RAPID COMMUNICATION Jpn. J. Cancer Res. 85, 1189–1192, December 1994

High Frequency of Cancer Patients with Abnormal Assembly of the T Cell Receptor-CD3 Complex in Peripheral Blood T Lymphocytes

Yoshio Gunji,[1] Seiji Hori,[1] Tomohiko Aoe,[2] Takehide Asano,[1] Takenori Ochiai,[1] Kaichi Isono[1] and Takashi Saito[2,3]

[1]*Department of Surgery (II) and* [2]*Division of Molecular Genetics, Center for Biomedical Science, Chiba University School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260*

Structural abnormality of T cell receptor (TCR)-CD3 complex on the cell surface was investigated in peripheral blood lymphocytes (PBL) from 55 cancer patients. In 24 of the 68 tests done on these patients, the CD3ζ chain was not detected by immunoprecipitation with anti-CD3ε monoclonal antibody (mAb), but was observed with anti-CD3ζ mAb, suggesting that a high frequency of cancer patients possesses abnormal T cell receptor (TCR) complex in PBL. On the other hand, the total ζ chain was missing in several advanced cases. During follow-up of several patients, the ζ chain became undetectable after two or three months of cancer progression. It appears that immunosuppressive status can be monitored by analyzing the TCR-CD3 complex on the cell surface of PBL.

Key words: T cell receptor — CD3ζ chain — Abnormal assembly — Immunosuppression

Tumors escape from host immune surveillance through various mechanisms, such as changing tumor epitopes and secretion of suppressive cytokines. T lymphocytes are responsible for recognition of tumor antigens and are mediators of antitumor immunity. Recently, it has been demonstrated that the TCR[4] complex and the associated machinery for signal transduction are impaired in tumor-bearing mice,[1] suggesting that the immune suppression under tumor-bearing conditions may be at least partially attributed to impairment of the signaling machinery in T cells. The TCR-CD3 complex is composed of 8 chains, divided into three groups: TCR $\alpha\beta$, CD3 $\gamma\delta\varepsilon$ and ζ dimer. The assembly of all the chains is required to be expressed on the cell surface in normal T cells. The lack of the ζ chain results in impairment of the surface expression of TCR-CD3 complex.[2,3] Therefore, the ζ chain is important for the assembly and cell surface expression of the TCR-CD3 complex as well as for signal transduction. The ζ, η and FcRγ chains compose the ζ family and play important roles in signal transduction in TCR and Fc receptors. T cells utilizing FcRγ instead of ζ were discovered in epithelia,[4] and may have distinct functions.[5] Tumor-bearing mice demonstrated the loss of expression of the ζ chain and replacement by the FcRγ chain, and impaired expression of ζ has recently been reported in TIL of cancer patients.[6,7] We have investigated the structure of the TCR-CD3 complex on the cell surface of peripheral T cells, rather than TIL, in patients with carcinomas of various tissue origins.

PBL from cancer patients and healthy donors were isolated by Ficoll-density centrifugation. The cells were surface-labeled by biotinylation and lysed in a lysis buffer containing 10 m*M* Tris (pH 7.6), 150 m*M* NaCl, 4 m*M* EDTA, 0.05% NaN_3, 1 m*M* phenylmethylsulfonyl fluoride, 0.15 U/ml aprotinin, 5 μg/ml leupeptin, 10 m*M* iodoacetamide and 1% digitonin as previously described.[2] The cell lysates were immunoprecipitated with 5 μg of mAb against CD3ε (OKT3), CD3ζ (H146-466) or FcRγ (4D8, kindly provided by Dr. J. Kochan, Hoffmann-La Roche Inc., Nutley, NJ). The precipitates (equivalent to $2–4\times10^7$ PBL) were analyzed on a nonreducing (13%)-reducing (15%) two-dimensional SDS-PAGE gel and transferred onto a PVDF membrane, which was then incubated with avidin-peroxidase and developed by using the ECL system (Amersham).

We have analyzed 68 specimens from 55 cancer patients. Most of the patients belonged to stage IV of the TNM classification (II, 7; III, 10; IV, 51) (Table I). The PBL from the patients were surface-biotinylated and lysed with 1% digitonin-containing buffer. Cell lysates of PBL were immunoprecipitated with anti-CD3ε mAb OKT3. Representative results are shown in Fig. 1. Twenty-four specimens (35%) showed loss of the CD3ζ chain in the TCR complex and another 21 (31%) showed reduced expression compared with normal controls (Table II). In all cases, the expressions of other CD3 chains (γ, δ and ε), as well as TCR $\alpha\beta$ chains, were not altered. In accord with the precipitation results, the

[3] To whom requests for reprints should be addressed.

[4] The abbreviations used are: TCR, T cell receptor; mAb, monoclonal antibody; PBL, peripheral blood lymphocytes; FcRγ, the γ chain of high-affinity Fc receptor for IgE; TIL, tumor-infiltrating lymphocytes.

expression level of TCR-CD3 complex on the cell surface as evaluated by surface staining with anti-CD3ε mAb was not changed even in the cases where ζ was not detected (data not shown). The percentages of T cells expressing CD4, CD8 and CD16 were not significantly changed in these patients (data not shown). The patients were divided into four groups according to the configuration of the TCR-CD3 complex on the cell surface of PBL (Fig. 1). The TCR complex in group 1 (Gr. I) was normal, as in healthy controls. The patients in Gr. II, III and IV exhibited loss of the cell surface ζ chain, when analyzed with OKT3. They were further divided on the basis of immunoprecipitation results with anti-ζ and anti-FcRγ mAb. In Gr. II, the ζ chain was not detected by anti-CD3ε but was detected with anti-ζ mAb. Since such dissociation between anti-CD3 and anti-ζ precipitation was not observed in healthy controls, this is not the result of an experimental artifact (e.g., presence of detergent). In Gr. III and IV, neither anti-CD3ε nor anti-ζ mAb could detect the ζ chain. The FcRγ dimer was co-precipitated with the TCR-CD3 complex only in Gr. IV but not in Gr. III (Fig. 1). This was confirmed by precipitation with anti-FcRγ mAb (data not shown), indicating that FcRγ replaced ζ in the TCR complex in PBL of Gr. IV patients, as in a murine cancer model.[1] Since we screened patients by immunoprecipitation with OKT3, and analysis with anti-ζ and anti-FcRγ mAb was performed only for selected patients, the exact frequency of each group could not be determined. However, most of the cases belonged to Gr. II, several to Gr. III, and only a single case to Gr. IV.

Table I. Correlation between Disappearance of the ζ Chain and Cancer Stage[a]

Expression of ζ chain	No. of tests	Cancer stage (TNM classification)			
		II	III	IV	rIV[b]
Normal	24	5[c]	4	14	1
Reduced expression	21	2	3	10	6
Disappeared	23	0	3	10	10
Total of number	68	7	10	34	17

a) Tests were performed as described in the footnote to Table II.
b) r: recurrence with distant metastasis after removal of primary tumor.
c) The numbers are the numbers of specimens that showed normal or reduced levels or the absence of the cell surface CD3ζ chain when immunoprecipitated with anti-CD3ε mAb.

Our findings suggest that the abnormal association between the ζ dimer and other CD3 chains takes place at the first stage of immunosuppression in cancer patients (Gr. II), and that the disappearance of ζ from the cell surface may then follow (Gr. III). Since ζ was not replaced with FcRγ in most of the cases, it remains to be determined whether the exchange of ζ by FcRγ (Gr. IV) represents a step in the progression of the cancer stage or only occurs in some special cases. No clear correlation was found between the loss of the ζ chain and the tissue specificity of cancer in these patients in this screening study (Table II). However, the disappearance of ζ seems to correlate with the stage of cancer patients (Table I). Although there were no difference between stage III and IV, a low percentage in stage II and a high frequency in stage IV patients with recurrent tumor were observed. Furthermore, in 5 patients who showed normal or reduced levels of the ζ chain expression at the first test, the chain became undetectable in 4 at the second test after an interval of more than 2 months. One of these cases is depicted in Fig. 2 (patient T.O). The patient received

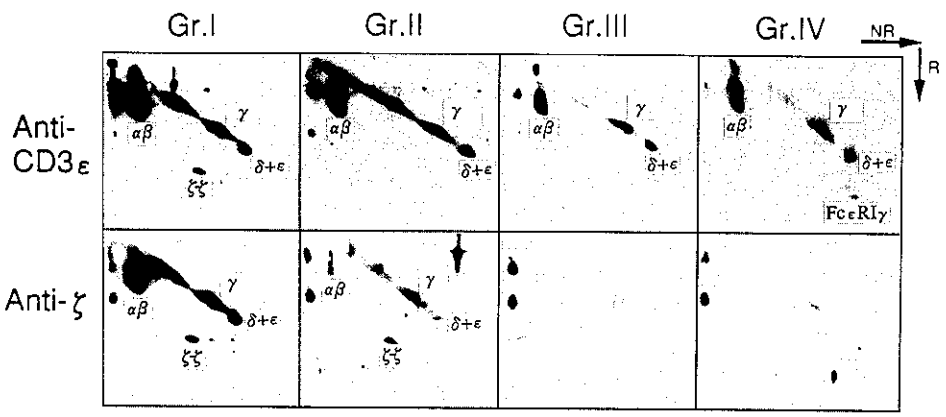

Fig. 1. Structure of the TCR-CD3 complex on the cell surface of peripheral blood T cells in four different groups of cancer patients. Cell lysates of PBL were precipitated with anti-CD3ε or anti-ζ mAb and analyzed on 13% nonreducing (NR)-15% reducing (R) two-dimensional SDS-PAGE. Gr: group.

Table II. Frequency of Cancer Patients with Disappearance of the ζ Chain in PBL[a)]

Origin of cancer[b)]	No. of tests (No. of patients)	No. of tests[c)]		
		Normal	Reduced ζ	Loss of ζ
Colon and rectum	24 (19)	8	7	9
Esophagus	16 (11)	4	5	7
Liver (hepatocellular)	9 (8)	4	3	2
Stomach	7 (6)	4	1	2
Pancreas	3 (3)	1	2	0
Extrahepatic bile ducts	3 (2)	1	1	1
Liver (cholangio)	2 (2)	0	1	1
Gall bladder	1 (1)	0	1	0
Lung	1 (1)	0	0	1
Breast	1 (1)	1	0	0
Ovary	1 (1)	0	0	1
Total	68 (55)	23	21	24

a) Lysates of PBL of cancer patients were immunoprecipitated with anti-CD3ε mAb and analyzed by diagonal SDS-PAGE.
b) The tissue containing the primary tumor is indicated.
c) The numbers are the numbers of specimens that showed normal or reduced levels or the absence of the cell surface CD3ζ chain when immunoprecipitated with anti-CD3ε mAb.

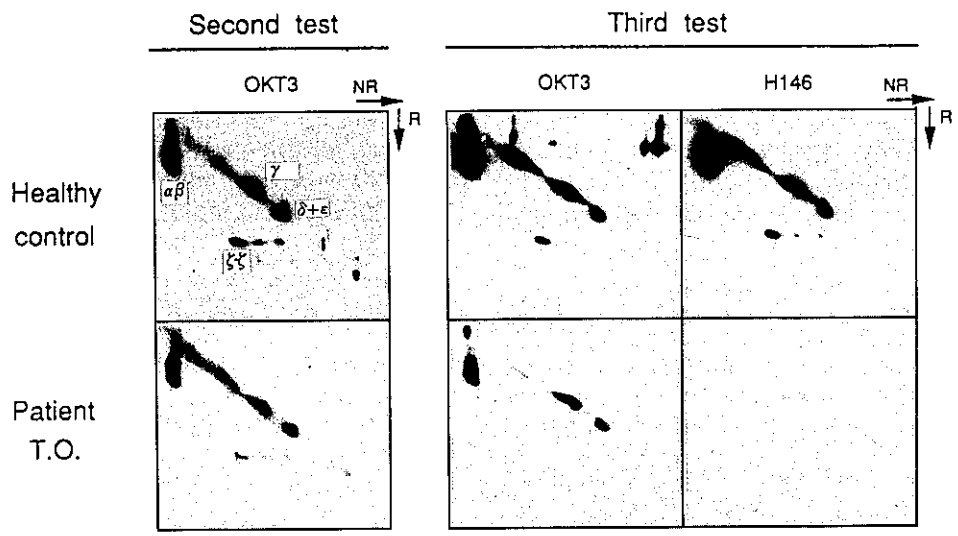

Fig. 2. Correlation between disappearance of the CD3ζ chain and progression of tumor stage. Analysis was performed as described in the legend to Fig. 1 with an interval of three months between the first and the second tests on the same patient, T.O. Similar results were obtained with four other cancer patients.

abdominoperitoneal rectal amputation due to rectal cancer 9 months before the first test, which was performed just after lymph node metastasis in the hepatoduodenal ligament was found. While the expression of the TCR-CD3 complex was normal in the first test, the level of ζ was reduced in the second test which was performed one month later. The third test was done a further two months later when the patient had developed multiple lung metastases and lymphangitis carcinomatosa. There was no ζ detectable by immunoprecipitation with either anti-CD3ε or anti-ζ mAb, indicating that the status of the patient had changed from Gr. I to Gr. III. A similar result was obtained in another test after an additional one month. On the other hand, there was one case in which the ζ chain reappeared at a significant level, at 3 months after being undetectable (data not shown). Since this patient had received radiation and chemotherapy during the intervening period and his condition had improved, the reappearance might be related to recovery from the immuno-suppressed condition. These results

suggest that the disappearance of the ζ chain from the cell surface TCR-CD3 complex may be related to the progression of cancer stage, and may be reversible.

Recently, the loss of the ζ chain in TIL in cancer patients was reported.[6,7] In those cases, the loss of the ζ chain was clearly observed in TIL but was hardly observed in PBL. However, those analyses, because the total ζ was analyzed either by Western blotting[6] or by staining after permeabilization of the cells,[7] may have failed to detect structural difference of the TCR-CD3 complex on the cell surface of PBL. In contrast, our analysis demonstrated that the majority of the patients who showed lack of ζ as detected with anti-CD3ε mAb exhibited abnormal assembly of the TCR-CD3 complex (Gr. III), and a relatively small number of patients exhibited total loss of ζ in peripheral blood T cells (Gr. III and IV). It is clear that ζ was replaced by FcRγ in only very rare cases. Successive analyses on several patients demonstrated that the total ζ chain disappears with tumor progression. Taking this finding together with the results on TIL, it seems likely that the disappearance of the CD3ζ chain starts to take place in TIL and occurs later in PBL, and that before the total ζ disappears in PBL, there is a stage in which the TCR complex has impaired assembly. Although the dissociation of the assembly within the TCR-CD3 complex has been reported in an immature thymocyte population,[8,9] the molecular basis of the dissociation of CD3ε- and CD3ζ-immunoprecipitation and also of the mechanism of the normal cell-surface expression of the TCR-CD3 complex in the absence of any detectable ζ family molecules in the present study are not yet clear. It has been shown that T cells bearing TCR-CD3 complex in the absence of ζ failed to respond to specific antigens in a variant T cell line.[2] In accordance with this, T cells lacking ζ from tumor-bearing mice exhibited impaired function, especially in cytotoxicity against specific targets (T. Aoe and T. Saito, manuscript in preparation).

Our results have practical implications because we analyzed PBL instead of TIL. Our finding that the initial change in the TCR complex in the tumor-bearing condition is an alteration of the assembly of the TCR-CD3 complex should allow monitoring of the immunosuppressive status of cancer patients by the structural analysis of the TCR-CD3 complex of PBL. This is based on the hypothesis that the structural disorder of the TCR complex takes place in conjunction with tumor progression. Molecular analysis of the mechanism of the disappearance of ζ may lead to the development of promising strategies for the prevention of T cell immunosuppression in cancer patients.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We thank Drs. R. Kubo, D. Presky and J. Kochan for providing antibodies, Ms. C. Ogata for technical assistance and Ms. H. Yamaguchi for assistance in preparing the manuscript.

(Received July 12, 1994/Accepted September 19, 1994)

REFERENCES

1) Mizoguchi, H., O'Shea, J. J., Longo, D. N., Loeffler, C. M., McVicar, D. W. and Ochoa, A. C. Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science*, **258**, 1795–1798 (1992).
2) Sussman, J. J., Bonifacino, J. S., Lippencott-Schwartz, J., Weissman, A. M., Saito, T., Klausner, R. D. and Ashwell, J. D. Failure to synthesize the T cell CD3-ζ chain: structure and function of a partial T cell receptor complex. *Cell*, **52**, 85–95 (1988).
3) Ohno, H., Aoe, T., Taki, S., Kitamura, D., Ishida, Y., Rajewsky, K. and Saito, T. Developmental and functional impairment of T cells in mice lacking CD3ζ chains. *EMBO J.*, **12**, 4357–4366 (1993).
4) Ohno, H., Ono, S., Hirayama, N., Shimada, S. and Saito, T. Preferential usage of the Fc receptor γ in the T cell antigen receptor complex by γ/δ T cell localized in epithelia. *J. Exp. Med.*, **179**, 365–369 (1994).
5) Ohno, H., Aoe, T., Ra, C., Yamamoto, T. and Saito, T. TCR isoform containing the Fc receptor γ chain exhibits structural and functional differences from isoform containing CD3ζ. *Int. Immunol.*, **5**, 1403–1411 (1993).
6) Finke, J. H., Zea, A. H., Stanley, J., Longo, D. L., Mizoguchi, H., Tubbs, R. R., Wiltrout, R. H., O'Shea, J. J., Kudoh, S., Klein, E., Bukowski, R. M. and Ochoa, A. C. Loss of T-cell receptor ζ chain and p56^{lck} in T-cell infiltrating human renal cell carcinoma. *Cancer Res.*, **53**, 5613–5616 (1993).
7) Nakagomi, H., Petersson, M., Magnusson, I., Juhlin, C., Matsuda, M., Mellstedt, H., Taupin, J. L., Vivier, E., Anderson, P. and Kiessling, R. Decreased expression of the signal-transducing ζ chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. *Cancer Res.*, **53**, 5610–5612 (1993).
8) Finkel, T. H., Cambier, J. C., Kubo, R. T., Born, W. K., Marrack, P. and Kappler, J. W. The thymus has two functionally distinct populations of immature α/β^+ T cells: one population is deleted by ligation of α/β TCR. *Cell*, **58**, 1047–1054 (1989).
9) Kuwabara, I., Ohno, H., Punt, J. A., Hashimoto, Y. and Saito, T. Transition from TCRβ dimer- to TCR$\alpha\beta$-expressing cells by introduction of an α chain in an immature thymocyte cell line. *J. Immunol.*, **52**, 2148–2156 (1994).