Decreased Expression of the Signal-transducing ζ Chains in Tumor-infiltrating T-Cells and NK Cells of Patients with Colorectal Carcinoma

Hiroshi Nakagomi, Max Petersson, Inger Magnusson, Claes Juhlin, Masanori Matsuda, Håkan Mellstedt, Jean-Luc Taupin, Eric Vivier, Paul Anderson, and Rolf Kiessling

Microbiology and Tumor Biology Center, Laboratory of Immunology, Karolinska Institute [H. N., M. P., M. M., R. K.]; Department of Surgery, Södersjukhuset [L. M., H. M.]; Radiumhemmet, Karolinska sjukhuset [L. M., H. J.]; and Dana Farber Cancer Institute, Boston, Massachusetts 02115 [J-L. T., E. V., P. A.]

Abstract

An impaired immune response is frequently observed in cancer patients and tumor-bearing mice. T-cells from mice with an experimental colon carcinoma were recently shown to express T-cell receptors that completely lacked the signal-transducing molecule CD3 ζ. Here, we have investigated the expression of the signal-transducing molecule ζ on lymphocytes from 14 patients with colorectal carcinomas using flow cytometric analysis of permeabilized cells with a monoclonal antibody (TIA-2; IgG1) specific for the cytoplasmic domain of the ζ chain as well as with immunoprecipitation and analysis on diagonal gel electrophoresis. We demonstrate that T-cells isolated from the tumors of the patients express significantly less CD3 ζ than T-cells in the peripheral blood of the same patients and that the peripheral blood of the patients express decreased levels of ζ chains, as compared to the levels found in lymphocytes from healthy controls. This decreased expression was also observed on ζ chains associated with the low affinity Fc receptor for IgG found in tumor-infiltrating NK cells (FcγRIIIα; CD16).

Introduction

A multitude of different mechanisms have been suggested to account for the poor immune response of lymphocytes from cancer patients, including an active inhibition by suppressor cells, decreased production of cytokines, or the presence of tumor of cytokines with known suppressive effects on T-cell functions (1). TILs from human or experimental tumors often have impaired effector functions which could be normalised upon culture in recombinant IL-2 (2). Mizoguchi et al. (3) recently reported alterations in the signal transduction molecule CD3 ζ associated with TCR in mice bearing an experimental colon carcinoma. Since alterations in the CD3 ζ chain may disrupt the internal signaling of the TCR, leading to a deficient T-cell activation, this suggests a fundamentally new way of explaining immune defects in tumor-bearing hosts. We have investigated the expression of CD3 ζ in T-cells and of CD16 ζ in NK cells isolated from the tumors of patients with human colorectal carcinoma as well as from the peripheral blood of the same group of patients. Our results demonstrate for the first time reduced expression of ζ chains in lymphocytes from human cancer patients.

Materials and Methods

Tumor samples were obtained from 14 patients during surgery for their primary colorectal carcinoma (three of Dukes stage A; six of B; four of C; and one of D). Single cell suspensions of the tumors were prepared by enzymatic digestion (4). This "bulk" preparation of tumor-derived cells contained 33 ± 27% (Mean ± SD; n = 14) of CD3+ cells, and the remaining cells were mainly composed of tumor cells. Peripheral venous blood was taken from the patients during or shortly after the surgery, and PBMC were isolated by Ficoll density centrifugation. The expression of CD3 ζ and CD16 ζ was investigated by flow cytometric analysis of permeabilized cells using a monoclonal antibody (TIA-2; IgG1) specific for the cytoplasmic domain of the ζ chain (5) and followed by staining with a rabbit-anti-mouse-fluorescein isothiocyanate antibody as described (6). Cells were double stained with phycoerythrin (FL2)-conjugated mAbs to CD3, CD16, CD4, or CD8 (Dako, Glostrup, Denmark) and then subjected to flow cytometric analysis using a FACSscan flow cytometer (Becton Dickinson). The mean FL1 of PBMC and TIL samples from cancer patients was expressed as the percentage of the average mean FL1 value of PBMC from healthy donors (n = 9). Statistics were calculated as probability for Student’s t test (different variance cases). For immunoprecipitations, lymphocytes were permeabilized with digitonin and subjected to radio-iodination with 125I by the lactoperoxidase method and immunoprecipitated as described (6). The immunoprecipitated samples were analysed with diagonal gel electrophoresis (6).

Results and Discussion

The expression of CD3 ζ was investigated with flow cytometric analysis comparing T-cells from TIL and PBMC of patients with T-cells from PBMC of healthy controls (Fig. 1A; n = 9). A markedly reduced CD3 ζ expression was detected in TIL cells of all investigated patients (Fig. 1C; n = 14; 23 ± 11% (SD) of control PBMC; P < 0.001). The T-cells in the tumors expressed, on the average, one-half as many CD3 ζ chains as those in PBMC from the same group of patients (P < 0.01). Also, the peripheral T-cells from the patients showed decreased expression of ζ compared to T-cells from healthy controls (Fig. 1B; n = 12; 47 ± 22%; P < 0.001). The decrease was selective for the ζ chain, as expression of the CD3 ε determinant was unaffected in the TIL population as well as in the PBMC from this group of patients (data not shown). There was considerable variation among different patients, ranging from only a moderate decrease (patients 3 and 9) to an almost absent expression (patients 5, 10, and 13) of CD3 ζ. Also, PBMC from healthy donors had varying expression of CD3 ζ (Fig. 1A).

Arguing against the possibility that the low CD3 ζ expression in the TIL cells may be an artifact due to the procedure involved in isolating TIL was the observation that the same enzymatic treatment did not significantly affect CD3 ζ expression of control PBMC (n = 5; 92 ± 13% of nontreated PBMC). The CD4+ as compared to the CD8+ TIL cell subset demonstrated the same degree of decreased CD3 ζ expression (data not shown).

Since the low affinity Fc receptor for IgG on human NK cells (FcγRIIIα; CD16) also is expressed in association with a ζ:ε heterodimer and a ζ:γ heterodimer (5), the expression of CD16 ζ was also investigated in NK cells from TIL and PBMC in this patient group. Compared to the expression of CD16 ζ in NK cells from PBMC of
Fig. 1. The expression of CD3 \( \zeta \) or CD16 \( \zeta \) as analyzed by FACScan. The mean FL1 values obtained with PBMC and TIL from cancer patients (Panels B, C, E, and F) were expressed as the percentage of the average mean FL1 value obtained with PBMC from healthy donors (Panels A and D). The expression of CD3 \( \zeta \) (Panels A, B, and C) as well as CD16 \( \zeta \) (Panels D, E, and F) is shown.

healthy controls (Fig. 1D), a significantly decreased expression of CD16 \( \zeta \) was found in the TIL as well as in the PBMC derived from the patients (Fig. 1E, \( n = 8, 24 \pm 13\% \), \( P < 0.001 \) for TIL; Fig. 1E, \( n = 12, 39 \pm 17\% \), \( P < 0.001 \) for PBMC; and TIL:PBMC, \( P < 0.05 \)) in the same range as that seen for CD3 \( \zeta \). Thus, decreased expression of the \( \zeta \) molecule was apparent in the T-cell as well as the NK cell compartment of these patients. Biochemical confirmation of the low CD3 \( \zeta \) expression in the TIL was done in a representative patient with colorectal carcinoma by separating anti-CD3e immunoprecipitates on nonreducing/reducing diagonal gels as shown in Fig. 2. The TCR complex from PBMC of a control individual included the \( \zeta: \zeta \) homodimer (Fig. 2A), while the TCR complex from TIL cells (Fig. 2B) as well as PBMC (data not shown) of a patient with colorectal carcinoma did not. Mizoguchi et al. (3) found the two \( \zeta \) chains of the CD3 complex in tumor-bearing mice to be replaced by two structurally related proteins from the Fc receptor for IgE expressed on mast cells and basophils (FceR1). Although the \( M_r \) 8,000–10,000 off-diagonal spot corresponding to the \( \gamma: \gamma \) homodimer is poorly resolved in the gel shown in Fig. 2, one-dimensional electrophoretic analysis indicated that both CD16 and CD3:TCR were associated with \( \gamma: \gamma \) homodimers from FceR1 in TIL isolated from patients with colorectal carcinoma (data not shown).

It was not clear whether the reduced expression of \( \zeta \) chains was causally related to the malignant disease or was dependent upon some factor(s) unrelated to the tumor in this group of patients. Colorectal carcinoma is a malignancy of advanced age. No correlation was, however, observed between the CD3 \( \zeta \) expression and the age of the patients (linear regression; \( r^2 = 0.0927; n = 12 \)). The strong reduction of CD3 \( \zeta \) observed in TIL is particularly interesting in relation to the comparatively moderate decrease of this molecule on peripheral lymphocytes from the same group of patients. This may be interpreted as an effect of a locally produced factor affecting, although to a lesser degree, peripheral cells as well. Alternatively, lymphocytes located in the gut may generally express lesser amounts of this molecule, and the low expression in TIL may be unrelated to a tumor-host interaction. In support of this interpretation, Malissen et al. (7), studying the T-cell...
LOW EXPRESSION OF SIGNAL-TRANSDUCING ζ CHAINS IN TIL CELLS

Fig. 2. Diagonal gel electrophoresis of anti-CD3 ζ immunoprecipitates of PBMC from a healthy control (A) and TIL from a patient with colorectal carcinoma (B). The molecular components of the TCR complex are indicated.

development in mice lacking the CD3 ζ gene, found that gut intraepithelial lymphocytes present in these mice expressed normal levels of TCR complexes which were associated with γ-γ homodimers from FceR1. The possibility that the human TIL population studied by us corresponds to the murine thymus-independent gut intraepithelial lymphocytes in their report should therefore be considered. If so, however, the lower expression of ζ in PBMC reported here and by Mizoguchi et al. (3) must still be a phenomenon associated with cancer and distinct from the suppression observed locally in the tumor. The functional consequences of reduced ζ expression on TIL and what role this has in the deficient proliferation and cytotoxicity of freshly isolated TIL (2) is not clear. Preliminary results indicate that reduced CD3 ζ expression is not unique to malignant diseases and that this phenomenon occurs in peripheral blood as well as locally in some autoimmune diseases, indicating that this may reflect a dysregulation of immune function common to various conditions characterized by immune deficiency.

References


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