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Alterations in Signal Transducing Molecules on T-cells in Cancer Patients.

—Decreased CD 3 ζ Chain on T Cells in Cancer Patients—

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Recent evidence suggests that T cells in cancer patients exhibit abnormalities in signal transduction that related to unresponsiveness to appropriate activation signals. Here we review the evidence that the expression of CD 3 ζ molecules were reduced in tumor-bearing host, and discuss the mechanism behind these alteration.

key word: CD 3 ζ, T cell, cancer

Introduction

Cancer is a disease in which frank systemic immunosuppression, which typically occurs only in the advanced stages, causes considerable problems manifested by opportunistic infections and possibly also progression of the malignant disease. The possibility of finding subtle alterations in the immune system of cancer patients before these are clinically manifest is increasing as methods of measuring immune functions are getting more refined and quantitative. These methods are shedding light upon mechanisms underlying escape from immune surveillance of tumors. The mystery behind the frequent observation of tumor growth in the face of seemingly normal systemic immune response is slowly being elucidated.

Recently there has been a shift in the focus of the research interest from systemic to local events during tumor establishment and subsequent growth. Tumor-infiltrating lymphocyte (TIL) are consistently devoid of cellular effector function unless cultured *in vitro* in the presence of abnormally high concentration of cytokines such as interleukin-2 (IL-2)[1]. The lethargic activity of freshly isolated TIL has been ascribed to suppressor lymphocytes or macrophages[2,3,4] or to the secretion of suppressor factors by tumor cells[5].

We will here focus on recent efforts directly pertaining to understanding the mechanisms responsible for decreased immune responses in cancer patients. This review will be the recently described alterations in signal transducing molecules in patient T cells and Natural Killer (NK) cells.

Alterations of signal transducing molecules on lymphocytes from cancer host.

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An exciting field, with the potential of explaining the mechanism behind many of the described defects in TIL and peripheral blood lymphocyte (PBL) functions is now rapidly developing. The T-cell receptor (TCR) complex is composed of heterodimeric antigen receptor α and β chains together with a set of invariant subunits that constitute CD 3 (Figure-1). Signal transducing molecules linked with TCR/CD 3 complex include the ζ chain, which is defined primary as a disulfide-linked homodimer of 16-KD each[6]and might contribute to the assembly of the CD 3 complex[7]. The cytoplasmic domain of the CD 3 ζ subunit is involved in signal transduction which is related to activation of T cells[8]. The low affinity Fcγ for IgG found on human NK cells has also been found associated with ζ-ζ homodimers and ζ-γ heterodimers[9], which might induce NK cell-mediated antibody dependent cellular cytotoxicity[10].

Mizoguchi et al.[11] were first to establish a correlation between alterations in intracellular signal transducing molecules and suppressed immune responses in mice bearing an experimental colon cancer. Only long term tumor bearing mice, for periods exceeding 26 days, had CD 8+cells with decreased cytotoxic activity, TNF-α and Granzyme B expression[11]. These T cells had altered TCR/CD 3 complexes, in which the ζ chain was replaced by FcεRI-γ. Reduced levels of the src family protein tyrosine kinases (PTK), p 56^{lck} p 59^{fyn}, were also found[11]. Others have not found similar changes in T cells from the majority of mice carrying various types of chemically induced sarcomas[12], arguing that the dissociation of ζ chains from the TCR/CD 3 complex is not the only mechanism underlying poor anti-tumor cytolytic responses.

Several studies, however, support the notion that alterations in signal transducing molecules in T cells and NK cells is a common phenomenon in a variety of human tumor types (Table-1).

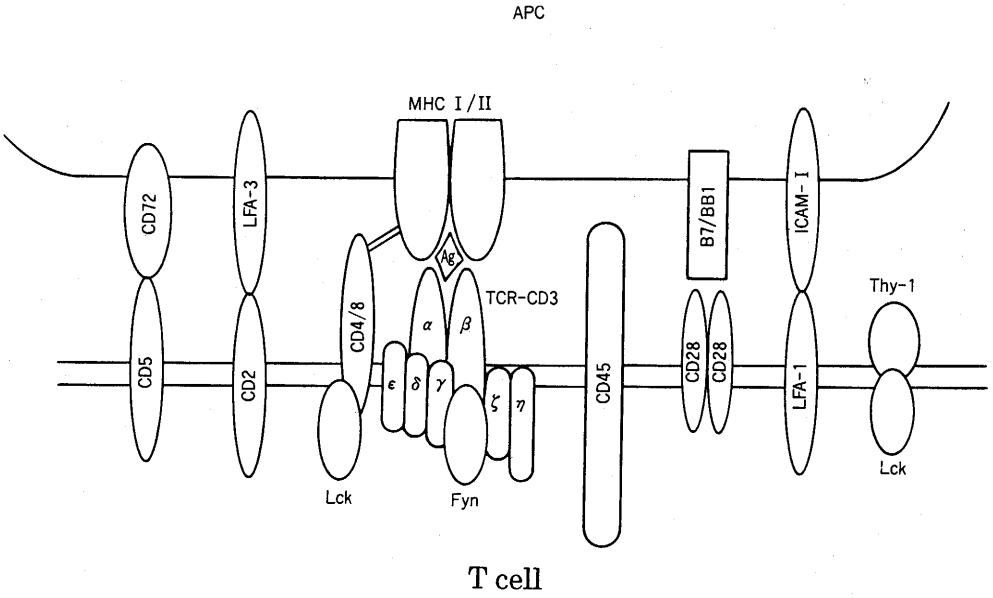

Figure-1 *Schematic model of structure of T cell receptor/CD 3 complex and interaction between T cell and target cell.*
T cell receptor/CD 3 complex is composed of heterodimeric antigen receptor α and β chains together with a set of invariant subunits (ε, δ, γ, ζ, η) that constitute CD 3. And CD 3 ζ molecules is related to protein tyrosine kinase, p 59 fyn, which is involved in the signaling into nuclei.

Table 1 T cell signaling defects in cancer

Human renal cell carcinoma[14]	Decreased level of ζ chains and $p56^{lck}$ in TILs
Human colorectal carcinoma[13]	Decreased level of ζ chains in TILs and NK cells
Human melanoma[18]	Decreased level of ζ chains and $p56^{lck}$ in PBL
Human ovarian cancer[15]	Decreased level of ζ chains in TALs
Human cervical cancer[16]	Decreased level of ζ chains in PBL
Human renal cell carcinoma	Abnormalities of the NF-κ B family in TILs and PBLs
Murine colon and renal cell carcinoma[11]	Decreased level of ζ chian, $p56^{lck}$ and $p59^{fyn}$ in splenic T cells
Murine fibrosarcoma	Decreased level of ζ chian, $p56^{lck}$ and $p59^{fyn}$ in splenic T cells

TIL, tumor-infiltrating lymphocytes TAL, tumor-associated lymphocytes PBL, peripheral blood lymphocytes
NK, natural killer cells

Reduced CD 3 ζ chain levels on TIL and PBL of patients with solid tumors.

Fresh T cells derived from TIL in colorectal cancer contained significantly fewer ζ chains per cell than patient PBL, which also had less ζ than normal PBL[13]. In addition, this pattern was simultaneously reported for renal cell carcinoma (RCC) infiltrating T cells[14]. These findings have subsequently been confirmed and extended also to a variety of other human tumors, including ovarian cancer[15], cervical cancer[16], and carcinoma of the esophagus, liver, stomach[17] and melanomas[18]. Several different techniques have been used to demonstrate decreases in ζ chain levels, including flow cytometric analysis of permeabilised cells using a mAb against intracellular domains of the ζ chain[13,16], immune precipitation of the TCR/CD 3 complex followed by 2 D gel identification of individual CD 3 components[11,13] or SDS-gel electrophoresis of surface labelled whole cell lysate followed by Western blot identification of individual components[17] or ELISA[18].

CD 3 ζ levels might also be prognostic indicators. Zea et al.[18] recently demonstrated that the overall survival rate of melanoma patients with low TCR ζ levels was significantly lower than that of patients with normal TCR ζ levels. TCR ζ deficient patients also tended to have faster growing tumors.

Mechanisms behind alterations in signal transducing molecules.

When discussing the mechanism behind the alterations in signal transducing molecules, it should be stressed that we do not think that this phenomenon is unique to malignant diseases. Preliminary findings indicate that T lymphocytes from leprosy patients present alterations in nuclear transcription factors and in CD 3 ζ chain expression[19]. Perhaps analogous to the tumor situation, chronic inflammatory processes might involve the same mechanism.

Literature reports in mouse tumor models in which ζ chain decreases are less common[12] may be explained by the fact that the experimental tumors grow rapidly *in vivo*, while human neoplasm typically develop over a long period of time. Again, Mizoguchi et al.[11] did not detect alterations in signal transduction until 26 days of the tumor growth. Therefore, tumor growth in human, chronic stimuli associated with microbial infections, or autoimmune conditions are conductive for alterations in signal transduction associated with immunosuppression.

One attractive possibility to explain alterations in the expression and functions of signal proteins is that this phenomenon reflects an anergic state in T and NK cells. Anergic Th 1 cells contain reduced levels of p 56^{lck} and p 59^{fyn}[20], altered phosphorylation of ζ and lack Zap 70[21]. Taupin and Anderson[22] have shown that T cell activation by phorbol ester or Con A can induce proteolysis of ζ cytoplasmic domains, and perhaps activation through the TCR results in ζ ubiquitination, suggesting a likely negative feedback mechanism. Repeated stimulation with anti-CD 3 markedly decrease expression of CD 3 ζ without affecting cell surface TCR/CD 3 expression[23]. Thus, chronic overstimulation within tumors by unknown stimuli is another interesting possibility.

Macrophages with the capacity to suppress immune responses in tumor-bearing host have been extensively described[3,4]. The possibility that suppressor macrophages induce alterations in signal transducing molecules has recently emerged from studies in murine and human systems. Splenic macrophages from tumor-bearing mice, or normal macrophages activated with zymosan A+LPS but not residential macrophages were found to induce the loss of CD 3 ζ as well as other structural components of the CD 3 complex[24]. This phenomenon was apparently contact dependent, but the proteins involved were not defined. These results have now been confirmed and extended by us in melanoma patients, and we have proposed that the responsible mechanism involves secretion of H_2O_2 [25]. Thus, macrophages isolated from metastatic lymph nodes of patients with malignant melanoma down-modulated CD 3 ζ levels on autologous peripheral blood T cells in a contact dependent manner. In addition, normal peripheral blood monocytes activated with LPS or PMA also induced down regulation of CD 3-and CD 16-associated ζ chains[25]. We also found that co-culture with these activated monocytes strongly inhibited melanoma specific CTL and anti-tumor NK activity *in vitro*[25]. Therefore, in murine tumors and in melanoma patients, tumor-associated macrophages can suppress anti-tumor responses paralleled by decreased expression of signal transducing molecules.

Macrophages are known to secrete a plethora of potentially immunosuppressive factors, including cytokines such as IL-10, prostaglandins and free oxygen radicals[26]. Monocytes recovered by centrifugal elution can inhibit *in vitro* human NK cell-mediated cytotoxicity via secretion of H_2O_2 [27]. The addition of selective scavengers of reactive oxygen species, such as catalase, can counteract the inhibition by oxidant stress on human lymphocyte cytotoxicity[27]. We also found that pre-treatment of CTL or NK cells with non-toxic concentrations (1×10^{-5} M) of H_2O_2 severely reduced their cytotoxic activity, whereas the presence of catalase during co-culture almost totally abrogated the inhibitory effect of LPS-activated monocytes[25]. Intriguingly, for melanoma specific CTL, catalase addition almost totally abrogated the contact dependent immunosuppressive effect of tumor-associated macrophages[25]. H_2O_2-induced suppression of cytolytic activity was directly correlated with reductions in CD 3-and CD 16-associated ζ levels[25]. In addition, these reductions in ζ levels were also prevented by catalase.

The possibility that monocyte/macrophage-derived reactive oxygen metabolites contribute directly to alterations in signal transducing molecules of T and NK cells and the mechanism of immunosuppression in individuals with cancer should therefor be considered. And these signaling changes might be related to induction of apoptosis in lymphocyte[28].

Clinical implications

Regardless of the underlying mechanism, alterations in signal transduction in patients with cancer have important clinical implications regarding the monitoring of patient immune status during therapy. Since it has been reported that levels of CD 3 ζ correlate with the stage of disease[29] and survival rate[18], measuring ζ expression might provide vital information that can be used to optimize therapy by preserving immune functions within cancer patients. Flow cytometric assay might provide a particularly convenient way of doing this on a routine basis. Indeed, during the course of immunotherapy, it might be particularly relevant to investigate if the restoration of altered signal transducing molecules within patients correlate with a favorable outcome.

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