studies suggest that mice readily tolerate at least 2.5 times this amount (*16*). The reason for this difference in susceptibility of Tax-transformed and normal cells to NF-κB ODNs is unclear. It may represent a difference in the requirement of intracellular NF-κB for survival or a difference in the ability to internalize the ODNs. Previous studies have correlated the activation or growth state of cells with their ability to take up oligonucleotides by receptor-mediated endocytosis (*18*). The Tax- or virus-transformed tumor cells appear highly activated, which may cause them to take up more ODNs (*8*).

Histologic analysis showed marked and widespread tumor necrosis, and even this short window of treatment was apparently sufficient to prevent tumor recurrences. This suggests that these ODNs may provide a valuable approach to therapy of HTLV-I–associated adult T cell leukemia, which has proved largely refractory to other modalities.

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19 May 1992; accepted 19 September 1992

Alterations in Signal Transduction Molecules in T Lymphocytes from Tumor-Bearing Mice

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Impaired immune responses occur frequently in cancer patients or in tumor-bearing mice, but the mechanisms of the tumor-induced immune defects remain poorly understood. In an in vivo murine colon carcinoma model (MCA-38), animals bearing a tumor longer than 26 days develop CD8$^+$ T cells with impaired cytotoxic function, decreased expression of the tumor necrosis factor–α and granzyme B genes, and decreased ability to mediate an antitumor response in vivo. T lymphocytes from tumor-bearing mice expressed T cell antigen receptors that contained low amounts of CD3γ and completely lacked CD3ζ, which was replaced by the Fc$_\epsilon$ γ-chain. Expression of the tyrosine kinases p56^{lck} and p59^{fyn} was also reduced. These changes could be the basis of immune defects in tumor-bearing hosts.

Cancer patients and tumor-bearing mice have impaired immune functions manifested by decreased delayed-type hypersensitivity, decreased lymphocyte lytic function, and a decreased lymphocyte proliferative response (*1*). A variety of mechanisms, including the action of suppressor cells, the production of suppressor factors by the tumor cells, deletion of tumor-specific clones, and diminished production of lymphokines, have been proposed to explain the altered immune response in cancer patients (*2*). The precise nature and prevalence of these defects has not been elucidated. In an in vivo tumor model, the progressive growth (>26 days) of a subcutaneous implant of MCA-38 resulted in decreased lytic function by the CD8$^+$ T lymphocytes that was associated with decreased expression of mRNA for tumor necrosis factor–α and granzyme B and the complete loss of the ability of adoptively transferred cells to mediate an antitumor effect in vivo (*3*). However, proliferation, lymphokine production, and lymphokine receptor up-regulation in CD4$^+$ T cells were comparable in normal and tumor-bearing mice. Cells with suppressor function were not detected, nor was the production of transforming growth factor–β detected in the lymphocytes from tumor-bearing mice or in the MCA-38 tumor cells.

In an effort to understand the molecular basis of these functional defects, we assessed the structure and function of the T cell antigen receptor (TCR) (*4*). No differences were detected between normal, purified splenic T cells and those from tumor-bearing mice in fluorescence intensity (an indicator of receptor number) or in the percentage of cells expressing the TCRαβ heterodimer (Fig. 1A) or the CD3 complex (Fig. 1B). Flow cytometry demonstrated that splenic T cells from tumor-bearing mice express Thy-1.2 (98%) and TCRαβ (98%) and have a normal CD4:CD8 ratio. They did not express natural killer (NK) cell markers: NK1.1 was <1%, and CD16 (Fc receptors) was <1%. No skewing of the T cell

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receptor repertoire was detected when V_β gene usage was examined in splenic T cells (5). Binding of ligand to the TCR causes Ca^{2+} mobilization (6). In contrast to T cells from normal mice, those from tumor-bearing mice had a blunted Ca response when stimulated with monoclonal antibody to CD3 (anti-CD3). The response did not improve with prolonged times of incubation (400 s) (Fig. 2A). However, stimulation with Ca ionophore resulted in equivalent maximum Ca^{2+} flux in normal T cells and in those from tumor-bearing mice. Thus, the cells from tumor-bearing mice had adequate intracellular Ca^{2+} stores and loading of Indo-1 (5). Analysis of purified $CD4^+$ and $CD8^+$ T cell subsets showed quantitatively similar defects in each subset. These T cells did not optimally release intracellular Ca^{2+} stores in response to signaling through the TCR even though surface TCR density was normal.

Protein tyrosine phosphorylation is the earliest demonstrable event in TCR-mediated signaling, preceding phosphatidylinositol hydrolysis, Ca^{2+} mobilization, and later functional events such as cytokine secretion and cytokine receptor expression (7). Because TCR-dependent Ca^{2+} flux was impaired, we sought to determine whether TCR-dependent protein tyrosine phosphorylation was also altered. The basal pattern of protein tyrosine phosphorylation was altered in T cells from tumor-bearing mice, as compared with normal cells (Fig. 2B). However, stimulation with anti-CD3 did induce protein tyrosine phosphorylation, but the pattern of proteins phosphorylated was not normal (Fig. 2B). The abnormal pattern of basal protein tyrosine phosphorylation in these T cells may have resulted from alterations in the expression of cellular protein tyrosine kinases or in the synthesis of the substrates. Therefore, expression of $p56^{lck}$ and $p59^{fyn}$, two Src family protein tyrosine kinases (PTKs) expressed in T lymphocytes (8, 9), was assessed. Immunoblotting of proteins from whole-cell extracts (Fig. 2C) demonstrated a reduction in $p56^{lck}$ and $p59^{fyn}$ in T cells from tumor-bearing mice, as compared with normal mice.

The structure of the TCR was evaluated by surface iodination of purified T cells (Fig. 3A) from normal and tumor-bearing mice. Proteins in the TCR complex were then immunoprecipitated with anti-CD3 and separated by two-dimensional nonreducing-reducing (NR-R) SDS–polyacrylamide gel electrophoresis (PAGE) (10). Both the TCRαβ heterodimer (which migrated below the diagonal because of interchain disulfide bonds) and CD3ε (which migrated above the diagonal because of intrachain disulfide bonds) were present in approximately normal amounts in T lymphocytes from tumor-bearing mice. In contrast, alterations in other elements of the TCR-CD3 complex were apparent in T cells from tumor-bearing mice. CD3ζ was absent, and the amount of CD3γ was reduced. The failure to detect CD3ζ in association with the TCR expressed on the cell surface might result from reduced steady-state amounts of CD3ζ, aberrant assembly and transport, or instability of detergent-solubilized receptor complexes. To deter-

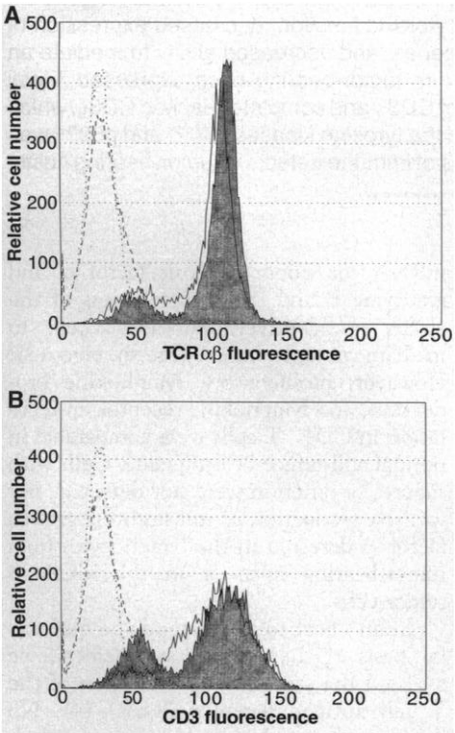

Fig. 1. Surface expression of the TCR-CD3 complex measured by flow cytometry. Isolated T cells from normal and tumor-bearing mice (dotted and dashed lines, respectively) were incubated with control fluorescein-labeled immunoglobulin G2A (IgG2A) and (**A**) antibody to mouse TCRαβ (clone H57-597, Pharmingen, San Diego, California) or (**B**) anti-CD3ε (145-2C11). Normal mice, shaded; tumor-bearing mice, unshaded. These single-parameter histograms reflect the number of cells with a particular level of fluorescence as a function of level of fluorescence. Fifty thousand cells were analyzed. Fluorescence intensity less than 75 represents background levels of fluorescence.

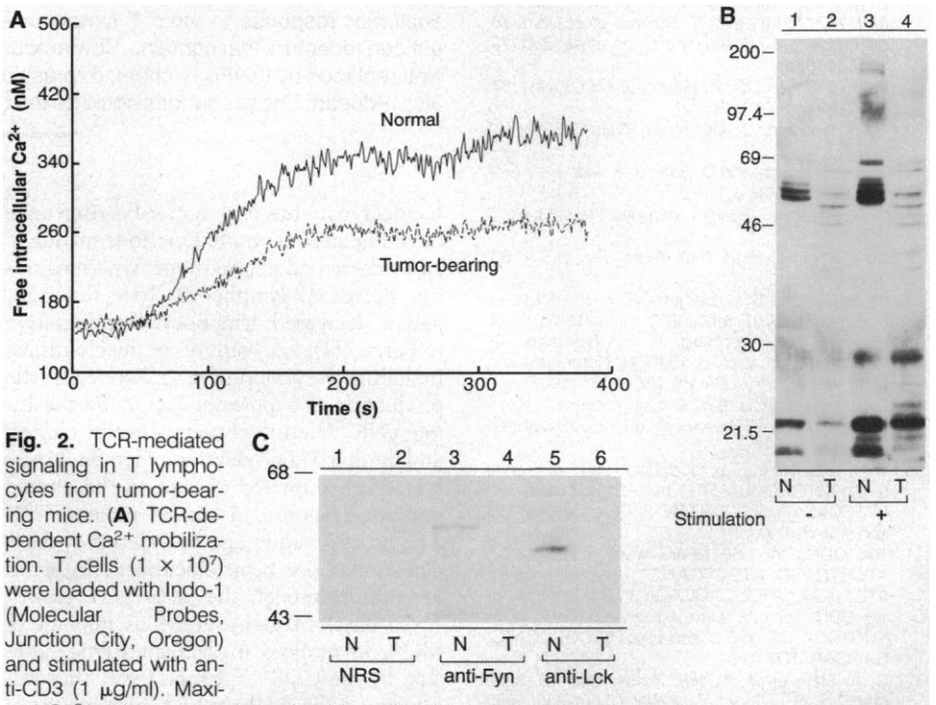

Fig. 2. TCR-mediated signaling in T lymphocytes from tumor-bearing mice. (**A**) TCR-dependent Ca^{2+} mobilization. T cells (1×10^7) were loaded with Indo-1 (Molecular Probes, Junction City, Oregon) and stimulated with anti-CD3 (1 μg/ml). Maximal Ca^{2+} release was determined in cells stimulated with ionomycin. Maximal fluorescence was determined in cells lysed with Triton X-100, and minimum fluorescence was measured after chelation of Ca^{2+} with EGTA. The concentration of free intracellular Ca^{2+} was measured as described (20). (**B**) Protein tyrosine phosphorylation. Purified T cells (1×10^7) from normal (N) or tumor-bearing (T) mice were stimulated with purified anti-CD3 (1 μg/ml) or left untreated in serum-free medium for 2 min. We terminated the reaction by washing the cells twice in ice-cold phosphate-buffered saline containing 400 μM sodium orthovanadate and 1 mM EDTA. We lysed and centrifuged the T cells to remove nuclei. Proteins in supernatants were separated by SDS-PAGE, transferred to Immobilon P filters (Millipore), and immunoblotted with antibody to phosphotyrosine, MAb-4G10 (40 ng/ml). Molecular size standards are shown at left (in kilodaltons). (**C**) Expression of $p56^{lck}$ and $p59^{fyn}$ in T lymphocytes from normal and tumor-bearing mice. Proteins in cell lysates were separated by SDS-PAGE (14% gel), transferred to Immobilon P, and immunoblotted with antiserum to Lck and Fyn peptides (UBI, Lake Placid, New York) or normal rabbit serum (NRS). Molecular size standards are shown at left (in kilodaltons).

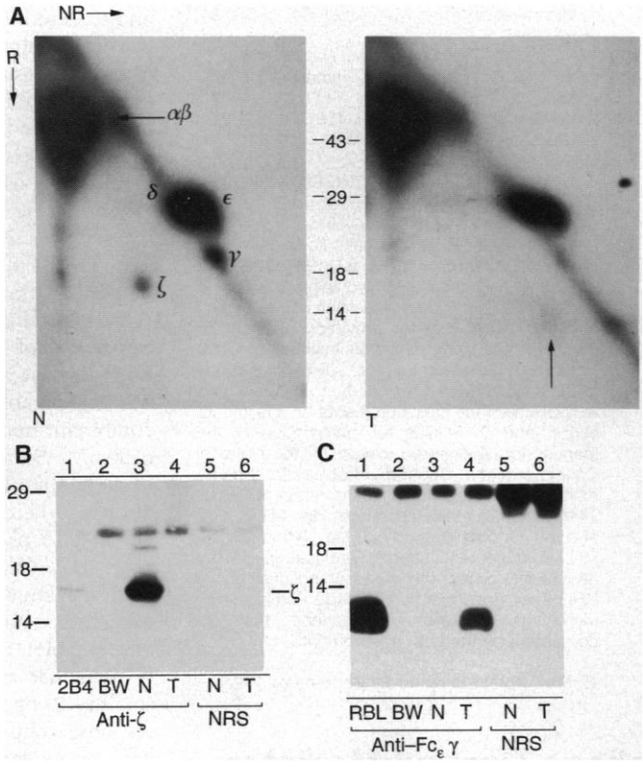

Fig. 3. Alterations in the structure of the TCR in T lymphocytes from tumor-bearing mice. (**A**) T lymphocytes (5×10^7) were labeled with $Na^{125}I$ by the lactoperoxidase–glucose oxidase method (*21*). Cells were then lysed in 1 ml of lysis buffer [25 mM tris (pH 7.4), 300 mM NaCl, 0.5% Triton X-100, 1 mM sodium orthovanadate, aprotinin (10 μg/ml), leupeptin (10 μg/ml), and 5 mM EDTA] for 5 min on ice. The TCR complexes were immunoprecipitated from supernatants of cell lysates with anti-CD3 adsorbed to protein G–Sepharose (Pharmacia, Gaithersburg, Maryland) and resolved by two-dimensional NR-R SDS-PAGE (14% gel). Subunits are indicated in the N panel. The arrow in the T panel indicates a disulfide-linked protein smaller than CD3ζ, similar to $FC_\epsilon\gamma$ in size. The specificity of the subunits immunoprecipitated was confirmed with a thymoma cell line (BW5147) that lacks the TCR (*6*). Molecular size standards are shown in the middle (in kilodaltons). Immunoblot analysis of CD3ζ (**B**) and $Fc_\epsilon\gamma$ (**C**) expression. T cells (2×10^7) from normal (N) mice, tumor-bearing (T) mice, BW5147 (BW) lysates, and 2B4.11 (2B4) hybridoma were solubilized as described (Fig. 1), and proteins from supernatants of cell lysates were immunoprecipitated with rabbit antiserum to ζ (anti-ζ; 387), antiserum to $Fc_\epsilon\gamma$ (anti-$Fc_\epsilon\gamma$) or NRS, as indicated. The 2B4.11 hybridoma was used as positive control for CD3ζ. The RBL-2H3 (rat basophilic leukemia, RBL) cell line was used as positive control for $Fc_\epsilon\gamma$. Blots were developed with peroxidase-conjugated monoclonal antibody to mouse IgG by enhanced chemiluminescence (Amersham, Arlington Heights, Illinois). Molecular size standards are shown at left (in kilodaltons).

mine if detectable amounts of CD3ζ were present, we immunoblotted proteins from whole cell lysates of splenic T cells (Fig. 3B). Again, in contrast to normal T cells, CD3ζ was absent from T cells of tumor-bearing mice. This result was paradoxical because the TCR is transported to the cell surface inefficiently in the absence of CD3ζ (*11*). However, the TCR can be associated with other ζ family members (*12*). The Fc_ϵ γ-chain is a ζ-related protein that subserves ζ-like function in Fc receptor assembly and transport and in signal transduction (*13*). A protein of smaller molecular size than CD3ζ was present in the surface-labeling experiment (Fig. 3A, indicated by the arrow) that was similar in size to $Fc_\epsilon\gamma$. We confirmed the presence of this chain in tumor-bearing T cells by immunoblotting with antibody to $Fc_\epsilon\gamma$ (anti-$Fc_\epsilon\gamma$) (Fig. 3C). Thus, the T lymphocytes from tumor-bearing mice expressed unusual TCR complexes that lacked CD3ζ but contained the Fc_ϵ γ-chain in the majority of T cells. Abnormal TCR complexes were noted in both $CD4^+$ and $CD8^+$ subsets (*5*). The steady-state amounts of mRNA for Lck and CD3ζ were normal or elevated, suggesting that the defect may be at the posttranscriptional level.

$CD8^+$ T lymphocytes in tumor-bearing mice have impaired lytic and therapeutic function (*3*) and alterations in the expression and function of signaling molecules. CD3ζ is not only a critical signal-transducing component of the TCR complex (*14*) but also the limiting subunit in the assembly and membrane expression of the TCR-CD3 complex in T cell hybridomas (*15*). Lytic function is reduced in cells lacking TCRα or CD3δ (*16*). The effect of the absence of either CD3ζ or CD3γ from T cells in tumor-bearing mice is not clear. A subpopulation of large granular T lymphocytes (LGLs) characterized as $CD3^+$, $NK1.1^+$, $CD16^+$, $CD4^-$, and $CD8^-$ and expressing the Fc_ϵ γ-chain instead of CD3ζ has been described (*17*). These lymphocytes differ phenotypically from those seen in tumor-bearing mice. However, these LGLs do have lytic function. Thus, target cell lysis is not dependent on the presence of CD3ζ. Moreover, chimeric molecules made with Fc_ϵ γ expressed in cytotoxic T cell lines maintain their lytic function. This suggests that Fc_ϵ γ and CD3ζ may be interchangeable in coupling to lytic mechanisms, especially in view of the degree of homology between these two chains (*18*).

In addition to the structural changes in the TCR, the T cells from tumor-bearing mice have a decrease in $p56^{lck}$ and $p59^{fyn}$. Studies with PTK inhibitors have demonstrated the function of such enzymes in TCR-mediated signal transduction (*7*). However, proliferation in response to anti-CD3, up-regulation of interleukin-2 receptors, and production of lymphokines (interleukin-2, interleukin-6, and interferons) remained normal in T cells from tumor-bearing mice. Thus, lytic function may be more dependent on PTK activity than other lymphocyte functions. Interleukin-2 secretion can be normal in the absence of TCR-dependent Ca^{2+} flux (*19*).

It is not clear what fraction of the immune defects seen in cancer-bearing animals and humans is related to the phenomena described here. However, supernatants from a variety of tumor cell lines inhibit anti-CD3–induced cytotoxicity by normal T cells in vitro (*5*). We have found precisely the same functional abnormalities and TCR structural abnormalities in T cells from BALB/c mice bearing the Renca renal cell adenocarcinoma (*5*). Thus, these findings are not unique to MCA-38–bearing mice. We believe that these findings are related to the interaction of the tumor and the host in these animals. We have not examined the T cells of mice made chronically ill through other nonneoplastic disease processes, but the molecular changes described here occur during stages of the disease in both MCA-38– and Renca-bearing mice when mice are not cachectic or moribund.

In addition, in 7 of 12 human cancer patients, we found that peripheral blood T cells lacked expression of ζ and Fyn protein. Thus, structural and functional alterations are also noted in T cell signal transduction molecules from tumor-bearing humans. Not only might the ability to reverse this defect be useful in the treatment of cancer, but also the ability to induce the defect might be useful in the treatment of autoimmunity or as an adjunct to organ transplantation.

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12 May 1992; accepted 21 September 1992

Interleukin-8 as a Macrophage-Derived Mediator of Angiogenesis

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Angiogenic factors produced by monocytes-macrophages are involved in the pathogenesis of chronic inflammatory disorders characterized by persistent angiogenesis. The possibility was tested that interleukin-8 (IL-8), which is a cytokine that is chemotactic for lymphocytes and neutrophils, is also angiogenic. Human recombinant IL-8 was potently angiogenic when implanted in the rat cornea and induced proliferation and chemotaxis of human umbilical vein endothelial cells. Angiogenic activity present in the conditioned media of inflamed human rheumatoid synovial tissue macrophages or lipopolysaccharide-stimulated blood monocytes was equally blocked by antibodies to either IL-8 or tumor necrosis factor–α. An IL-8 antisense oligonucleotide specifically blocked the production of monocyte-induced angiogenic activity. These data suggest a function for macrophage-derived IL-8 in angiogenesis-dependent disorders such as rheumatoid arthritis, tumor growth, and wound repair.

Angiogenesis is important in wound healing, tumor growth, and inflammatory disorders such as rheumatoid arthritis, and activated monocyte-macrophages are key angiogenesis effector cells in these settings (*1*, *2*). Although angiogenesis is critical to the initiation and progression of angioproliferative disease processes, the mediators responsible for this angiogenic activity remain to be precisely defined. IL-8 is a cytokine that has chemotactic activity selectively for neutrophils and lymphocytes at nanomolar and picomolar concentrations, respectively (*3*). This cytokine may be involved in leukocyte-vascular endothelial interactions such as the invasion of neutrophils though a vessel wall model via β_2 integrin attachment (*4*). Moreover, we and others have implicated IL-8 in angiogenic disease states such as psoriasis and rheumatoid arthritis (*5*). We have shown that rheumatoid synovial tissue macrophages are a major source of angiogenic mediators and IL-8 in the inflamed rheumatoid synovial milieu (*6*, *7*). IL-8 binds to heparin, a characteristic of many angiogenic cytokines such as acidic and basic fibroblast growth factor (aFGF and bFGF, respectively) and vascular endothelial growth factor (*1*). To test the hypothesis that IL-8 is angiogenic and to determine its contribution to monocyte-macrophage–associated angiogenic activity, we examined the ability of IL-8 to mediate angiogenesis in in vitro and in vivo models and compared its activity to that of tumor necrosis factor–α (TNF-α), a known macrophage-associated angiogenic mediator in murine systems (*8*).

First, we showed that recombinant human IL-8 stimulated chemotaxis of human umbilical vein endothelial cells (HUVECs) (Fig. 1A). IL-8 was chemotactic at a concentration of 0.125 nM. Chemotaxis toward IL-8 at a concentration of 1.25 nM was comparable to chemotaxis toward recombinant human bFGF at a concentration of 6 nM. Similarly, bFGF induced a doubling in the number of HUVECs (proliferation), whereas IL-8 at concentrations above 1.25 pM induced a similar response (Fig. 1B). We next determined whether IL-8 was angiogenic in vivo. IL-8 was incorporated into Hydron (Interferon Sciences) pellets and implanted in the normally avascular rat cornea (*9*). Amounts as small as 10 ng induced a corneal angiogenic response (ten out of ten positive corneas) with no evidence of nonspecific inflammation (Fig. 2, A and B).

We then determined whether IL-8 contributed to the angiogenic activity liberated by normal human monocytes activated in vitro or by macrophages isolated from diseased human synovial tissues (*10*), which are presumably activated in the inflamed joint milieu (Table 1). Antibodies to TNF-α and IL-8 (anti–TNF-α and anti–IL-8, respectively) reduced the chemotactic activity for HUVECs present in conditioned medium of lipopolysaccharide (LPS)-stimulated blood monocytes by 37% ± 9% (SE) and 34% ± 7%, respectively. Similar results were found when conditioned medium from inflammatory rheumatoid synovial tissue macrophages was incubated with anti–TNF-α or anti–IL-8: the chemotactic activity was reduced by 55 ± 1% and 39 ± 2%, respectively.

Neutralization of IL-8 in the LPS-activated monocyte-conditioned medium resulted in either diminution or complete

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