Systemic and Local Immunosuppression in Pancreatic Cancer Patients

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Abstract

Pancreatic cancer is characterized by an extremely poor prognosis. For the development of more effective immunotherapies, the systemic and local immunological escape mechanisms need to be further elaborated. These mechanisms may include the secretion of immunosuppressive cytokines, the local hindrance of tumor-infiltrating lymphocytes (TILs), or the loss of the signal transducing CD3 ζ-chain of TILs. In this study, we have analyzed these parameters in 116 patients suffering from pancreatic ductal adenocarcinoma. Mean concentrations of interleukin (IL)-10 and transforming growth factor-β were considerably higher than in control sera (P < 0.0001). Disseminated tumor cells were found in 16 of 39 cases. In 28 of 33 surgical specimens, TILs did not reach tumor cells in significant numbers, being “trapped” in the peritumoral tissues. We suggest this as a simple but highly effective tumor escape mechanism. In cases of a TIL/tumor cell contact, CD3 ζ was mostly lost. Overall, 27 of 33 surgical specimens, 9 of 19 peritumoral lymph nodes, and 13 of 25 peritoneal lavage specimens showed significant loss of CD3 ζ (P < 0.02). Elevated concentrations of IL-10/TGF-β1/2 were, in all but one of three cases, correlated with a CD3 ζ loss in corresponding specimens. Patients with disseminated tumor cells also showed a CD3 ζ loss in all but two corresponding tumor specimens. These results present strong evidence for an active systemic immunosuppression in pancreatic cancer, as shown by elevated IL-10 and TGF-β1/2 serum levels as well as the presence of disseminated tumor cells. Killing of tumor cells by potentially cytotoxic TILs is obviously suppressed by the prevention of a direct TIL/tumor cell contact and the inactivation of TILs, as shown by a severe loss of CD3 ζ. In addition to active immunization strategies, successful immunotherapies have to focus on restoring in vivo T-cell function to improve the almost always fatal prognosis of pancreatic cancer.

Introduction

Pancreatic cancer is one of the most aggressive malignancies with a 5-year survival rate of 3–8% (1). Surgical as well as nonsurgical therapies have widely failed to improve patient outcome. To develop more effective therapies, particularly immunotherapies, against pancreatic cancers, their systemic and local immunological escape mechanisms need to be elaborated.

Malignant tumors often show massive infiltration by potentially cytotoxic immune cells, in particular T-cells (TILs). However, most tumors have developed mechanisms to escape immune surveillance. Among many other mechanisms, we and others have shown that tumors may express only low levels of or even lose important costimulatory molecules that are necessary for optimal T-cell function, such as intercellular cell adhesion molecule-1 or B-7 (2–4). In addition to the loss of functional death receptors such as Fas (5) or blockade mechanisms in apoptotic signaling (6), we and others have suggested that pancreatic cancer cells may also actively suppress immune cells by factors such as TGF-β, IL-10, glycoproteins, or FasL (5–9). Serum levels of these molecules, potentially secreted by tumor cells, may be elevated and could indicate a generalized state of immunosuppression (8, 10, 11). This may promote tumor growth and the formation of disseminated disease, as evidenced by disseminated tumor cells (12, 13). The lack of activation of TILs is one indicator of local immunosuppression by malignant tumors. One particular marker of T-cell function and activation is the ζ-chain of the TCR complex. The TCR-ζ-chain is a disulfide-linked homodimer associated with the CD3 complex. Its cytoplasmic domain is essential for signal transduction and subsequent activation of T cells (14, 15). A loss of this molecule will lead to impaired T-cell function, destroying an important part of the immune system of the tumor host to kill tumor cells effectively.

To assess the extent of possible immunosuppression in ductal pancreatic adenocarcinoma, we have studied the concentrations of the potentially immunosuppressive molecules IL-10, TGF-β1, and TGF-β2 by ELISA in tumor sera of 116 patients suffering from pancreatic ductal adenocarcinoma. Thirty nine patients were tested for disseminated tumor cells in sera, peritoneal lavages, and bone marrow specimens by immunocytochemistry or RT-PCR. To assess the local state of immunosuppression, 33 surgical specimens and 19 peritumoral lymph nodes

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4 The abbreviations used are: TIL, tumor infiltrating lymphocyte; TGF, transforming growth factor; IL, interleukin; mAb, monoclonal antibody; TCR, T-cell receptor; FasL, Fas ligand; RT-PCR, reverse transcription.
of pancreatic cancer patients were tested for: (a) evidence and
distribution of T cells (as stained for the CD3 ε-chain) in
relationship to surrounding tumor cells within the specimens;
(b) the expression of the CD3-ζ-chain; and (c) expression of
CD4 and CD8. In addition, T cells of 25 peritoneal lavage
samples were investigated by cytospin preparations for their
expression of CD3 ζ.

Materials and Methods

Patients. A total of 116 patients (55 male and 61 female
patients; age range, 39–81 years; mean age, 63.7 years) with
pancreatic ductal adenocarcinoma entered this study at Kiel
University Hospital. Patients had been thoroughly informed
and gave written consent for all investigations. The study had been
approved by our internal review board commission. Patients
(n = 107) were tested for serum levels of IL-10, TGF-β1, and
TGF-β2. Thirty-one patients suffering from acute pancreatitis
(21 males and 10 females; age range, 18–79 years; mean age,
52.2 years), 39 patients with benign illnesses (18 males and 21
females; age range, 27–78 years; mean age, 56.6 years), and 77
healthy individuals (49 males and 28 females; age range, 19–67
years; mean age, 40.6 years) served as controls for serum
cytokine levels.

Thirty-nine patients were evaluated for the presence of
disseminated tumor cells in bone marrow, blood, and peritoneal
lavage specimens. Thirty-three tumor specimens, 19 peritumoral
lymph node specimens, and 25 peritoneal lavage specimens
were investigated for the presence, distribution, activation,
and/or phenotype of CD3+ T cells.

Samples. Bone marrow (10 ml, aspirated from the right
anterior superior iliac spine with a Jamshidi needle) and blood
samples (20 ml) were collected just before surgery. Peritoneal
lavages were performed immediately after laparotomy before
any manipulation of the tumor was done. The preparation of
samples was done as described previously (12). Analyses of
samples for the presence of disseminated tumor cells by immuno-
cytochemistry (12, 13) or RT-PCR (16) have been reported as
well. Three compartments were assessed: bone marrow, perito-
neal cavity, and blood. A patient with one positive finding in at
least one compartment was considered as having disseminated
tumor cells. Primary pancreatic cancer specimens including
adjacent lymph nodes were obtained after resection, immedi-
ately snap frozen in liquid nitrogen, and stored at −80°C until
further use.

Cytokine Concentrations in Patient Sera. After thawing
serum samples, protein concentrations of IL-10 and active
TGF-β1 and TGF-β2 were determined by ELISA, using kits
commercially available, following the manufacturer’s protocol.
The lower threshold of sensitivity of the different kits were as
follows: IL-10, 3.9 pg/ml (R&D Systems, Wiesbaden, Ger-
many); TGF-β1, 7 pg/ml (R&D Systems); and TGF-β2, 7 pg/ml
(R&D Systems).

Antibodies. Staining of cytopsin for disseminated tu-
mor cells (blood, bone marrow, and peritoneal lavage) was
performed with the monoclonal antibodies as described previ-
ously (12): C1P83 (17); CA19-9 (DAKO, Carpinteria, CA; Ref.
18); 17-1-A (Centocor, Leiden, the Netherlands; Ref. 19); RA96
(13); C54-0 (20), only used for peritoneal lavage samples; and
KL-1 (Coulter-Immunotech, Hamburg, Germany), only used in
bone marrow samples. More recently, we have also used the
commercially available EPIMET Epithelial Cell Detection kit
(Baxter, Unterschleissheim, Germany) to detect disseminated
tumor cell. This kit contains a conjugate of the Fab fragment of
the pan-cytokeratin mAb A45-B/B3 with alkaline phosphatase
and was used following the manufacturer’s recommendations.

For the expression of the corresponding cellular molecules
in tumor, lymph node, and peritoneal lavage specimens, the
following antibodies were used: CD3 ζ-chain, mAb clone
TCR-ζ (10 μg/ml; Coulter Immunotech); CD3 ε-chain, clone
UCHTT-1 (10 μg/ml; Ancell Corporation/Alexis, Grünberg,
Germany); CD4 and CD8, mAbs CD4 (5 μg/ml) and CD8 (5
μg/ml; both Ancell Corporation/Alexis); immunoglobulin
matched mAbs (10 μg/ml; all PharMingen, Hamburg, Ger-
many); and cytokeratin, KL-1 (5 μg/ml; Coulter-Immunotech).

Immunohistochemistry. Serial sections of tumor and
lymph node tissues were stained with the Vectastain ABC kit,
the Vector Biotin/Avidin blocking kit, and the 3,3′-diaminoben-
zidine substrate kit for peroxidase (all Vector Laboratories Inc.,
Burlingame, CA) according to the manufacturer’s recommen-
dations. Briefly, frozen tissues were serially cut at 5 μm,
mounted on poly-t-lysine-coated glass slides, and air dried.
Immediately before staining, sections were fixed with acetone.
Each step was followed by one wash with PBS, and unspecific
binding sites were successively blocked with normal horse
serum, avidin, and biotin and then incubated with primary
antibodies for CD3 ζ-chain, CD3 ε-chain, CD4, CD8, or with
IgG-matched negative control antibodies, and KL-1, respec-
tively. After a wash with PBS, sections were incubated with
biotinylated secondary antibody, washed, and incubated with an
avidin/biotinylated horseradish peroxidase substrate. Subse-
quently, sections were incubated with the 3,3′-diaminobenzi-
dine substrate kit until the desired staining intensity developed.
Sections were then rinsed in tap water, counterstained with
hemalaun, cleared, and coverslipped.

Cytospin slides of peritoneal lavage specimens were pro-
cessed and stained similarly using an alkaline phosphatase kit
(Vector Laboratories, Inc.), following the manufacturer’s rec-
ommendations.

In all specimens, the CD3 ε-chain served as the positive
control, and immunoglobulin-matched unspecific mouse anti-
bodies served as the negative control. Also, all tumor and lymph
node specimens were stained with the epithelial cell marker
KL-1.

Evaluation of Lymphocyte Staining. All specimens
were cut in serial sections for better comparison between spec-
imens. In tumor specimens, the distribution of CD3+ TILs in
relationship to surrounding tumor cells was assessed to evaluate
whether potential cytotoxic effector T cells had contact with
their tumor target cells.

Expression of CD3 ζ- and ε-chains, CD4, or CD8 was
scored as follows. Staining intensity of infiltrating lymphocytes
significantly stronger than background staining was interpreted
as positive for the evaluated marker. ζ-chain, CD4, and CD8
expression were evaluated semiquantitatively using CD3 ε ex-
pression as a positive control. Significant loss of ζ-chain staining,
in particular in lymphocytes in the close proximity of tumor
cells, was regarded as ζ-chain loss only if in parallel controls

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(normal lymph node sections) an equivalent staining intensity with ε and ζ mAbs was observed. The relative amount of CD3ε+/CD4−/CD8− T cells was estimated by adding the numbers of CD4+ and CD8+ T cells and comparing this with the number of CD3ε+ T cells.

**Statistical Analysis.** For all statistical analyses, SPSS software (SPSS, Inc., Chicago, IL) was used. To test for statistical differences in IL-10 levels, the variance analysis or the Dunnett T3 test was used; for TGF-β1/2 statistics the Dunnett T3 test was used. To assess correlations between elevated cytokine levels and the occurrence of disseminated tumor cells, between elevated cytokine levels and loss of CD3ζ, and between the occurrence of disseminated tumor and CD3ζ loss, we used the χ² and Fisher’s exact test. The occurrence of CD3ζ loss was assumed of being normal in up to 20% of the normal population as a very conservative assumption. With this assumption, statistical significance of CD3ζ loss was assessed by a binomial distribution.

**Results**

**Serum Levels of IL-10, TGF-β1, and TGF-β2.** The potentially immunosuppressive molecules IL-10, TGF-β1, and TGF-β2 were measured in tumor sera of overall 107 patients by ELISA (Fig. 1). We found that the mean of IL-10 levels (7.3 pg/ml; SD, 8.7 pg/ml) was significantly higher (P < 0.0001) in pancreatic cancer patients than in healthy controls (2.5 pg/ml; SD, 2.4 pg/ml). Interestingly, when compared with benign disease (6.1 pg/ml; SD, 9.5 pg/ml) or pancreatitis (6.8 pg/ml; SD, 9.6 pg/ml), there was no significant difference between serum levels.

Mean levels of TGF-β1 and TGF-β2 were also significantly higher (P < 0.0001) in cancer patients (TGF-β1: 35.0 pg/ml and SD, 48.6 pg/ml; TGF-β2: 6.4 pg/ml and SD, 8.8 pg/ml) than in normal controls (TGF-β1: 7.5 pg/ml and SD, 10.5 pg/ml; TGF-β2: 1.2 pg/ml and SD, 3.7 pg/ml) as well as in patients suffering from benign diseases (TGF-β1: 16.3 pg/ml and SD, 26.5 pg/ml; TGF-β2: 1.6 pg/ml and SD, 4.3 pg/ml; P < 0.0001). However, there was no statistical difference between cancer disease and pancreatitis (TGF-β1: 34.9 pg/ml and SD, 41.6 pg/ml; TGF-β2: 6.1 pg/ml and SD, 9.6 pg/ml).

**Disseminated Tumor Cells.** In 39 patients with pancreatic carcinoma, blood, bone marrow, and peritoneal lavage samples were investigated for the presence of disseminated tumor cells (Table 1 and Fig. 2A). Tumor cell dissemination was seen in 16 of 39 (41%) patients. Ten patients displayed tumor cells in bone marrow only, 3 patients in peritoneal lavages only, and 1 patient in serum samples only. Two patients were positive for tumor cells both in bone marrow and peritoneal lavage specimens.

By comparing elevated cytokine levels and the presence of disseminated tumor cells, we found no significant correlation between elevated IL-10, TGF-β1, and TGF-β2 levels on one side and tumor cell dissemination on the other side.

**T-Cell Tumor Cell Contact.** Altogether, 33 tumor specimens of ductal pancreatic adenocarcinoma were immunohistochemically investigated for the presence and distribution of TILs staining positively with a mAb against the CD3 ε-chain (Table 1 and Fig. 2A). CD3ε+ TILs were found in all specimens. In the majority of tumor specimens, i.e., in 29 of 33 cases (88%), TILs did not reach the tumor cells in significant numbers, thereby being “trapped” within the peritumoral, mostly fibrous tissues.

**Expression of CD3ζ-Chain.** In addition to the same 33 tumor specimens as above, 19 peritumoral lymph node specimens and 25 peritoneal lavage specimens were immunohistochemically tested for expression of the CD3ζ-chain, again using CD3ε as a positive control (Table 1 and Fig. 2, A–J). For better comparison of antigens within one specimen, serial sections were used. Overall, 27 of 33 (82%) tumor specimens had a significant decrease or loss of CD3ζ compared with corre-

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**Table 1** Results of immunohistochemical and PCR data

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Result</th>
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<tr>
<td>Disseminated tumor cells</td>
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<td>ζ loss in</td>
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Fig. 2 Immunohistochemical specimens, serial sections, using mAbs against the TCR-CD3 ε- or ζ-chains of T cells. Specimens stained for CD3 ε (A) and stained for CD3 ζ (B) show pancreatic ductal adenocarcinoma with obvious T-cell trapping around the tumor and almost complete loss of CD3 ζ (×200). C and D, a similar specimen with some T cells reaching the tumor cells in C but complete loss of CD3 ζ in D (×400). E (CD3 ε) and F (CD3 ζ) show no differences in staining intensities of T cells of peritumoral lymph node specimens as well as in a case of a non-Hodgkin’s lymphoma infiltrating the pancreas in G (CD3 ε) and H (CD3 ζ; ×400). J (CD3 ε) and J (CD3 ζ) show complete loss of CD3 ζ in T cells of peritoneal lavage specimens (×400). K shows a specimen with a disseminated tumor cell in a bone marrow sample (×400). In this specimen, a mixture of different mAbs was used to detect tumor cells.

In cases of a TIL/tumor cell contact, CD3 ζ was mostly lost (Fig. 2, A–D). Four of the six tumor specimens without a loss of CD3 ζ exhibited peritumoral trapping of TILs; only two specimens showed neither loss of CD3 ζ nor trapping of peritumoral TILs.

Peritumoral lymph node specimens exhibited a significant decrease of CD3 ζ in 9 of 19 (47%) specimens (P < 0.02). One of these lymph node specimens displaying a CD3 ζ loss also had an intranodal micrometastasis. In this specimen, lymph node T cells did not reach the tumor cells either.
Peritoneal lavage specimens showed a significant decrease or loss of CD3 \( \xi \) in 13 of 25 (52\%) cases \((P < 0.001)\). In cases of a CD3 \( \xi \) loss in lymph nodes or peritoneal lavages, the corresponding tumor specimens always displayed a similar loss.

In 21 patients in which we could analyze T-cell trapping, the expression of CD3 \( \xi \) and serum levels of IL-10 and TGF-\( \beta \)-1 were parallel. In all but two cases, elevated serum levels of IL-10 and TGF-\( \beta \)-1 were associated with T-cell trapping. By comparing elevated individual IL-10 levels and TGF-\( \beta \)-1 levels with loss of CD3 \( \xi \), we found that in all but one case, elevated IL-10 levels were associated with loss of CD3 \( \xi \). This was found in all but three cases when elevated individual TGF-\( \beta \)-1 values were compared with CD3 \( \xi \) expression. However, statistically, these results were not significant. By comparing loss of CD3 \( \xi \) and the presence of disseminated tumor cells, we found that in all but two cases, the presence of tumor cell dissemination was associated with a loss of CD3 \( \xi \) in corresponding tumor specimens.

**CD4 and CD8 Expression.** In tumor specimens, CD4\(^+\) T cells were more frequently (48\%) of cases; 14 of 29) found than CD8\(^+\) T cells. In 9 of 29 (31\%) cases, there was no difference between the expression of CD4 and CD8, and in 6 of 29 (21\%) cases, CD8 was the predominantly expressed antigen on CD3 \( \epsilon \) lymphocytes. Remarkably, most lymphocytes reaching tumor cells expressed CD8.

In lymph node specimens, the number of CD4\(^+\) lymphocytes was always much higher than that of CD8\(^+\) lymphocytes, both with a rather equal distribution within the lymph node. Here, the number of CD3 \( \epsilon \) lymphocytes always equaled the sum of CD4\(^+\) and CD8\(^+\) lymphocytes.

**Discussion**

In the present study, we have shown that the immune system in pancreatic cancer patients is severely impaired. Systemically, we have found elevated serum levels of the potentially immunosuppressive cytokines IL-10 and TGF-\( \beta \)-1, as well as the presence of disseminated tumor cells in the bone marrow, the blood, and the peritoneal cavity in many cancer patients. By investigating tumor specimens, we have found that in most cases, TILs do not reach their malignant cellular targets; they get "trapped" within the peritumoral, mostly fibrous tissues. In cases of a T-cell/tumor cell contact, most lymphocytes are clearly inactivated, as evidenced by a loss of their CD3 \( \xi \) chains.

The immunosuppressive actions of IL-10 and TGF-\( \beta \) have been described extensively \((8, 10, 11)\). Elevated serum levels of IL-10 have also been found by De Vita et al. \((21)\) in gastric and colorectal carcinoma patients. Whereas in their report significantly higher levels of IL-10 in patients with solid metastatic disease were described, we do not find such a correlation in patients with disseminated tumor cells compared with tumor patients without dissemination. This group as well as others \((22)\) have also reported a worse prognosis of patients with elevated IL-10 levels before chemotherapy or surgery, respectively. However, these findings may not be tumor specific because patients, suffering from pancreatitis, also showed elevated IL-10 levels.

Chau et al. \((22)\) also showed that IL-10 levels decrease after surgery. We have made similar observations (data not shown). In this context, it is important to note that IL-10 may not be secreted by tumor cells but may be indirectly regulated by tumor cells. In contrast to Bellone et al. \((23)\), who found elevated IL-10 levels in pancreatic cancer cell lines, we could not detect any IL-10 in pancreatic cancer cell lines. High amounts of IL-10 were detected in monocytes cocultured with colon cancer cell lines producing high levels of TGF-\( \beta \)-1 and prostaglandin E\(_2 \) \((24)\). This suggests that colon cancer cells may enhance the ability of peritumoral monocytes and macrophages to produce IL-10, therefore further promoting immunosuppression. This may also hold true for pancreatic cancer; despite the lack of IL-10 in pancreatic cancer lines as determined by RT-PCR and highly sensitive ELISA tests, we found a positive signal for IL-10 mRNA and protein in pancreatic cancer tissues. With respect to prostaglandin E\(_2 \) possibly being immunosuppressive in colon cancer \((24)\), we cannot make a definitive statement because the ELISAs used did not give reproducible results in serum samples.

Increased serum levels of TGF-\( \beta \) have been found in renal cell cancer \((25)\), invasive prostate cancer \((26)\), hepatocellular carcinoma \((27)\), colorectal cancer \((28)\), and more recently, pancreatic cancer \((23)\). Also, tumor progression and poorer prognosis in patients with elevated plasma levels or increased tumor expression of TGF-\( \beta \) have been shown \((29-31)\). Interestingly, Wunderlich et al. \((25)\) reported significantly higher TGF-\( \beta \) plasma levels in renal cell cancer patients than in cases of inflammation. Our results show significant worse difference between cancer patients and patients treated for benign diseases only (excluding pancreatitis), whereas patients with pancreatitis do not differ in TGF-\( \beta \) serum levels. It has been suggested that TGF-\( \beta \) may play a role in the repair process after the onset of pancreatitis \((32)\).

Neopterin, a marker for cellular immune activation \((33)\), has been suggested as a prognostic factor in pancreatic cancer by Birk et al. \((34)\). By using the same methods, we have also found elevated neopterin levels in sera of pancreatic cancer patients compared with healthy controls. However, in contrast to Birk's results, we have been unable to find a significant difference between the two groups. We did not detect any correlation between serum levels and survival, and neopterin did not serve as a prognostic factor. Also, in our study, neopterin levels in patients with pancreatitis were even higher than those in cancer patients. Therefore, larger numbers of patients may be necessary to re-evaluate the data.

Previously, we have shown that disseminated tumor cells can be detected in pancreatic cancer patients in >70% \((12)\) and that the detection of disseminated tumor cells serves as a prognostic factor \((13)\). Although in this study we have detected tumor cell dissemination in 41% of patients, we have failed to show a correlation between tumor cell dissemination and ele-
vated cytokine levels. This is in contrast to the findings of De Vita et al. (21), who showed increased formation of metastases in gastric and colon cancer patients with elevated IL-10 levels.

To effectively eradicate tumor cells, TILs obviously need to reach their tumor cell targets. In a previous study, the CD8+ infiltrate in cancer cell nests was proposed as a prognostic factor in human colorectal cancer (35). The prognosis improved with the amount of CD8+ T cells infiltrating colorectal cancer cell nests. In contrast, TILs of pancreatic cancer specimens do not reach in significant numbers pancreatic cancer cells in the vast majority of tumor specimens. These T cells, mostly CD8+, are “trapped” within the peritumoral, mostly fibrotic tissues, unable to attack the cancer cell. Therefore, we suggest T-cell trapping as a novel, simple, but highly effective tumor escape mechanism in pancreatic ductal adenocarcinoma. It remains to be shown whether this mechanism is caused by “repetent” chemokines, as described recently (36).

In tumor specimens where TILs reached the tumor cells, these TILs were mostly inactive, as indicated by their loss of the signal transducing CD3 ζ-chain. Altogether, in 94% of our investigated tumor specimens, TILs did not reach tumor cells and/or had down-regulated or lost CD3 ζ-chains. These findings suggest a highly immunosuppressive local environment. In our study, we have failed to show a correlation between elevated serum levels of IL-10 or TGF-β. Gastmann et al. (37) have suggested a caspase-mediated degradation of the CD3 ζ-chain in FasL-expressing tumor cells. This may also be true for pancreatic cancer cells because all pancreatic cancer cell lines and specimens tested thus far express FasL (5, 6). However, at least soluble FasL does not seem to play a role because it was not detectable in significant amounts in tumor sera. Furthermore, it is unlikely that FasL is the only molecule involved in CD3 ζ loss because in our study, most TILs are not in the close vicinity of tumor cells with their membrane-bound FasL. Also, we have observed a CD3 ζ loss in peritoneal lavage specimens as well as in peritumoral lymph node specimens, often without evidence of disseminated tumor cells. This points to soluble factors being responsible for this impairment of T-cell function.

Interestingly, although it appears widely accepted that many different tumors can induce a loss of CD3 ζ in T cells (38–42), this phenomenon has been doubted. Other authors have questioned that tumor specific factors may be responsible for a loss of TCR ζ, attributing it to contaminating monocyte/macrophage protease activity (43–45). This would lead to a false significant loss of TCR ζ in tumor patients (46). More recently, no loss of CD3 ζ in colorectal carcinoma was observed (47). However, by treating all tissue sections and cell preparations with the same techniques and by including positive (CD3 ε staining, lymph node specimens, non-Hodgkin’s lymphoma) and negative control specimens in all our experiments, we do have strong evidence for a severe loss of CD3 ζ in pancreatic cancer.

IL-10 and TGF-β are probably important factors in inhibiting a T helper 1 response and promoting a T helper 2 response in TILs of pancreatic cancer (23). An increase in T helper 2 cells would lead to a reduced number of CD8+ TILs and a relative increase of CD4+ cells. We have found predominantly CD4+ T cells in almost 50% of tumor specimens, perhaps reflecting pathological alterations of the CD4/CD8 ratio as proposed (48). This may contribute to a loss of effective cytotoxic T cells.

Taken together, our results reveal a severe state of immunosuppression in pancreatic cancer patients. By further identifying immunosuppressive molecules and mechanisms, the wide range of immunotherapeutic intervention strategies to counteract tumor-induced immunosuppression needs to be further explored. Ideally, surgery to resect the primary tumor or at least to lower the tumor burden should be combined with a multimodal immunotherapy. This could include active immunization protocols, the use of antibodies directed against immunosuppressive molecules, the use of immunoprotective stimulatory cytokines, or adoptively transferred activated immune cells. These therapies, able to reach minimal residual disease, should be explored preferentially in an adjuvant setting.

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