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Prognostic Significance of Activated CD8$^+$ T Cell Infiltrations within Esophageal Carcinomas

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ABSTRACT

The purpose of this study was to explore whether there is a linkage between the infiltration of CD8$^+$ T cells and the risk of death from esophageal cancer. Cases of 70 consecutive patients in whom esophageal squamous cell carcinomas ($n = 33$) or adenocarcinomas ($n = 37$) were R0-resected between 1993 and 1999 were reviewed. The presence of activated CD8$^+$ T cells was evaluated by quantitative real-time PCR and immunohistochemistry and compared to clinical and pathological stages. The primary end point analyzed was overall survival, and a multivariate analysis was performed to distinguish any factors conferring an improved survivorship. Intratumoral (i.t.) CD8$^+$ T cells accumulating within the epithelial complexes were detected in 11 of all (16%) cases: in 9 of 25 (36%) patients with Union Internationale Contrele Cancer stage I or II *versus* 2 of 45 (4%) patients with Union Internationale Contrele Cancer stage III or IV ($P = 0.001$). Intratumoral CD8$^+$ T cell infiltrations showed proliferative activity and also IFN-γ secretion. The presence of i.t. CD8$^+$ T cell infiltration more than peritumoral infiltration was associated with a good prognosis in both squamous cell and adenocarcinomas. Multivariate analysis showed that i.t. CD8$^+$ T cell infiltration was an independent prognostic factor (hazard ratio, 0.5; $P = 0.0004$) indicating favorable outcome. In conclusion, the presence of CD8$^+$ T cell infiltration in esophageal carcinomas is a favorable prognostic factor that should have diagnostic and therapeutic implications.

INTRODUCTION

The existence of CTLs recognizing tumor antigens provide strong evidence that natural immune response can exist against cancer in humans. This can be illustrated by the multitude of melanoma-specific TILs[2] that have been isolated (1). It has been shown in animal models that infiltration of tumors by tumor-reactive T lymphocytes is required for efficient tumor regression (2, 3). Therefore, it seemed that local cytokine effects or modulation of host cells are essential for adoptive immunotherapy rather than systemic effects. Various human carcinomas can be infiltrated by TILs (4, 5). In colorectal carcinomas, immunohistochemical identification of CD8$^+$ T lymphocytes could be correlated to an improved overall survival (6, 7). In contrast, the presence of activated CTLs was positively correlated with aggressive disease and with reduced overall survival in patients suffering from Hodgkin's disease (8) or non-Hodgkin's lymphomas (9). Esophageal carcinoma is an aggressive tumor with a poor prognosis. Despite improved rates of complete resection and decline in postoperative mortality, metastatic relapse remains the most frequent cause of cancer-related deaths attributable to the presence of disseminated tumor cells in lymph nodes (10). Current treatment strategies including adjuvant chemo- and radiotherapy after complete surgery were associated with increased comorbidity and 5-year survival rates still range between 20 and 36% (11). Obviously, new approaches for adjuvant treatment and knowledge on factors influencing prognosis are needed.

Several studies on carcinomas of the upper gastrointestinal tract indicate significant differences between the two major histological subtypes, adenocarcinomas and SCCs. Immunohistochemical analysis on infiltrating CD8$^+$ T cells in EBV-associated gastric cancer showed significantly higher levels of proliferative activity and perforin granules in these tumors (12). Histological analysis on the presence of inflammatory cells infiltrating esophageal SCCs correlated positively with prognosis (13–15). Immunostaining of esophageal carcinomas showed decreased levels of MHC class I antigen expression as compared to normal tissues that could be correlated to significantly reduced survival rates in SCCs but not in adenocarcinomas (16). Here, we show that infiltration of CD8$^+$ T cells within esophageal tumors is a reliable positive prognostic factor in both adenocarcinomas and SCCs.

MATERIALS AND METHODS

Tissue Samples. Specimens of esophageal tumors and corresponding normal tissue samples were obtained from 70 patients through surgery. All samples were divided into two parts: one for histopathological examination, the other was frozen in liquid nitrogen immediately after removal and stored at −80°C until RNA extraction or immunohistochemistry.

Quantitative Analysis of mRNA Expression. Total cellular RNA was extracted according to standard procedures using the InViScript PCR systems kit (InViTek, Berlin, Germany). cDNA was synthesized according to standard protocols. The presence of each cDNA was detected by PCR amplification. Primers and *Taq* Man probes were as described elsewhere (17, 18): CD8 (forward) 5′-CCCTGAGCAACTCCATCATGT-3′; CD8 (reverse) 5′-GTGGGCTTCGCTGGCA-3′; and CD8 (probe) FAM-TCAGCCACTTCGTGCCGGTCTTC-TAMRA; IFN-γ (forward) 5′-AGCTCTGCATCGTTTTGGGTT-3′; IFN-γ (reverse) 5′-GTTCCATTATCCGCTACATCTGAA-3′; and IFN-γ (probe) FAM-TCTTGGCTGTTACTGCCAGGACCCA-TAMRA. Quality of RNA preparations was determined with primers specific for β-actin as an internal control. Thermal cycler parameters included 2 min at 50°C, 10 min at 95°C, and 40 cycles involving denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Gene expression was determined with the use of the Applied Biosystems Prism 7700 Sequence Detection System (Perkin-Elmer, Norwalk, CT). Standard curves were generated for both CD8 and IFN-γ. Real-time monitoring of fluorescent emission from cleavage of sequence-specific probes by the nuclease activity of *Taq* polymerase allowed definition of the threshold cycle during the exponential phase of amplification (19). All PCR assays were performed in triplicates and reported as the average. For quantification of CD8 mRNA, data were adjusted for β-actin mRNA copies. Data for IFN-γ mRNA were adjusted for CD8 mRNA copies on the assumption that stimulation with HLA class I-restricted epitopes leads to proliferation of CD8$^+$ T cells as the relevant population (18, 20). Finally, PCR products were also separated in a 2% agarose gel and semiquantified from video images by densitometry using a Fluorimager SI (Molecular Dynamics, Wiesloch, Germany). cDNA samples obtained from hyperplastic lymph node tissue as well as from an influenza matrix peptide recognizing the CD8$^+$ cytotoxic T cell line (compare "Immunohistochemistry") served as positive controls.

Immunohistochemistry. Acetone-fixed cryostat sections were stained with H&E and the following monoclonal antibodies: rat antihuman CD8

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[2] The abbreviations used are: TIL, tumor-infiltrating lymphocyte; i.t., intratumoral; p.t., peritumoral; SC, scattered; SCC, squamous cell carcinoma; DAPI, 4′,6-diamidino-2-phenylindole; PBMC, peripheral blood mononuclear cell; UICC, Union Internationale Contrele Cancer; MACS, magnetic cell sorting.

(IgG1; Serotec, United Kingdom), mouse antihuman IFN-γ (IgG1; PharMingen, San Diego, CA), mouse anti-Mib-1 (IgG1; Dako, Glostrup, Denmark), and rat IgG1 and murine IgG1 isotype control antibodies (PharMingen). Alexa Fluor 488-goat antirat IgG (H + L) antibody (Molecular Probes, Eugene, OR) was used as second-step reagent for detection of $CD8^+$ T cells. Alexa Fluor 568-goat antimouse IgG (H + L) antibody (Molecular Probes) was used as a second-step reagent for detection of Mib-1 and IFN-γ-positive cells, respectively. In single-color immunohistochemical analysis, nucleic counterstaining was performed with propidium iodide (Dako) whereas DAPI (Dako) was used for nucleic counterstaining of double immunofluorescence stainings. The slides were mounted with antifading reagent (Dako). Positive controls included hyperplastic lymph node tissue and a $CD8^+$ T cell line. The latter was established after weekly restimulation of peripheral blood lymphocytes with influenza matrix peptide p58–66 according to standard protocols (21). Negative controls included cytospin preparations from unstimulated $CD8^+$ T cells that were derived from PBMC and negatively enriched by MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) as described elsewhere.[3] Control stainings also included incubation with isotype control antibodies as first-step reagents as well as incubation with the second-step reagent only.

Histological Analysis. $CD8^+$ T cells, proliferating $CD8^+$ T cells, and IFN-γ-secreting $CD8^+$ T cells were quantified by systematically screening the entire cancer area of at least two sections obtained from different areas of the tumor using a modification of the method of Naito *et al.* (7) as follows: For the quantitative evaluation of i.t. and p.t. $CD8^+$ T cell presence in representative sections, a minimum of 10 microscopic fields (area, 0.0625 mm^2) was chosen and examined by fluorescence microscopy at ×400 magnification with a BX50 microscope (Olympus, Hamburg, Germany) and excitation cubes for UV (330–385 nm), blue (460–490 nm), and green (520–550 nm) fluorescence as well as a triple-band filter cube (DAPI/FITC/propidium iodide). Average numbers of >50 accumulating $CD8^+$ TILs per 3 high-power fields were scored as infiltration. Then, individual cases were classified into the following four groups by their location and quantification: i.t. infiltration (group i.t.), $CD8^+$ TILs are located in the epithelial compartment of the tumor accumulating within cancer cell nests and complexes; p.t. infiltration (group p.t.), $CD8^+$ T cells localize predominantly in the mesenchymal stroma, which surrounds the epithelial compartment of the tumor; SC (group SC), $CD8^+$ T lymphocytes are found sparsely and evenly distributed between the epithelial and stromal compartments and/or along the invasive margin of the tumor; and nil (group 0), no $CD8^+$ T cells are detectable in the epithelial and stromal compartments or along the invasive margin of the tumor. All counting was performed independently by two investigators (K. S. and W. H.) without knowledge of clinical information. Variations in the percentage of stained cells enumerated by the two investigators were within a range of 5%. The remaining slides were reevaluated and a consensus decision was made. Additionally, results obtained by quantitative mRNA analysis were compared to the immunohistochemistry data.

Clinicopathological Data. The mean age among the patients (58 males and 12 females) at the time of diagnosis was 60.2 years. Staging distribution of the tumors revealed 8 stage I patients, 17 stage II patients, 29 stage III, and 16 stage IV (pM1a(LYM)) patients according to the classification of the UICC. All patients were locoregionally R0-resected. Thirty-three samples were obtained from SCCs and 37 samples were obtained from adenocarcinomas. All data including gender, age, stage of disease, and pathological factors were obtained from the clinical and pathological records. Patients were monitored every 2–6 months.

Statistical Analysis. The statistical analysis was performed using the χ^2 test or Fisher's exact probability test. For recurrence-free interval and survival, Kaplan-Meier curves were generated and the log rank statistic was used. Multivariate analysis of $CD8^+$ T cell presence adjusting for pathological markers was performed using the Cox proportional hazard regression model. The significance level was set at $P < 0.05$. All analyses were performed using the SPSS statistical software (SPSS version 9.0 for Windows; SPSS Inc., Chicago, IL).

[3] K. Schumacher, W. Haensch, C. Röefzaad, W. Kemmner, G. C. Spagnoli, and P. M. Schlag. Heterogeneous expression pattern of cancer-testis antigens of the MAGE-A family and NY-ESO-1 on primary and related metastatic tumor sites of upper GI tract neoplasms depends on histotype and tumor stage, submitted for publication.

RESULTS

Analysis of $CD8^+$ T Cells. In tumor samples, 11 of 70 (16%) analyzed primary tumors showed i.t. $CD8^+$ T cell infiltration accumulating within the epithelial compartments of the tumors. In 22 of 70 (31%) cases, $CD8^+$ T cells were localized p.t. with infiltrates predominantly in the mesenchymal stroma around but not within the epithelial complexes. In 37 of 70 (53%) cases, only SC or no $CD8^+$ T cells could be detected within the epithelium or the surrounding stromal compartments. It is important to note that all 11 tumors belonging to the group i.t. also contained $CD8^+$ T cell accumulations within the surrounding stromal compartments. Examples for i.t., p.t., or SC $CD8^+$ T cell presence are shown in Fig. 1. No significant differences were noted between the expression levels obtained by mRNA analysis and immunohistochemistry.

Interestingly, there seems to be an association between the nodal status at the time of tumor resection and the presence of i.t. $CD8^+$ T cell infiltrations (Table 1). The frequency of i.t. $CD8^+$ T cell infiltrations declined from nodal-negative (30% i.t. and 30% p.t. $CD8^+$ T cells) to nodal-positive stages (9% i.t. and 32% p.t. $CD8^+$ T cells; $P = 0.015$). In parallel, increasing size of the primary tumor (T stage) inversely correlated with $CD8^+$ T lymphocyte presence ($P = 0.008$). All patients with i.t. $CD8^+$ T cell infiltrations belonged to the group with the smaller T_1 and T_2 tumors. There was also a tendency of a higher frequency in i.t. $CD8^+$ T cell infiltrations within well-differentiated tumors ($P = 0.040$). There was no difference between the presence of i.t. $CD8^+$ T cells within the histological subtypes ($P = 0.112$).

Activity of $CD8^+$ T Cells at the Primary Tumor Sites. To estimate the activation and proliferation status of $CD8^+$ T cells in esophageal tumors *in vivo* without the knowledge on defined tumor antigens, we performed double immunohistochemical staining for CD8 and IFN-γ or CD8 and Mib-1, respectively. Intratumoral $CD8^+$ T cell infiltrations contained proliferating areas that also included IFN-γ-secreting cells, which could be detected by both quantitative mRNA analysis (Table 2) and immunohistochemistry (Fig. 1).

Follow-Up of Esophageal Cancer Patients with $CD8^+$ T Cells at Different Locations. At the end of the follow-up period, 40 of 70 (57%) patients were still alive. Median follow-up after R0 resection of primary tumors for the 40 censored patients was 17 months (range, 6–85 months). Median overall survival was 15.5 months. One-year overall survival was 70%; 3-year overall survival was 58%. Monovariate analysis revealed that $CD8^+$ T cells within the tumors had a significant impact on the recurrence-free and overall survival of the patients (Table 3). These differences were significant when the Breslow-Gehan-Wilcoxon test (stressing the first 3-year postoperative period) and also when the Cox-Mantel log rank test (stressing the end points) were applied (Fig. 2; Table 4). Especially i.t. $CD8^+$ T cell infiltration resulted in long-term disease-free survival. As shown in Fig. 2, the patients showed a longer disease-free and overall survival as the presence of $CD8^+$ T cells within the tumors increased.

Multivariate Regression Analysis of Survival in Esophageal Carcinomas. In the multivariate regression analysis using the Cox proportional hazards model, the significant risk factor was UICC staging with a relative risk of 2.3, indicating that this factor is a risk factor (Table 4). $CD8^+$ T cell presence within esophageal tumors is also significant with a relative risk of 0.5, indicating favorable prognosis. In this model, variables were included that have been shown in previous studies to be of prognostic value (10).

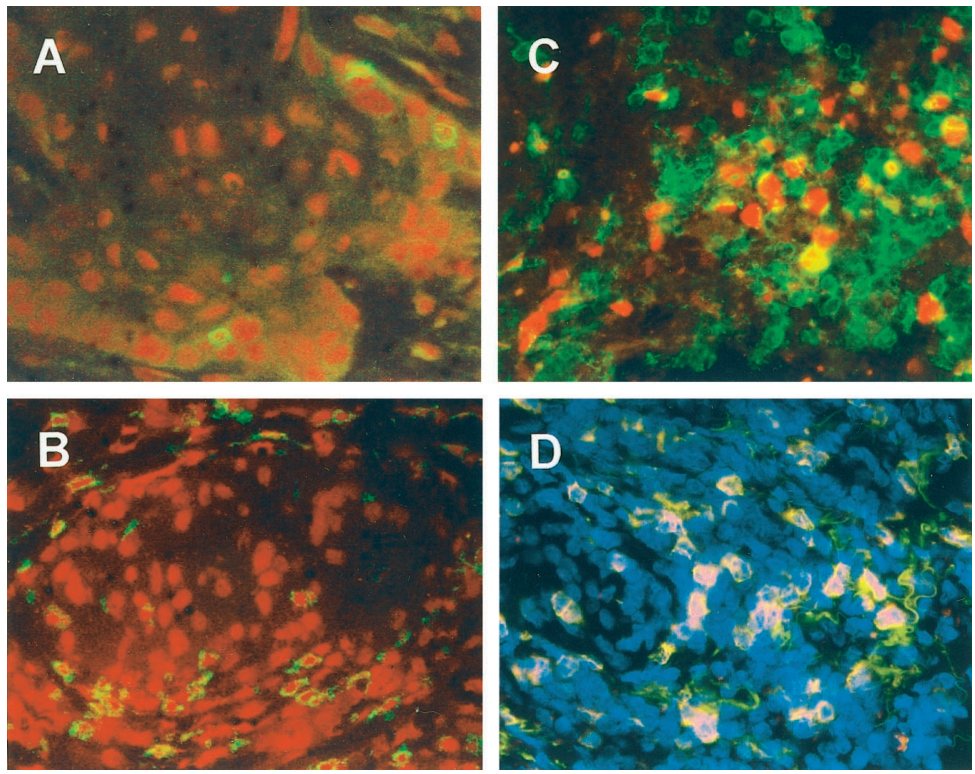

Fig. 1. Presence of $CD8^+$ T cells within esophageal carcinomas is often scattered (*A*) or $CD8^+$ T lymphocytes are located mainly in the stroma surrounding epithelial complexes (*B*), whereas infiltration within the epithelial tumor cell nests can be colocalized with proliferation markers (*C*) and IFN-γ immunoreactivity (*D*). Immunofluorescence analysis of frozen sections was performed with CD8 (*A–D*), Mib-1 (*C*), and IFN-γ (*D*) as described in "Materials and Methods." Alexa Fluor 488-conjugated goat antirat IgG (green) was used as a second-step reagent for CD8 cell detection. Alexa Fluor 568-conjugated goat antimouse IgG (red) served for detection of IFN-γ and Mib-1 immunofluorescence, respectively. Nuclear counterstaining was performed with propidium iodide (red, *A* and *B*) or DAPI (blue, *D*) or was not performed (*C*), and overlays of the stainings are shown. *A*, SC presence of $CD8^+$ T cells within a UICC stage IV SCC. *B*, p.t. localization of $CD8^+$ T cells within a UICC stage III SCC. *D*, colocalization of IFN-γ secretion and CD8 T cell presence in a UICC stage II SCC patient with i.t. $CD8^+$ T cell infiltrations. Results obtained by quantitative mRNA analysis of this patient (P1) are shown in Table 2. Note also the presence of proliferative $CD8^+$ T cells within the tumor (*C*). Original magnifications: *B*, ×200; *A*, *C*, and *D*, ×400.

DISCUSSION

Tumor-related antigens can be recognized by CTLs in the context of MHC class I-expressing tumors. Important prognostic factors for successful active antigen-specific immunotherapy are therefore (*a*) homogeneous expression of target antigens in tumors; (*b*) conservation of MHC class I-restriction elements throughout tumor progression; and (*c*) induction of CTLs (22).

In the present study, we analyzed $CD8^+$ TILs within different esophageal neoplasms to demonstrate that i.t. infiltration of activated $CD8^+$ T cells can be a prognostic factor related with favorable survival in both SCCs and adenocarcinomas. Our findings are in accordance with earlier reports about the expression of $CD8^+$ T cells in other human tumor systems including colorectal adenocarcinomas that also showed a positive prognostic significance with regard to patient survival (7). Similar analyses in colon and stomach tumors morphologically showed proliferation of i.t. $CD8^+$ T cells by the description of Mib-1 immunoreactivity (7, 12). However, immunohistochemical evaluation of proliferating $CD8^+$ T cells does not allow conclusions concerning their functional proliferation status. Therefore, in addition, IFN-γ secretion of $CD8^+$ TILs in esophageal tumors was quantified by real-time PCR and localized immunohistochemically, indicating that the esophageal tumor epithelium may contain activated TILs.

Analysis of our cohort of 70 R0-resected esophageal cancer patients reveals survival data that are comparable to previous studies (10, 11, 23). Two-year survival in a series of 63 patients was 51% (10) (present cohort, 62%), 3-year survival was reported to be 77.5% in a cohort of 40 tumor patients without lymph node involvement (present cohort, 82%) and between 28 and 64.8% in 49 patients with lymph node-positive disease (present cohort, 49%) (23). Therefore, selection

Table 1 *Presence of $CD8^+$ T cells within esophageal tumors decreases with tumor progression*

Levels of $CD8^+$ T cells were estimated in 70 patients after complete resection of primary esophageal carcinomas as described in "Materials and Methods." Statistic analysis was performed using the χ^2 test. Statistical significance was set at $P < 0.05$. Data in parentheses are percentages.

Total primary tumors (70 cases)	No. of cases	$CD8^+$ T cells			*P*
		i.t.	p.t.	SC/nil	
pT Stage[a]					0.008
pT_1	13	4 (31)	7 (54)	2 (15)	
pT_2	23	7 (30)	4 (17)	12 (52)	
pT_3	28	0 (0)	9 (32)	19 (68)	
pT_4	5	0 (0)	1 (20)	4 (80)	
Lymph node metastasis[a]					0.015
pN_0	23	7 (30)	7 (30)	9 (40)	
pN_+	47	4 (8)	15 (32)	28 (60)	
Tumor grading[a]					0.040
Low	6	3 (50)	2 (33)	1 (17)	
Moderate	27	3 (11)	9 (33)	15 (56)	
High	37	5 (13)	11 (30)	21 (57)	
Histotype					0.112
Adenocarcinoma	37	8 (22)	8 (22)	21 (56)	
SCC	33	3 (9)	14 (43)	16 (48)	

[a] Classified according to Tumor-Node-Metastasis classification. pT, tumor size; pN, lymph node involvement.

Table 2 *Reactivity of $CD8^+$ T cells within and around esophageal tumors*

Representative examples for quantitative PCR on tumor (Tm) and adjacent normal mucosa (Nm) samples from patient P1 with i.t. $CD8^+$ T cell infiltrations and patient P2 with p.t. location of $CD8^+$ T cells. Mean values of triplicate analysis are shown. Comparative immunofluorescence staining for patient P1 is shown in Fig. 1.

	Patient	CD8	IFN-γ	β-actin	CD8/β-actin[a]	IFN-γ/CD8[b]
$CD8^+$ i.t.	P1:Tm	30.85	34.40	20.23	1.52	1.12
	P1:Nm	29.72	32.11	21.83	1.36	1.08
$CD8^+$ p.t.	P2:Tm	33.35	35.72	27.79	1.20	1.07
	P2:Nm	33.37	35.92	21.78	1.53	1.08
PBMC[c]		32.19	34.97	25.22	1.28	1.09
$CD8^+$ flu[d]		24.18	27.99	20.15	1.20	1.16

[a] Values represent copies of CD8 mRNA per copies of β-actin mRNA.
[b] Values represent copies of IFN-γ mRNA per copies of CD8 mRNA.
[c] Controls included unstimulated $CD8^+$ T cells from PBMC.
[d] Controls included influenza-matrix peptide-specific $CD8^+$ T cells 2 h after peptide restimulation (see also Refs. 17, 18, and 20) that were generated from PBMC as described in "Materials and Methods."

bias with the gathering of patients for the present investigation seems unlikely as cause for the observed effect of CD8+ T cells within esophageal tumors on survival.

Despite several immunohistological analyses on tumor-infiltrating effector cells in various types of carcinoma (24–31), little information was available about the distribution and significance of CD8+ T cells in the different esophageal tumor types. There are several indicators that the two major esophageal histological subtypes, adenocarcinomas and SCCs, vary in terms of their functional molecular genetics, which also become obvious by alterations in their tumorigenesis. For example, there has been reported a frequent loss of the *p16* gene in esophageal SCC but not in adenocarcinomas of the esophagus or stomach (32). We recently found a higher expression of cancer testis antigens in primary esophageal SCCs than in adenocarcinomas.[3] Immunohistological analysis of esophageal carcinomas revealed reductions in MHC class I antigen expression as compared to normal tissues that could be correlated to significantly reduced survival rates in SCCs in contrast to adenocarcinomas (16). Also, in colorectal adenocarcinomas, MHC class I is usually quite homogeneously expressed within the tumors and there is no evidence for survival impairments in patients with decreased levels of MHC class I immunoreactivity (33). The presence of CD8+ T cell infiltrations could therefore indicate the importance of specific immune effector cells within several tumor systems despite etiopathological differences. However, it is important to note that the frequency of i.t. CD8+ T cell infiltrations usually was low in both esophageal tumor entities.

Metastatic relapse attributable to the presence of tumor cells within lymph nodes is the most frequent cause leading to cancer-related deaths in patients with esophageal tumors (10). Our data suggest that CD8+ T cells within esophageal tumors function not only locally but also systemically in tumor-draining lymph nodes to suppress micrometastasis. Previous reports on immunohistochemical analysis of infiltrating CD8+ T cells in EBV-associated gastric cancer showed significantly higher levels of proliferative activity and perforin granules in these tumors (12). The occurrence of IFN-γ-secreting cells in our analyzed esophageal tumors suggests that parts of the CD8+ T cells within the tumors are activated CTLs (17, 18, 20). Recently, it was shown for chronic myelogenous leukemia patients that CTLs could be antigen specific and functional. Their presence can correlate with cytogenetic responses and these CTLs can persist *in vivo* over several years (34). However, precursor frequencies of antigen-specific T cells without previous immunotherapeutic treatment might be below detectable limits. Future studies will have to address the question on the existence of naturally occurring T cell responses in those patients with i.t. CD8+ T cell infiltrations who simultaneously express known tumor-related antigens. In conclusion, CD8+ TILs within esophageal tumors can serve as important criteria for selection and monitoring of suitable patients for adjuvant immunotherapeutic strategies and can be a reliable prognostic marker to predict favorable outcome.

Table 3 *Time-to recurrence and time-to death dates in esophageal cancer patients depend on localization and amount of CD8+ T cells within the tumors*

Presence of i.t., p.t., or SC or no CD8+ T cells in 70 primary carcinomas of the esophagus was compared to clinical follow-up. Median time to recurrence and median time to death dates are listed. Data in parentheses are ranges. As a control follow-up data for nodal negative (pN_0) *vs* nodal positive patients (pN_+) are also shown.

	No. of Patients	Time to recurrence (mo)	Recurrence (no.)	Log rank	Time to death (mo)	Death (no.)	Log rank
CD8+ i.t.	11	–	0		–	0	
CD8+ p.t.	22	20 (7–32)	13	0.0003	50 (10–89)	9	0.0004
CD8+ SC/0	37	16 (12–20)	25		18 (10–25)	21	
pN_0[a]	23	44 (35–55)	6		45 (37–51)	5	
pN_+	47	16 (13–18)	32	0.0004	33 (23–44)	25	0.0029

[a] Classified according to Tumor-Node-Metastasis classification.

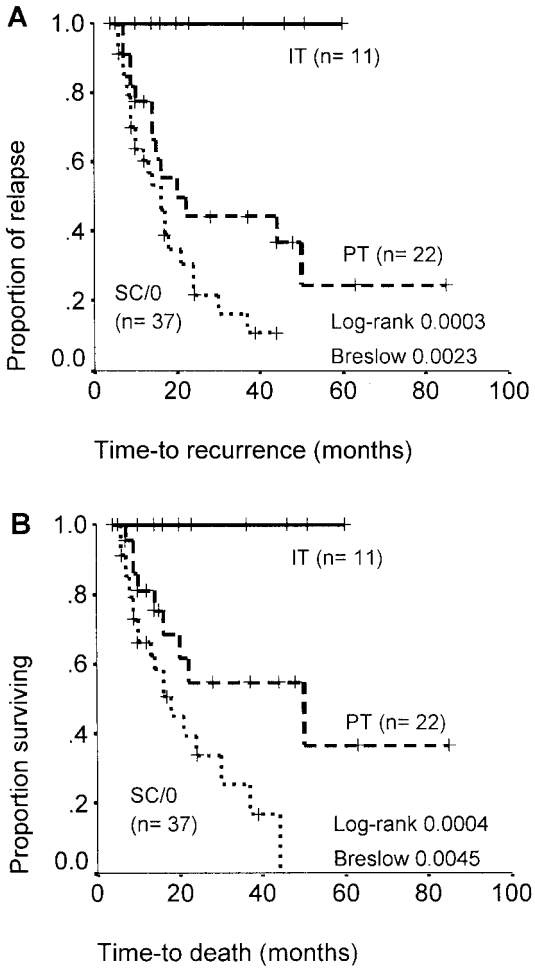

Fig. 2. *Time-to recurrence* (*A*) and *Time-to death* (*B*) rates are improved in patients as amounts of CD8+ T cells within the tumors increase. For details in the determination of CD8+ T cells within the 70 completely resected primary esophageal tumors, see "Materials and Methods." +, censored cases; *IT*, i.t.; *PT*, p.t.; *SC/0*, scattered or no presence of CD8+ T cells within esophageal carcinomas.

Table 4 *Univariate and multivariate analyses of prognostic factors for survival*

	Univariate analysis		Multivariate analysis		
	Log rank	Breslow	Relative risk	95% Confidence interval	*P*
i.t. CD8+ T cells (present)[a]	0.0004	0.0045	0.5	0.34–0.73	0.0004
UICC stage (I–IV)	0.0010	0.0032	2.3	1.50–23.53	0.0001
Nodal stage (pN_+)	0.0029	0.0020	3.9	1.47–10.41	0.0060
Histotype (adenocarcinoma/SCC)	0.7738	0.7302	1.1	0.54–2.29	0.7768

[a] CD8+ T cell infiltration within the tumor is a factor (variable) related to a favorable survival with the relative risk below 1.0.

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