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Alteration of signal-transducing molecules in tumor-infiltrating lymphocytes and peripheral blood T lymphocytes from human colorectal carcinoma patients

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Abstract Tumor development or growth is accompanied by impaired immune responses, such as a poor proliferative response or down-regulated cytolytic T lymphocyte activity. Although recent reports have suggested that modification of the signal-transducing molecule is responsible for impaired immune responses in tumor-bearing hosts, the causes of defective immune function are not yet completely understood. Furthermore, the clinical significance of the findings is not yet clear. In this study, we investigated the alteration of several signal-transducing molecules in peripheral blood T lymphocytes (T-PBL) as well as in tumor-infiltrating lymphocytes (TIL) from human colorectal carcinoma patients and their relationship with the impaired host immune responses. A greater reduction in CD3ζ chain level was observed in TIL than in T-PBL from tumor-bearing hosts. CD3ζ chain reduction in T-PBL correlated with the clinicopathological stage of a tumor, especially with the status of lymph node metastasis. The levels of p56*lck* and p59*fyn* protein tyrosine kinase in T-PBL were also compared between tumor-bearing hosts and normal healthy volunteers. In T-PBL from tumor-bearing hosts, expression of protein tyrosine kinase p59*fyn* was significantly lower than that of p56*lck*. However, the level of CD3ζ chain expression did not correlate with T lymphocyte functions such as T lymphocyte proliferative response or allogeneic target cell lysis.

Key words TCR-CD3 · TIL · Colorectal cancer · Protein tyrosine kinase (PTK) · Immune suppression

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Introduction

Malignant tumors express specific antigens which may be recognized as foreign substances by the host immune system. However, the host immune surveillance frequently fails to block the outgrowth of some tumors, resulting in development of the advanced tumor state. Although down-regulated immune responsiveness has been demonstrated in cancer patients with a variety of tumors [6, 13, 17, 18, 21, 38], it is not clear whether this defective immune function leads to the development of the tumor or if these alterations are a consequence of tumor growth. The immune response against tumors is primarily cell-mediated and circulating antibodies tend to play a minor role [25]. Among the components of cell-mediated immunity, the effector cells are, in some cases, $CD8^+$ cytolytic T lymphocytes (CTL) while, in other cases, $CD4^+$ T helper cells behave as major effectors by amplifying CTL responses and acting in consort with macrophages and other accessory cells [7, 11, 14].

The six different protein chains (α, β, γ, δ, ε, ζ) of the T cell receptor (TCR) complex are required for both efficient transport of assembled TCR complexes to the cell surface and signal transduction [4]. The cytoplasmic parts of the various CD3 chains, which contain a recurrent motif of approximately 20 amino acids, are responsible for connecting the antigen-recognition segments of TCR to intracellular signaling pathways [20]. This motif is triplicated in the CD3ζ chain and is present as a single copy in the CD3α, δ and ε chains. Following antigen recognition, the two

tyrosines found in each motif are phosphorylated and, in this form, they are likely to function as the docking site for paired SH2 domains present in some lymphocyte-specific protein tyrosine kinases (PTK) involved in signal transduction [8]. In this respect, CD4, CD8 [35, 36] and NK cells' FcγRIII [28, 29, 35, 37] are tightly associated with PTK $p56^{lck}$. While the TCR-CD3 complex and FcγRII are associated with $p59^{fyn}$ [31, 33], the FcγRI receptor is associated with $p56^{lyn}$ or $p62^{yes}$ [10]. Specifically, $p56^{lck}$-deficient mice do not have significant anti-viral effector functions against two different viruses [24], and a $p56^{lck}$-deficient CTL line displays a profound reduction in TCR-dependent cytolytic effector functions [16]. However, the effect of $p59^{fyn}$ on the killing capability of effector cells has not yet been studied. Alterations of the TCR signal transduction molecules in human patients with colon carcinoma [21], renal cell carcinoma [12], ovarian carcinoma [17], and melanoma [38] have recently been reported. According to these reports, changes in the CD3ζ chain in tumor-bearing subjects may play a significant role in the induction of an immunodeficient host response. However, the possibility that a reduction in levels of other chains such as γ, δ, or ε may also contribute to tumor development cannot be excluded.

Since some signal-transduction pathways in T lymphocytes might be deleted or changed in tumor-bearing subjects, it is essential that an antitumor strategy finds an efficient way of normalizing immunodeficient effector cells. In fact, the level of mRNA in the altered $p56^{lck}$ and CD3ζ chain was not reduced [23]; the molecules involved in signal transduction may thus be post-transcriptionally influenced and reversibly react in vivo against suppressive or activating factors. There have been several reports describing the alteration of signal-transducing molecules in T lymphocytes from tumor-bearing hosts [12, 17, 21, 26, 38]. Matsuda et al. [21] reported a correlation in colorectal carcinoma patients with the stage of the disease. This report was, however, based on a relatively low number of patient's samples and did not present data based on the relationship between the alteration of signal-transducing molecules in T lymphocytes and functionally defective T lymphocytes. Some authors [17, 22, 38] have reported a correlation between altered signal-transducing molecules and reduced cellular functions such as proliferation and cytokine production. By contrast, our experimental results show no significant correlation between reduction of the CD3ζ chain and functionally defective T cells in patients, indicating the involvement in colorectal carcinoma patients of molecules other than the CD3ζ chain in eliciting impaired T cell function.

A better understanding of alterations occurring in T lymphocytes from tumor-bearing hosts will help develop a therapeutic strategy for the impaired immune system of advanced cancer patients.

Materials and methods

Preparation of cells

All chemicals and reagents used in this study were purchased from the Sigma Chemical Co. (St. Louis, Mo., USA), unless otherwise specified. Tumor-infiltrating lymphocytes (TIL) were isolated from surgical specimens of 44 colorectal carcinoma patients who had been operated on at Korea Cancer Center Hospital. The patients were categorized as follows: Astler-Coller stage B, 20; stage C, 13; and stage D, 11. Single-cell suspensions from tumors were prepared by triple enzymatic digestion as previously described [15]. Tumor tissues were minced and explanted into six-well tissue-culture plates (Nunc, Roskilde, Denmark) with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco), pen/strep (Gibco), 20 μg/ml DNase, 200 U/ml collagenase, and 10 μg/ml hyaluronidase. The following day, mononuclear cells were harvested from the interface of the Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient (100%/80%) of the "bulk" tumor-derived cells. Peripheral blood samples from patients were obtained just before surgery and peripheral blood mononuclear cells (PBMC) were isolated by the Ficoll-Hypaque density gradient method. Monocyte-depleted mononuclear cells were obtained after overnight culture at 37 °C in a 5% CO_2 incubator. In an attempt to purify T lymphocytes negatively from PBMC (T-PBL), cells were applied to a Dynabead cell-separation system using microbeads (Dynabead, Dynal) coupled with anti-CD16, anti-CD19 and anti-CD14 monoclonal anitbodies. The percentage of $CD3^+$ T lymphocytes from the T-PBL of patients (CD4, 72.1 ± 11.3%; CD8, 27.6 ± 11.4%) and of normal volunteers (CD4, 66.7 ± 5.9%; CD8, 33.3 ± 5.2%) was above 96%, whereas the range for TIL was 53%–75%. The ratios of $CD4^+$:$CD8^+$ T cells ($CD3^+$) among the T-PBL of patients (n = 44) was normal. Furthermore, there were no significant differences of $CD4^+$:$CD8^+$ T cell ratios between the T-PBL of patients and those of normal volunteers (n = 10). Since it was impossible to purify enough T lymphocytes from TIL, the whole mononuclear cell fraction harvested was used directly as a source of TIL.

Western blot analysis

TIL and T-PBL preparations were immediately lysed in lysis buffer (0.1% NP-40 in 100 mM TRIS buffer, pH 8.0, supplemented with 40 mM EDTA, 200 mM sodium orthovanadate, 20 μg/ml each of leupeptin and aprotinin, and 100 mM sodium fluoride) for 15 min on ice. Cell lysates were centrifuged at 12 000 rpm for 10 min and the postnuclear supernatant was mixed with an equal volume of 2×sodium dodecyl sulfate (SDS) sample buffer. Samples (equivalent to 1×10^6 cells) were resolved on 15% SDS/polyacrylamide gel electrophoresis and transferred to Immobilon polyvinylidene difluoride membrane (Millipore, Bedford, Mass.). The membranes were incubated with anti-CD3 antibody (Leu4, Becton-Dickinson), anti-TCRζ antibody (Coulter Zeta), anti-$p56^{lck}$ (UBI, Lake Placid, N.Y.) and anti-$p59^{fyn}$ (UBI). After washing, the membranes were incubated with appropriate secondary antibodies conjugated with horseradish peroxidase, and then developed using a chemiluminescence kit: ECL (Amersham). In other experiments, alkaline-phosphatase-labeled secondary antibodies were used.

Assays for proliferative activity

Anti-CD3 (OKT3) mAb or control mAb (5 μg/ml) were cross-linked to the surface of 96-well flat microtiter plates by incubation at 37 °C in 0.1 M sodium bicarbonate buffer (pH 9.6). After incubation for 3 h, the plates were washed three times with 0.2% Tween-20 in phosphate-buffered saline followed by incubation for 72 h with purified T-PBL expressing the CD3ζ chain (1×10^5 cells/well). Cultures were pulsed with 0.5 μCi [^{3}H]thymidine (2 mCi/mmol; New England Nuclear Research Products, Boston, Mass.) 8 h before harvesting onto glass-fiber filters (Whatman Ltd., Maidstone, UK). Results are expressed as mean radioactivity (cpm) incorporation of triplicates.

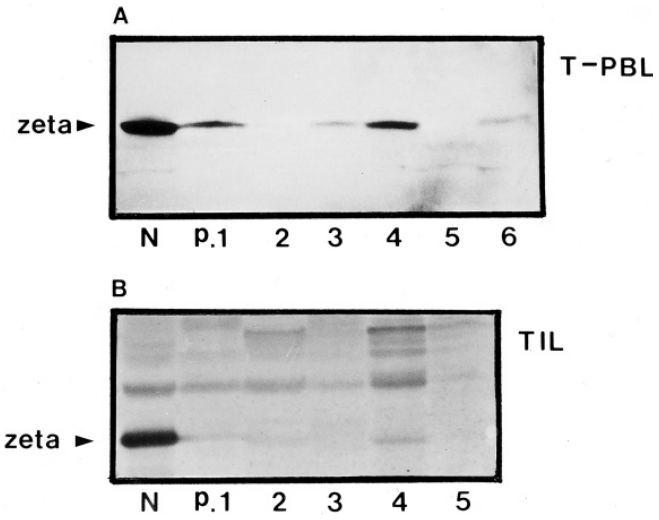

Fig. 1 Immunoblot analysis of CD3ζ chain expression in peripheral blood T lymphocytes (*T-PBL*) from a healthy donor (normal control) and in T-PBL and tumor-ilfiltrating lymphocytes (*TIL*) from colorectal carcinoma patients. A total of 20 μg cell lysate (equivalent to about 2×10^6 cells) from each sample was resolved on 15% sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) gel, transferred to Immobilon P, and immunoblotted with anti-CD3ζ (Coulter Zeta). 5-Bromo-u-chloro-3-indolyl phosphate (indolyl)/nitroblue tetrazolium was used for final color reaction. *N* normal control, *P* patient. A patient's sample number is indicated by Arabic numerals

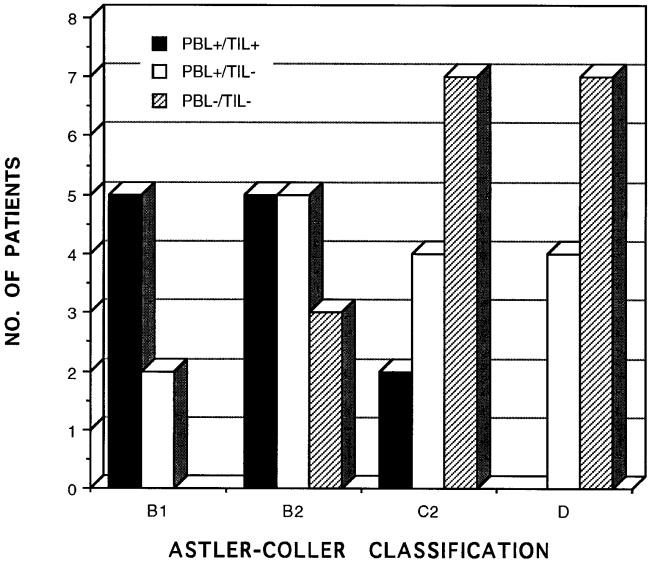

Fig. 2 The relationship between clinicopathological stage and CD3ζ chain expression in TIL and T-PBL of colorectal carcinoma patients. + presence of ζ chain; – complete loss of ζ chain. *PBL⁺/TIL⁺* 12/44 (27%), *PBL⁺/TIL⁻* 15/44 (34%), *PBL⁻/TIL⁻* 17/44 (39%). *B1* extending into muscularis propria but not penetrating through it (uninvolved nodes), *B2* penetrating through muscularis propria (uninvolved nodes), *C2* penetrating through muscularis propria (involved nodes), *D* distant metastatic spread

Generation of CTL and assays for cytotoxic activity

Nonspecific cytotoxic T cells were generated by culturing T-PBL with concanavalin A (5 μg/ml) alone or anti-CD3 antibody plus 2000 U/ml human recombinant IL-2 (rhIL-2) (kindly donated by Dr. K.S. Ham of the Genetic Engineering Research Institute, Daejeon, Korea) for 96 h [27]. Target cells from the colorectal cancer cell line HT29 (ATCC), donated by the Cell Line Bank at the Cancer Research Center, Seoul National University, Seoul, Korea, were labeled with 0.1 mCi $Na^{51}CrO_4$ (NEN) at 37 °C for 1 h, washed three times with Hank's balanced salt solution and adjusted to 2×10^4 cells/ml in 10% FBS/RPMI medium. Various numbers of effector cells in 0.1 ml 10% FBS/RPMI medium were added to a 0.1-ml target cell suspension containing 2×10^3 target cells in each well of 96-well round-bottom microtiter plates (Costar, Cambridge, Mass.) and to different E/T ratios in triplicate. Spontaneous-release wells contained target cells in culture medium only; maximum-release wells contained target cells in 5% SDS. Following incubation for 5 h at 37 °C in a 5% CO_2 incubator, 0.1 ml supernatant was harvested and radioactivity was determined in a γ-counter (LKB 1272). The percentage specific cytotoxicity was determined by the following formula: [experimental mean release (cpm)–spontaneous mean release (cpm)/maximal mean release (cpm)–spontaneous mean release (cpm)]×100 = cytotoxicity (%).

Results

T lymphocytes from a tumor-bearing host show the impaired CD3ζ chain expression

In order to investigate further the relationship between reduced expression of CD3ζ chain and down-regulated immune responsiveness in tumor-bearing hosts, the level of CD3ζ chain expression in T-PBL from several colorectal carcinoma patients was measured and compared with that of T-PBL from normal healthy volunteers. Of 44 patients tested, CD3ζ chain expression was barely detected in T-PBL from 17 patients (39%). Furthermore, in 10 patients (23%) the level of CD3ζ chain expression in T-PBL was significantly lower than that of a normal volunteer control (Fig. 1A for examples). If the reduction of CD3ζ chain expression in T-PBL from tumor-bearing hosts is mediated by certain factors and/or tumor mass itself, impaired CD3ζ chain expression might be more prominent in TIL than in T-PBL. The TIL from five samples (Figs. 1B) failed to express any detectable CD3ζ chain. In total, the TIL from 32 of 44 patients (73%; data not shown except for five samples) appeared not to express the CD3ζ chain. This result is consistent with previously reported findings [12, 17, 21, 26, 37]; the TIL from the remaining 12 patients (27%), however, appeared to be moderately or strongly CD3ζ chain-positive (data not shown).

Thirty samples of T-PBL and 30 of TIL, taken from 44 patients, were also immunoblotted and reacted with Leu4 antibody to determine whether the CD3δ chain was present. All of those with T-PBL samples from normal healthy volunteers (n = 7) showed a similar intensity of CD3δ chain expression regardless of the presence or absence of the CD3δ chain in the same samples (data not shown). In this study, T cells were not purified from TIL but, even so, the CD3δ chain was seen at normal levels in the 30 TIL samples (data not shown). The decrease of CD3ζ levels in TIL was not an in vitro artifact caused by enzymatic

degradation, since no difference in CD3ζ expression was observed between enzyme-treated and non-treated normal PBL (data not shown). Since the pattern of reduced CD3ζ chain expression in T-PBL and TIL from tumor-bearing hosts was not identical among the patients investigated, the clinicopathological stages of the patients were reviewed to determine whether there was any relationship between the reduction of CD3ζ chain expression and the stages of cancer development. In the early stages (B1 and B2) of tumor development, we observed a moderate decrease in CD3ζ chains; in patients at the later tumor progression stage (C2 and D), we found a severe reduction in total CD3ζ chain content of T-PBL and TIL (Fig. 2).

p59*fyn* was more closely related than p56*lck* to the defective signal transduction occurring in T lymphocytes from tumor-bearing hosts

Since CD3ζ chain expression was significantly lower both in T-PBL and TIL, the expression of immediately downstream signal-transducing molecules, such as p56*lck* and p59*fyn*, was monitored to clarify the mechanisms involved in tumor-mediated down-regulation of immune responses occurring in tumor-bearing hosts. In Fig. 3, we used the same samples from the same patients shown in Fig. 1. In T-PBL from 25 of 27 patients (93%), p59*fyn* expression was significantly reduced (Fig. 3A, data not shown, except for seven samples). The degree of reduction in p59*fyn* expression is likely to correlate with the level of CD3ζ chain expression (Fig. 1A, 3A) but, to confirm this result, further studies should be performed. In contrast, only p56*lck* expression in T-PBL from 12 of 27 patients (44%) was reduced (Fig. 3B). In addition, normal expression of p56*lck* was observed even in T-PBL with significant loss of CD3ζ chain expression (Fig. 1A, 3B).

CD3ζ itself is not solely responsible for T lymphocyte function

Significant reduction of CD3ζ chain and p59*fyn* expression in T-PBL and TIL from tumor-bearing hosts suggests that modification of the signal-transducing pathway mediated by these molecules could be responsible for the lack of immune responsiveness in tumor-bearing hosts. To test this possibility, the existence of a correlation between the level of CD3ζ chain expression and T lymphocyte function was investigated. After anti-CD3 antibody treatment, patients' T-PBL with and without CD3ζ chain expression was compared for proliferative activity of T lymphocytes. As shown in Fig. 4, even CD3ζ- chain-expressing T-PBL from cancer patients (n = 11) failed to proliferate efficiently after CD3 molecules were cross-linked with anti-CD3 antibody. However, T-PBL from healthy volunteers (n = 3) and other cancer patients (n = 4) with normal CD3ζ chain expression proliferated efficiently in response to anti-CD3 antibody treatment (data not shown). To confirm this phenomenon in cytotoxic T lymphocytes, allogeneic target cell lysis by

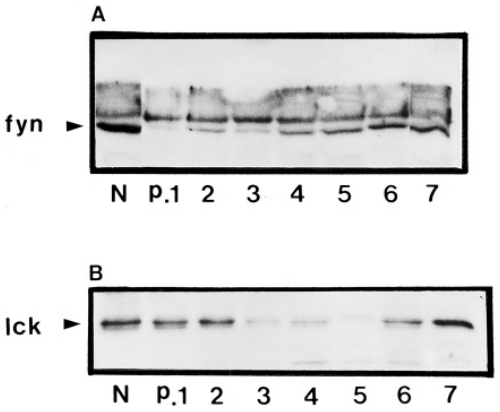

Fig. 3A, B Immunoblot analysis of p56*lck* and p59*fyn* expression in T-PBL from a healthy donor (normal control) and from colorectal carcinoma patients. A total of 10 μg cell lysate (equivalent to about 1×10^6 cells) from each negatively purified T-PBL sample was resolved on 10% SDS-PAGE gel, transferred to Immobilon P, and immunoblotted with anti-p59*fyn* (**A**) and anti-p56*lck* (**B**) (UBI). Blots were developed with peroxidase-conjugated GαRIgG by chemiluminescence (ECL)

T-PBL was measured after treatment with anti-CD3 antibody and recombinant human interleukin-2 (rhIL-2). As shown in Fig. 5, the T-PBL expressing CD3ζ chain from cancer patients failed to induce cytolytic activity against allogeneic target cell line HT29. Two different target cell lines, SNU C2A and SNU C4, were also tested for cytotoxicity, and the results were similar (data not shown). Concanavalin A, however, a T lymphocyte mitogen, restored the cytotoxicity of those T-PBL (Fig. 6). The experiment involved the activation of non-specific killer cells by culturing PBL in concanavalin A.

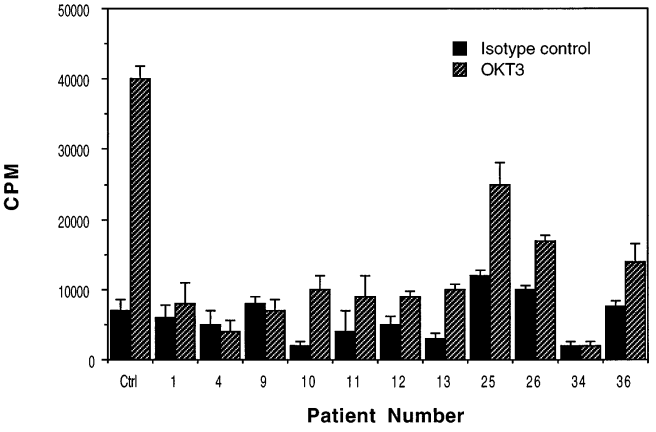

Fig. 4 Proliferation of T lymphocytes from healthy donors and patients by immobilized anti-CD3 mAb (OKT3). T cells from a healthy donor and patients (n = 11) were negatively purified by Dynabeads coupled with anti-CD16, anti-CD19 and anti-CD14 monoclonal antibodies. T cells strongly expressing the CD3ζ chain were selectively chosen. Wells of a 96-well flat-bottom plate were coated with OKT3 or control mAb (5 μg/ml) at 37 °C for 3 h. T-PBL (1×10^5/250 μl) were plated into each well. Thirty-six hours after incubation, the cells were pulsed with [^{3}H]thymidine for 8 h before harvesting. Error bars represent SD from the mean of quadruplicate wells

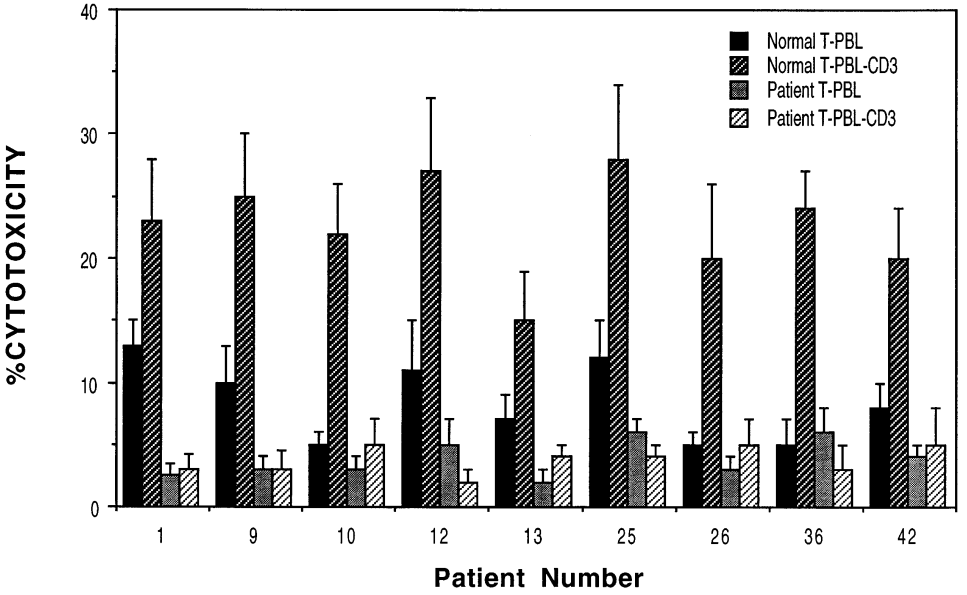

Fig. 5 Anti-CD3-mAb-induced cytotoxicity against a colon cancer cell line by normal or patients' T-PBL activated with immobilized anti-CD3 plus recombinant human interleukin-2 (rhIL-2). Isolated CD3ζ-positive T-PBL samples (n = 9) were cultured with immobilized OKT3 plus rhIL-2 for 96 h. After culture, cell cytotoxicity against an allogeneic tumor target cell line (HT29) was measured by ^{51}Cr-release assay in the presence or absence of OKT3 and rhIL-2. Error bars represent SD from the mean of triplicate wells

Discussion

In the present study, PBL and primary tumor tissues of 44 colorectal cancer patients were used to investigate the possible modification of signal-transducing molecules present in T lymphocytes. The level of CD3ζ chain expression in T-PBL from tumor-bearing hosts was significantly lower than that from normal healthy volunteers (Fig. 1A). In addition, reduced CD3ζ chain expression was more prominent in TIL than in T-PBL (Fig. 1B). The complex mediating antigen-specific signaling is a very dynamic ensemble consisting of the TCR/CD3 receptor complex, various accessory molecules such as CD4 or CD8, and tyrosine kinases and phosphatases, which determine the outcome of stimulation (i.e., changes in cell morphology, gene transcription or proliferation). T cells express two major *src*-family tyrosine kinases, p59^{fyn} and p56^{lck}, which are associated with the TCR/CD3 complex or CD4 and CD8 accessory molecules respectively [9, 32]. Finke et al. [12] and Mizoguchi et al. [23] have recently reported reduced expression of both PTK in T-PBL from tumor-bearing mice and a loss of p56^{lck} in CD3ζ chain-deficient T-TIL from human renal cell carcinoma. Our results, however, were somewhat different. The expression of p59^{fyn}, which mediates signal transduction from the TCR-CD3 complex, was reduced in T lymphocyte from tumor-bearing hosts, but the expression of p56^{lck} showed no such reduction. This suggests that p59^{fyn} rather than p56^{lck} PTK may be involved in transducing signals through the CD3 complex. In addition, the consistent reduction of p59^{fyn} expression implies that a down-regulated immune response in tumor-bearing hosts is based upon alterations in the signal-transducing pathway,

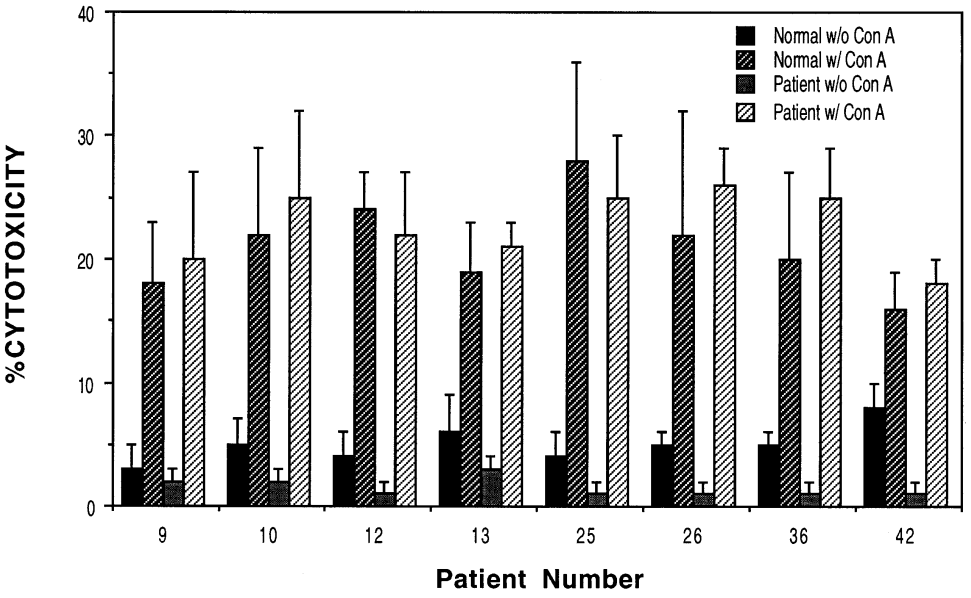

Fig. 6 Concanavalin-A-induced cytotoxicity against a colon cancer cell line by normal or patients' T-PBL. CD3-positive T-PBL samples (n = 8) were activated with concanavalin A (5 μg/ml) for 96 h. After washing off excess concanavalin A (*ConA*), the non-specific cytotoxicity of effector cells against an allogeneic tumor cell line (HT29) was measured by ^{51}Cr-release assay in the presence or absence of concanavalin A. Error bars represent SD from the mean of triplicate wells

which might be mediated by factor(s) secreted from the tumor mass, through the CD3ζ chain.

Since the pattern of reduced CD3ζ chain expression was not identical in T-PBL and TIL from different patients, we reviewed the clinicopathological stages of the patients to determine whether the extent to which CD3ζ chain expression is reduced depends on the stage of cancer development. We observed a correlation and inferred that tumor cell growth causes CD3ζ chain reduction. Our results strongly suggest, therefore, that CD3ζ chain expression in T lymphocytes of colorectal cancer patients is negatively influenced by tumors and that this effect might be mediated by factor(s) released from the tumor mass. In fact, it has previously been suggested that soluble factors in the serum of tumor-bearing mice are target molecules that influence CD3ζ reduction in T cells [23]. Moreover, Aoe et al. [2] recently reported that activated macrophages in tumor-bearing mice could induce alteration in TCR molecular structures by direct contact or through the mediation of short-acting substances. Our results concerning CD3ζ reduction in T-PBL, as well as in TIL from colorectal carcinoma patients in advanced stages, suggest that it would be worthwhile investigating whether a fraction of mononuclear cells behaving as suppressors in PBL can be found in such patients.

Although it has been reported that TIL from some human tumors display a poorer proliferative response than T-PBL [1, 8, 17], the functional consequences of reduced CD3ζ chain expression, and the role of this in the impaired proliferation and cytolytic activity of these cells are not clear. Considering the result of the T lymphocyte proliferative response and allogeneic target cell lysis after treatment with anti-CD3 antibody, It is unlikely, however, that T lymphocyte function is closely associated with the level of CD3ζ chain expression (Figs. 4, 5). In fact, Tartour et al. [34] reported a lack of proliferation in TIL from cancer patients with normal CD3ζ chain expression, and their findings to some extent support our observation. Our results suggest that there could be other critical factors that mediate the down-regulation of T lymphocyte function in tumor-bearing hosts.

As an example, decreased immune response in tumor-bearing hosts may be mediated through an abnormal costimulatory pathway involving CD28. In our preliminary data, the percentage of $CD28^+$ cells among $CD8^+$ T lymphocytes from colon carcinoma patients ($50.1 \pm 15.7\%$) was significantly lower than in normal donors ($79.9 \pm 1.7\%$) (unpublished data). Although the above finding could not be the main cause of immune dysregulation, we cannot exclude the possibility that the decreased number of $CD8^+CD28^+$ cells could prevent tumor-bearing hosts from generating effective CTL clones against the tumor. In addition, the increased number of $CD28^-$ cells among the $CD8^+$ cell population can act as immune suppressors [3, 19]. Salvador et al. [30], on the other hand, suggested that IL-2 could prevent immune suppression in tumor-bearing mice by maintaining normal signal transduction, as well as by maintaining the expression of CD3ζ in T cells. Exogenous IL-2 may strengthen the signal-transduction pathway through IL-2 receptors β and γ [5], and finally overcome the impaired TCR complex structure in T cells from tumor-bearing hosts. We investigated whether there are differences in protein tyrosine phosphorylation in T-PBL between tumor-bearing hosts and normal healthy volunteers, and an apparent discrepancy of phosphotyrosine protein band patterns was detected (unpublished data). This suggests that an alteration of other signal-transducing molecules, independent of chain variation, may contribute to the functional defect of T lymphocytes in tumor-bearing hosts. To understand the mechanisms involved in tumor-mediated down-regulation of the immune response in tumor-bearing hosts, further characterization of those molecules is necessary.

In summary, our data demonstrate that the altered signal-transducing pathways lead to impaired immune function in a tumor-bearing host. A better understanding of alterations in T lymphocytes from tumor-bearing hosts will help to develop a strategy for repairing defects in the immune system, and to monitor the course of therapy in advanced cancer patients.

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