DIVISION OF A Tγ SUBSET IN NORMAL AND LEUKEMIC CELLS BY USING ANTI-Tγ-CLL HETEROANTISERUM

ICHIRO KONISHI[1], TAKASHI MACHII[1], AKIRA HIRAOKA, YOSHIO KANAYAMA, NOBUHIRO TANIGUCHI[1], TOSHIHARU TAMAKI, TAKESHI YONEZAWA and TERUO KITANI[1]

The Second Department of Internal Medicine, Osaka University Medical School, Fukushima-ku, Osaka 553, Japan

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SUMMARY A specific heteroantiserum was prepared against the leukemic cells from a patient with T-derived chronic lymphocytic leukemia (T-CLL). The antiserum was absorbed with cells of a morphologically different type from another patient with T-CLL. Both the immunizing cells and absorbing cells had Fc receptor for IgG (FcγR), so the former case was named Tγ-CLL type 1, and the latter Tγ-CLL type 2. This antiserum, termed anti-Tγ-1, reacted with 19% of normal peripheral blood T lymphocytes, but not with non-T lymphocytes or monocytes. The T lymphocytes in the blood that reacted to anti-Tγ-1 were 72% of the Tγ cells. Anti-Tγ-1 also reacted to 60–78% of the thymocytes. Except for Tγ-CLL type 1 cells, anti-Tγ-1 did not react with various types of leukemia cells from lymphoid malignancies, myelogenous leukemias and monocytic leukemias.

Studies on the relation between anti-Tγ-1 and OKT8 monoclonal antibody revealed that anti-Tγ-1 reactive (anti-Tγ-1$^+$) cells and OKT8$^+$ cells largely overlapped, but they were different in part. More interestingly, OKT8 inhibited FcγR binding, but anti-Tγ-1 did not.

These results indicate that anti-Tγ-1 is useful for detecting a certain subset of T cells and for classifying lymphoproliferative disorders.

INTRODUCTION

Efforts to discover cell markers defining human T cell subsets have been made for the purpose of better analysis of T cell function and its differentiation.

By use of differential Fc receptor binding, Moretta et al. (1977) demonstrated that the T cell population can be dissected into two distinct subsets (Tμ and Tγ subsets), each with distinctive functional properties. However,

1 Present Address: Division of Internal Medicine, Department of Clinical Research, Research Institute for Microbial Diseases, Osaka University, 3–1, Yamadaoka, Suita, Osaka 565, Japan.

more recent evidence suggests that each subset is further heterogeneous with respect to its functions (Hayward et al. 1978; Shaw, Pichler and Nelson, 1979).

Attempts have been made to define antigenic markers specific to human T cell subsets. Some workers have reported the successful characterization of human T cell differentiation antigens, utilizing heteroantisera (Evans et al., 1977, 1978; Brouet and Chevalier, 1979) and hybrydoma antibodies (Kung et al., 1979; Reinherz et al., 1979, 1980b; Hynes et al., 1979).

T-CLL should represent a monoclonal expansion of a subset of mature T lymphocytes possessing their own antigenic markers. Therefore, T-CLL cells should be useful for immunization to obtain antisera specific to subsets of peripheral blood T lymphocytes. We studied 2 patients with T-CLLs. The leukemic cells from the two patients both had FcγR, and they were indistinguishable on May-Giemsa staining and in cytochemical features. But, ultrastructurally, one contained parallel tubular array (PTA) and other did not. Thus we suspected that the two FcγR$^+$ T-CLLs (Tγ-CLLs) were derived from distinct subsets of T cells. If this was the case, the cells might express different surface antigens. A heteroantiserum was prepared by immunizing a rabbit with the PTA$^+$ Tγ-CLL cells and then absorption of the antiserum with the PTA$^-$ Tγ-CLL cells, in the expectation that the antiserum would detect a certain population of normal peripheral blood Tγ cells and PTA$^+$ Tγ-CLL cells.

MATERIALS AND METHODS

1. *Cells*

Peripheral blood mononuclear cells (PBMC) were obtained from normal donors and patients with lymphoid malignancies in leukemic phase by Ficoll-Conray density gradient centrifugation. Leukemic cells from patients with acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML) and monocytic leukemia were obtained by dextran sedimentation. Thymocytes were prepared from normal thymus from two children in undergoing partial thymectomy to facilitate cardiac surgery, and from autopsy material with no pathologic changes.

2. *Sources of materials for immunization and absorption*

Leukemic cells from two patients with T-CLL were used. Both had Fc receptors for IgG. May-Giemsa staining showed that the cells had a homogenously clumped round nucleus and abundant cytoplasm with faint basophilia. Many azurophilic granules were seen in the cytoplasm. The cells also showed small disperse reaction products of both nonspecific esterase and acid phosphatase. Ultrastructurally, however, these T-CLL cells appeared different. One contained granules showing a peculiar parallel tubular array like that reported by Mckenna et al. (1977), whereas the other did not. We named the former case " Tγ-CLL type 1 ", and the latter " Tγ-CLL type 2 ". Preliminary reports concerning the cases were published elsewhere (Machii et al., 1979; Kitani et al., 1979).

3. *Antisera*

Heteroantiserum was prepared as follows: 1×10^8 Tγ-CLL type 1 cells suspended in 5 ml of saline were injected i.v. into a rabbit on day 1, 7 and 14. One day 21 the rabbit was bled. Then 1 ml of the serum was absorbed four times with an equal packed volume of human AB-type erythrocytes, twice with 100 mg human liver powder and once with 6×10^9 CML cells. Finally the antiserum was diluted 1 : 2 with phosphate buffered saline, and 0.5 ml of the diluted antiserum was absorbed with $1.0–3.0\times10^9$ Tγ-CLL type 2 cells.

Monoclonal antibodies OKT3 (Kung et al., 1979), OKT4 (Reinherz et al., 1979), OKT8 (Kung et al., 1980) and OKM1 (Breard et al., 1980) (Ortho Pharmaceutica Co. Raritan, N. J., U.S.A.) were used by the addition of 1 ml of isotonic saline.

4. *Indirect immunofluorescence*

The reactivity of anti-Tγ-1 or monoclonal antibodies with lymphocytes was detected by the indirect immunofluorescent method. Anti-Tγ-1: 1×10^6 cells were incubated at 4 C for 30 min with 50 μl of appropriately diluted anti-Tγ-1. The cells were washed three times with Hanks' balanced salt solution (HBSS), and reincubated at 4 C for 30 min with FITC-conjugated goat anti-rabbit γ-globulin

(Behringwerke, Marburg, West Germany). Then they were washed three times, and then fluorescence was examined in a fluorescence microscope. Monoclonal antibodies: 1×10^6 cells were incubated at 4 C for 30 min with 50 μl of 1:10 diluted monoclonal antibody. Then the cells were washed three times with HBSS, and the reactive cells were stained at 4 C for 30 min with Rhodamine-conjugated goat anti-mouse IgG (Cappel Laboratories Inc., Cochranville, PA., U.S.A.).

5. *Cytotoxicity test*

Complement (C) dependent cytotoxicity was determined by the trypan blue dye exclusion test. As a souce of C, normal rabbit serum (NRS) was used. The NRS was selected to avoid killing the cells by itself. First 4×10^5 target cells in 20 μl of serially diluted anti-Tγ-1 were incubated at room temperature for 30 min, then 20 μl of C was added and the cells were incubated at 37 C for 30 min. Dead cells were counted after addition of 40 μl of 0.2% trypan blue. The percentage of cells reacting with anti-Tγ-1 was determind by the following formula:

$$\%\ \text{Reactive cells} = \frac{\text{(Percentage of dead cells with anti-T}\gamma\text{-1+C)}-\text{(Percentage of dead cells in C control)}}{100-\text{(Percentage of dead cells in Ccontrol)}}\times100$$

6. *Surface markers*

T cells were identified by assay of E rosette formation. Equal volumes of lymphocytes suspension (1×10^7/ml), fetal calf serum and sheep erythrocytes (1.5×10^9/ml) treated with neuraminidase (Behringwerke, Marburg, West Germany) were mixed. The mixture was then incubated at 37 C for 5 min and centrifuged for 5 min at $200\times g$. After incubation overnight at 4 C, E rosette forming cells (ERFC) were counted. Fc receptors for IgG were detected by use of ox erythrocytes coated with rabbit anti-ox erythrocyte IgG (EAγ) (Ferrarini et al., 1975). Surface immunoglobulins were detected by the direct immunofluorescent method. FITC-conjugated goat anti-human immunoglobulin (polyvalent) (Behringwerke, Marburg, West Germany) was used. Lymphocytes were pretreated with pH 4.0 or 37 C incubation as described by Kumagai et al. (1975).

7. *Separation of T and non-T cells*

T and non-T cells were separated by density on Ficoll-Conray gradients following the procedure for E rosette formation. The ERFC-enriched fraction was obtained from the bottom and the ERFC-depleted fraction from the interface. The ERFC-enriched fraction contained over 96% of ERFC and the ERFC-depleted fraction contained less than 4%. After cell separation, sheep erythrocytes were lysed in 0.86% NH_4Cl-Tris buffer.

8. *Double marking assay*

ERFC-enriched fractions were treated with 1:10 diluted anti-Tγ-1 and stained with FITC-conjugated goat anti-rabbit γ-globulin. Then the EAγ rosette technique was performed to detect FcγR$^+$ cells. EAγ rosette$^+$/anti-Tγ-1$^+$ cells, EAγ rosette$^+$/anti-Tγ-1$^-$ cells, EAγ rosette$^-$/anti-Tγ-1$^+$ cells and EAγ rosette$^-$/anti-Tγ-1$^-$ cells were counted. The question of whether anti-Tγ-1 inhibited EAγ rosette formation was also examined.

The relation between OKT8$^+$ cells and Tγ cells was also examined by double marking assay. ERFC-enriched fractions were treated with OKT8 and then stained with Rhodamine-conjugated goat anti-mouse IgG, and the EAγ rosette procedure was performed.

9. *Double staining assay*

First, ERFC-enriched fractions were stained with anti-Tγ-1 and FITC-conjugated goat anti-rabbit γ-globulin. Then the fractions were stained with OKT8 and Rhodamine-conjugated goat anti-mouse IgG. Cell was examined individually for reactivity with anti-Tγ-1 or OKT8 by their color of fluorescence under a fluorescence microscope.

RESULTS

1. *Absorption and titration test*

After absorption with human AB-type erythrocytes, normal human liver powder and chronic myelogenous leukemia cells, the heteroantiserum was still reacted with almost all normal and leukemic lymphocytes. Then quantitative absorption tests with Tγ-CLL type 2 cells were performed to determine the effectiveness of removing cross-reactive antibodies from this antiserum. A sample of 0.5 ml of 1:2 diluted antiserum was absorbed with 1.0, 1.5, 2.0, 2.5, and 3.0×10^9 Tγ-CLL

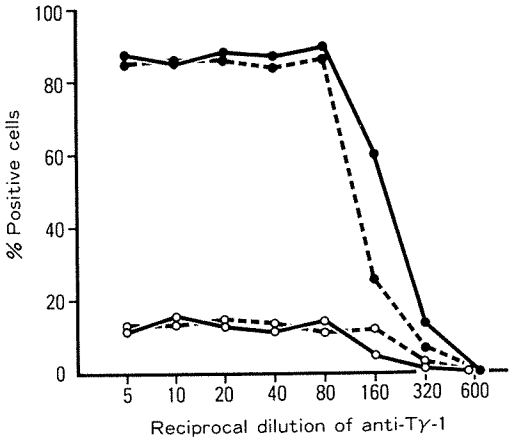

FIGURE 1. Titration test of anti-Tγ-1. Reactivity of serially diluted anti-Tγ-1 against PBMC from one normal donor (○) and Tγ-CLL type 1 patient (●) using two analytical methods: indirect immunofluorescence assay (——) and complement-dependent cytotoxicity (- - -).

type 2 cells, and was tested at a final of dilution 1:10 on PBMC from a normal donor, and Tγ-CLL type 1 and type 2 patients. When the antiserum was absorbed with 2.0×10^9 cells, no reactivity was left against Tγ-CLL type 2 cells, but 88% of the PBMC from the Tγ-CLL type 1 patient and 18% of normal were still stained. The number of absorbing cells was increased to 3.0×10^9, yet the percentage of positive cells among Tγ-CLL type 1 and normal PBMC did not diminish. In following experiments, therefore, the antiserum was used after absorption with 2.0×10^9 Tγ-CLL type 2 cells.

The reactivity of serially diluted anti-Tγ-1 was tested against PBMC from the Tγ-CLL type 1 patient and a normal donor by two analytical methods: indirect immunofluorescence assay and C dependent cytotoxicity. By both methods, the reactivity of anti-Tγ-1 reached equal plateaus at 1:5–1:80 dilution against both Tγ-CLL type 1 cells and normal PBMC (Fig. 1), suggesting that anti-Tγ-1 detected a certain spectrum of lymphocytes including Tγ-CLL type 1 cells. This cytotoxic activity of anti-Tγ-1 plus C rules out the possibility that the positively stained cells reacted non-specifically because of the attachment of antibodies to Fc receptors.

TABLE 1. *Reactivity of anti-Tγ-1 with normal PBMC*

Cell population	Percentage of anti-Tγ-1$^+$ cells
Unfractionated PBMC	14.5±3.4[a] (n=29)
ERFC-enriched fraction	19.0±6.5 (n= 9)
ERFC-depleted fraction	1.3±1.4 (n= 7)

[a] Mean±s.d.

2. *Specificity of anti-Tγ-1 for a T cell subset*

The reactivity of anti-Tγ-1 against normal PBMC was examined by indirect immunofluorescence assay (Table 1). Anti-Tγ-1 was used at 1:10 dilution. Anti-Tγ-1 reacted with 14.5% of the unfractionated PBMC, and 19.0% of the cells in the ERFC-enriched fraction. In contrast, only 1.3% of the cells in ERFC-depleted fraction reacted. This ERFC-depleted fraction contained 19–40% of the peroxidase positive monocytes and 22–38% of the surface immunoglobulin positive B cells. These results indicated that anti-Tγ-1 detected a certain subset of T lymphocytes, but not non-T lymphocytes or monocytes.

The relation between the anti-Tγ-1$^+$ cells and Tγ cells was investigated in the ERFC-enriched fraction from 7 normal donors by the double marking method. It was possible to perform double marking experiments with anti-Tγ-1 and EAγ rosette, because anti-Tγ-1 did not inhibit EAγ rosette formation (data not presented). As shown in Table 2, anti-Tγ-1$^+$ cells were all EAγ rosette positive T cells (Tγ cells), and accounted for 72% of the Tγ cells. Anti-Tγ-1 did not react with T cells not having FcγR (Tnon-γ cells). These results showed that anti-Tγ-1 was specific for a certain proportion of the Tγ cells, and also showed that Tγ cells were composed of antigenically heterogeneous subsets. Moreover, these results indicated that Tγ-CLL type 1 and Tγ-

TABLE 2. *Double marking experiments with anti-Tγ-1 and EAγ rosette in ERFC-enriched fractions*

Expt.	EREC (%)	EAγ rosette+		EAγ rosette−	
		Anti-Tγ-1+ (%)	Anti-Tγ-1− (%)	Anti-Tγ-1+ (%)	Anti-Tγ-1− (%)
1	98.0	21.0	4.5	0	74.5
2	98.0	6.0	4.0	0	90.0
3	96.0	15.5	9.5	0.5	74.5
4	97.0	17.0	8.0	0	75.0
5	98.0	18.0	6.0	0.5	75.5
6	96.0	13.0	6.0	1.0	80.0
7	97.0	28.5	9.0	0.5	62.0
Total		17.0±6.4[a]	6.7±2.0	0.4±0.4	75.9±7.7

[a] Mean±s.d.

CLL type 2 should be classified as distinct types of T-CLL.

3. *Reactivity of anti-Tγ-1 with thymocytes*

When normal thymocytes from 3 children were examined, 60–78% of the thymocytes reacted with anti-Tγ-1. An absorption test was performed to determine whether the antigen(s) detected with anti-Tγ-1 on PBMC and thymocytes were the same. When 0.5 ml of 1:2 diluted anti-Tγ-1 was absorbed with thymocytes from one child, 1×10^9 cells were required to remove 100% of the anti-Tγ-1 activity to the absorbing cells. The absorbed anti-Tγ-1 (abs. anti-Tγ-1) was then tested for reactivity with thymocytes from the two other children, PBMC from two normal donors and Tγ-CLL type 1 cells. In all cases, no reactivity of abs. anti-Tγ-1 was detected. This suggests that the antigen(s) detected with anti-Tγ-1 on thymocytes was shared by 72% of the normal peripheral blood Tγ cells.

4. *Reactivity of anti-Tγ-1 against various leukemic cells*

The reactivities of anti-Tγ-1 against leukemic cells from 64 patients with various haematopoietic malignancies were examined. Leukemic T cells were found in 21 patients.

TABLE 3. *Reactivity of anti-Tγ-1 with various haematopoietic leukemia cells*

Diagnosis	Number of cases tested	Number of anti-Tγ-1+ cases
T cell type leukemia		
T-ALL	4	0
T-CLL	4[a]	1[b]
Adult T cell leukemia	8	0
Sezary syndrome	3	0
Mycosis fungoides	1	0
T-lymphoma	1	0
Non-T cell type leukemia		
Non-T, non-B ALL	11	0
Pre-B cell leukemia	1	0
B-ALL	1	0
B-CLL	4	0
B-lymphoma	4	0
Hairy cell leukemia	4	0
AML	5	0
CML	8	0
Monocytic leukemia	5	0

[a] Two of 4 cases were Tγ-CLL type 1 and type 2, as described before, one of the other 2 was E receptor+, FcγR−, C receptor+ and Ia+, and the other had E receptor only.

[b] This cases was Tγ-CLL type 1.

Four patients had T-derived acute lymphocytic leukemia (T-ALL), 4 T-CLL, 8 adult T cell leukemia according to Uchiyama et al. (1977), 3 Sezary syndrome, 1 mycosis fungoides and 1 T-lymphoma. Of these leukemic T lymphocytes only two types of Tγ-CLL cells had FcγR. Anti-Tγ-1 did not react with any of the leukemic T lymphocytes except Tγ-CLL type 1 cells. As shown in Table 3, none of the other leukemic lymphocytes examined, or myelogenous leukemia cells or monocytic leukemia cells reacted with anti-Tγ-1.

5. *Comparison of anti-Tγ-1 and monoclonal antibodies*

Monoclonal antibodies OKT3, OKT4, OKT8 and OKM1 were examined for reactivities with Tγ-CLL type 1 cells and Tγ-CLL type 2 cells. The Tγ-CLL type 1 cells were OKT3$^+$, OKT4$^-$, OKT8$^+$ and OKM1$^-$, and the Tγ-CLL type 2 cells were OKT3$^+$, OKT4$^+$, OKT8$^-$ and OKM1$^+$. To examine whether the specificity of anti-Tγ-1 was identical to that of OKT8, double staining experiments were performed on normal peripheral blood T lymphocytes. First, cells were stained anti-Tγ-1 and FITC-conjugated goat anti-rabbit γ-globulin, and then with OKT8 and Rhodamine-conjugated goat anti-mouse IgG. The results are shown in Table 4. These results showed considerable overlap between anti-Tγ-1$^+$ T cells and OKT8$^+$ T cells. Then the relationship between OKT8$^+$ T cells and Tγ cells was examined by the double marking method with OKT8 and EAγ rosette. Most, but not all, OKT8$^+$ T cells did not form EAγ rosette, and the percentage of EAγ rosette forming cells was greatly reduced. This suggests that OKT8 inhibited EAγ rosette formation. To confirm the effectiveness of OKT8 on EAγ rosette formation, EAγ rosette inhibition tests were done on ERFC enriched fractions by adding serially diluted OKT8. At the same time titration tests of OKT8 were performed by the indirect immunofluorescence assay. As shown in Fig. 2, the percentage inhibition of EAγ rosette formation reached about 90%. It is also interesting that positive indirect immunofluorescence staining required a higher concentration of OKT8 than EAγ rosette inhibition. These comparisons of anti-Tγ-1 and OKT8 suggest that the specificity of anti-Tγ-1 should be different from that of OKT8.

TABLE 4. *Double staining experiments with anti-Tγ-1 and OKY8 in ERFC-enriched fractions*

	Expt. 1 (%)	Expt. 2 (%)	Expt. 3 (%)
Anti-Tγ-1$^+$, OKT8$^+$	23	20	18
Anti-Tγ-1$^+$, OKT8$^-$	2	2	1
Anti-Tγ-1$^-$, OKT8$^+$	4	3	4
Anti-Tγ-1$^-$, OKT8$^-$	71	75	77

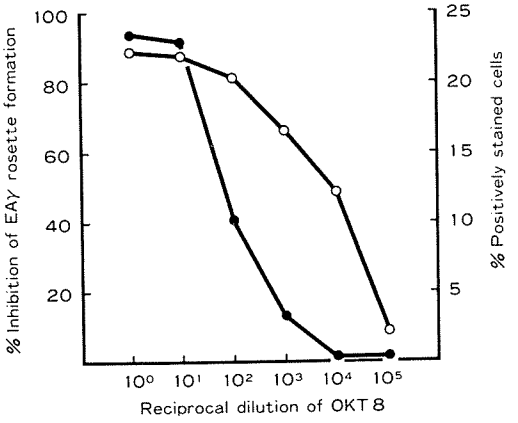

FIGURE 2. Effect of OKT8 on EAγ rosette formation, and titration of OKT8 by indirect immunofluorescence assay. An EAγ rosette inhibition test was done on the ERFC-enriched fraction by adding serially diluted OKT8 (○). At the same time, the percentage of OKT8$^+$ T cells was counted by the indirect immunofluorescence method (●). Similar results were obtained in two replicate experiments.

DISCUSSION

We prepared a heteroantiserum in a rabbit us-

ing Tγ-CLL type 1 cells and Tγ-CLL type 2 cells for immunization and absorption, respectively, expecting that the antiserum would detect a certain spectrum of Tγ cells. This expection was based on the following assumption: 1) T-CLL cells are likely to represent a clonal expansion of a certain subset of mature T lymphocytes possessing a specific antigen(s); 2) since Tγ cells have been shown to be heterogeneous with respect to their functions (Moretta et al. 1977; Shaw et al. 1979), there may be two or more distinct types of T-CLLs (Tγ-CLLs) each derived from a different subsets of Tγ cells. These leukemic cells should possess their own antigenic markers.

Quantitative absorption experiments with Tγ-CLL type 2 cells showed that the reactivity of the antiserum with Tγ-CLL type 2 cells was lost by its absorption with 2.0×10^9 cells. In contrast, its reactivity with normal PBMC and Tγ-CLL type 1 cells remained constant on absorption with 2.0×10^9 or more cells. These results indicate: 1) Tγ-CLL type 1 cells possess a different antigenic marker(s) from Tγ-CLL type 2 cells. 2) This antigen(s) is shared by Tγ-CLL type 1 cells and a certain proportion of normal PBMC.

Adequately absorbed antiserum (anti-Tγ-1) reacted with 19% of the normal peripheral blood T lymphocytes, but not with non-T lymphocytes or monocytes. Double marking experiments with anti-Tγ-1 and EAγ rosette in the ERFC-enriched fraction revealed that only 72% of the T cells were anti-Tγ-1$^+$ T lymphocytes. A study of various haematopoietic leukemia cells showed that the antigen(s) defined by this antiserum was expressed on Tγ-CLL type 1 cells, but not on various other types of leukemic cells. These results showed that anti-Tγ-1 was specific for a certain spectrum of normal and leukemic Tγ cells. Catovsky and his colleagues observed that anti-Tγ-1 was reactive with cells in their 2 cases of PTA$^+$ Tγ-CLLs (personal communication). Kanayama et al. demonstrated that 63.1–75.0% of normal peripheral Tγ cells contained PTAs (in preparation). These findings suggest that anti-Tγ-1 would detect a PTA$^+$ subset of Tγ cells.

Recently, monoclonal antibodies specific for subsets of T cells have been produced. We examined the reactivities of Tγ-CLL type 1 cells and Tγ-CLL type 2 cells with OKT3, OKT4, OKT8 and OKM1 monoclonal antibodies. Tγ-CLL type 1 cells were OKT3$^+$, OKT4$^-$, OKT8$^+$ and OKM1$^-$, and Tγ-CLL type 2 cells were OKT3$^+$, OKT4$^+$, OKT8$^-$ and OKM1$^+$. This reactivity of OKT8 was similar to that of anti-Tγ-1. In addition, OKT8$^+$ cells and anti-Tγ-1$^+$ cells were largely overlapped in normal donors. On the other hand, OKT8 inhibited FcγR binding, but anti-Tγ-1 did not. These comparative studies of anti-Tγ-1 and OKT8 suggest that the antigenic determinant(s) defined by anti-Tγ-1 should be different from the determinant defined by OKT8, though it is not clear whether the determinants are on the same molecule or not. Catovsky et al. found one case of T-prolymphocytic leukemia in which the phenotype was OKT3$^+$, OKT4$^+$, OKT8$^+$ and anti-Tγ-1$^-$ (personal communication). This difference in specificity between anti-Tγ-1 and OKT8 suggests that anti-Tγ-1 is useful for classify lymphoid malignancies.

By utilizing series of monoclonal antibodies, Reinherz et al. (1980a) reported that most Tγ cells did not react with OKT3, OKT4 or OKT5, which are thought to define all peripheral T cells. They also found that 50–90% of Tγ cells reacted with OKM1 monoclonal antibody (Breard et al. 1980) that defines an antigen shared by monocytes and granulocytes. They suggested, therefore, that Tγ cells might be of monocyte-myeloid lineage rather than T lineage. However, a different conclusion should be drawn from our study. Anti-Tγ-1 reacted with 72% of normal peripheral Tγ cells, but not with T non-γ cells, non T cells or monocytes. Anti-Tγ-1 also reacted with 60–78% of thymocytes. An absorption test with thymocytes showed that the antigen(s) detected by this antiserum on the thymocytes was shared by

72% of the peripheral blood Tγ cells. In addition, OKT8$^+$ cells and anti-Tγ-1$^+$ cells largely overlapped. These results indicated that Tγ cells defined by anti-Tγ-1 were of T lineage. Furthermore, the phenotype of Tγ-CLL type 2 cells showed the existence of OKM1$^+$ T cells, and suggested the possibility that Tγ cells might include a PTA$^+$, anti-Tγ-1$^+$, OKT4$^-$, OKT8$^+$, OKM1$^-$ subset and PTA$^-$, anti-Tγ-1$^-$, OKT4$^+$, OKT8$^-$, OKM1$^+$ subset.

The correlation between this anti-Tγ-1$^+$ subset and its function is an interesting subject. We have already found that the immunizing cells have antibody dependent cell-mediated cytotoxicity activity, and a functional study on this is under way.

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