Intratumoral CD8$^+$ T Lymphocytes as a Prognostic Factor of Survival in Endometrial Carcinoma

Svetlana Kondratiev,[1] Edmond Sabo,[1] Evgeny Yakirevich,[1] Ofer Lavie,[2] and Murray B. Resnick[1]

[1]Department of Pathology and [2]Division of Gynecologic Oncology, Carmel Medical Center and the Technion Rappoport Faculty of Medicine, Haifa, Israel

ABSTRACT

Purpose: **CTLs are a prominent immune component infiltrating many solid tumors. These cells are considered to be a manifestation of host-immune response to the tumor; however, their prognostic significance remains a subject of considerable debate. The objective of this study was to evaluate the distribution pattern and prognostic value of CD8$^+$ T cells in endometrial carcinoma.**

Experimental Design: **We studied 90 cases of endometrial carcinoma, including 75 endometrioid and 15 papillary serous carcinomas. Immunohistochemical staining for CD8 and granzyme B was performed on paraffin-embedded sections. The number of immunohistochemically staining CD8$^+$ T cells was enumerated in the following four regions: lymphocytes infiltrating the tumor epithelium at the invasive border, within the underlying tumor stroma, within the superficial tumor epithelium, and in the perivascular areas of the myometrium.**

Results: **Patients with >10 CD8$^+$ T lymphocytes/high-power field within the tumor epithelium at the invasive border displayed improved overall survival compared with patients with fewer intraepithelial CD8$^+$ T lymphocytes (87 and 50%, respectively; $P = 0.027$). Multivariate analysis revealed that stage, vascular invasion, grade, and the number of intraepithelial CD8$^+$ T lymphocytes at the invasive border were the only independent predictors of survival ($P < 0.0001$, $P = 0.001$, $P = 0.011$, and $P = 0.025$, respectively). Granzyme B$^+$ cytoplasmatic granules were detected in a high proportion of CTLs, confirming their activated cytotoxic phenotype.**

Conclusions: **Our study demonstrates for the first time that increased numbers of CTLs at the invasive border may be a reliable independent prognostic factor of survival in patients with endometrial carcinoma.**

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Requests for reprints: Murray Resnick, Department of Pathology, Carmel Medical Center, 7 Michal Street, Haifa, Israel. Phone: 972-4-825-0845; Fax: 972-4-825-0816; E-mail: mresnick@lifespan.org.

INTRODUCTION

Endometrial carcinoma is the most common malignancy of the female genital tract in the United States and Western Europe. Although endometrial carcinoma accounts for only 2% of all cancer-related deaths, the number of women dying of this disease is still significant (1). Traditional histopathological prognostic factors that correlate with endometrial carcinoma patient outcome include stage and grade of disease, histological subtype, and vascular invasion (2–5). Several studies have investigated the prognostic value of histopathological and molecular parameters in women with endometrial carcinoma. These non-traditional predictors of survival include DNA ploidy, estrogen receptor status, and expression of vascular endothelial growth factor, metallothioniens, p53, HER-2/neu, and bcl-2 (4, 6–10).

Tumor-infiltrating lymphocytes (TILs) are one of the major immune components infiltrating solid tumors. The majority of TILs in endometrial carcinomas express the CD8$^+$ suppressor/cytotoxic phenotype, and minor subsets express B-lymphocyte and macrophage markers (2, 11). Natural killer cells are virtually absent in endometrial tumors (11). Activated CTLs expressing TIA-1 and/or granzyme B cytotoxic granules have been demonstrated in certain human neoplasms, such as EBV-associated gastric cancer, medullary carcinoma of the breast, germ cell tumors of the testis, and carcinomas of the colon, cervix, esophagus, and kidney (12–18).

The impact of tumor-infiltrating CTLs on prognosis in endometrial carcinoma has not been addressed. The primary objective of this study was to determine the pattern of CTL distribution in endometrial carcinoma and evaluate whether the number of CTLs correlates with patient survival.

MATERIALS AND METHODS

Patient Selection. Paraffin blocks containing tissue samples that had been obtained from 90 patients with endometrial carcinoma between the years 1991 and 1999 were retrieved from the archives of the Carmel Medical Center. All tissues had been obtained by hysterectomy. None of the patients had undergone radiation or chemotherapy before surgery. Routine H&E staining was performed, and histological grade and subtype were reviewed by two pathologists (M. B. R. and S. K.). The histological grade, stage, and subtype of the tumor were determined according to the guidelines of the WHO/Fédération Internationale des Gynaecologistes et Obstetristes classification (19, 20). Histopathological types included 75 endometrioid and 15 papillary serous carcinomas. The cases of endometrioid carcinoma were stratified into the following groups: 42 low-grade (grade I), 25 intermediate-grade (grade II), and 8 high-grade (grade III) tumors.

Vascular invasion was recorded only if the tumor nests were within vascular spaces lined by endothelium. In cases where vascular invasion was equivocal on H&E-stained sec-

tions, endothelial cells were highlighted by CD31 immunohistochemistry (1:20 dilution; DAKO, Copenhagen, Denmark).

Immunhistochemical Staining. Immunohistochemical staining for mouse monoclonal anti-CD8 (clone C8/144B, 1:100 dilution; DAKO) was performed according to the following protocol: consecutive sections from formalin-fixed, paraffin-embedded tissue blocks were cut at 5 μm, deparaffinized, and dehydrated. Microwave pressure cooker pretreatment was performed in 1 mM EDTA buffer (pH 8.0), followed by cooling for 15 min at room temperature. Immunohistochemical staining was carried out with the Ventana ES automated staining system with the basic 3,3′-diaminobenzidine detection kit (Ventana Medical Systems, Tucson, AZ).

Immunohistochemical staining for granzyme B was performed manually with the primary antibody (clone Gr-7, 1:20 dilution; Monosan, Sanbio, the Netherlands). The labeled streptavidin-biotin-peroxidase method with the Histostain-Plus kit and 3-amino-9-ethylcarbazole substrate from Zymed Laboratories (South San Francisco, CA) was used. Sections were blocked with 10% goat serum for 60 min and then incubated with the primary antibody for 1 h at room temperature.

Quantification of $CD8^+$ and Granzyme B^+ T Lymphocytes. The number of $CD8^+$ T lymphocytes was determined separately in the following compartments: (*a*) within the superficial tumor epithelium; (*b*) within the tumor epithelium at the invasive border; and (*c*) within the underlying stroma (a distance of one microscopic field at ×200 magnification from the tumor–myometrium junction). The presence or absence of lymphocytic infiltrates in perivascular areas within the myometrium away from the tumor (more than one microscopic field at ×200 magnification away from the tumor–myometrial junction) was also noted. In addition, we enumerated granzyme B^+ cells in 31 tumors diagnosed between 1996 and 1999. Progressive loss of immunoreactivity for granzyme B, in contrast to well-preserved immunoreactivity for CD8, was noted in tissues from cases obtained before 1996.

The number of cells was counted with use of a computerized image analysis system composed of a Trichip RGB video camera (SONY, Tokyo, Japan), installed on an Olympus BX50 light microscope and attached to an IBM-compatible personal computer (Pentium III, MMX, 450 MHz, 125 MB RAM) equipped with a frame grabber. Histological images were captured, digitized, and displayed on a high-resolution color 17-inch monitor. Histomorphometrical measurements were performed with Image Pro Plus 4.5 software (Media Cybernetics, Baltimore, MD). Five microscopic fields (×200), representing the most dense lymphocytic infiltrates were selected for each case. The results were expressed as the mean (±SE) number of cells for one computerized ×200 microscopic field in different compartments or as the mean (±SE) number of cells surrounding one vascular space.

Statistical Analysis. The distribution of variables was tested with the Kolmogorov–Smirnov test for normality. Comparison among three nonparametric groups was performed with the Kruskal–Wallis nonparametric ANOVA test followed by the Dunn's *post hoc* test. Two groups were compared with the Mann–Whitney *U*-test. Associations were tested with the χ^2 test of the Fisher's exact test as appropriate. Univariate survival analysis included the construction of Kaplan–Meier curves for the different clinical and pathological variables, using the product limit method. Survival times were compared with the log-rank test. Multivariate analysis was performed with the Cox's proportional hazard model. A two-tailed $P \leq 0.05$ was considered statistically significant.

Table 1 Clinicopathological characteristics of the patients with endometrial carcinoma

	No. of patients (%)
Type	
Endometrioid	75 (83)
Papillary serous	15 (17)
FIGO[a] grade (endometrioid)	
G1	42 (56)
G2	25 (33)
G3	8 (11)
FIGO stage (endometrioid)	
I	50 (66)
II	17 (23)
III	8 (11)
FIGO stage (papillary serous)	
I	3 (20)
II	5 (33)
III	6 (40)
IV	1 (7)

[a] FIGO, Fédération Internationale des Gynaecologistes et Obstetristes.

RESULTS

Clinicopathological Parameters. The clinicopathological characteristics of the patients with endometrial carcinoma are represented in Table 1. The mean age of the patients was 71 years, and clinical follow-up was available for all cases. The median follow-up period was 60 months (range, 1–106 months). The endometrioid carcinoma group included 75 patients: 50 with tumor confined to the uterine corpus (stage I), 17 with uterine cervix involvement (stage II), and 8 with pelvic extension (stage III).

Correlation of the Distribution Pattern of $CD8^+$ Lymphocytes with Classical Prognostic Variables in Endometrial Carcinoma. The distribution pattern of $CD8^+$ lymphocytes was similar to that of lymphocytes by conventional H&E staining. Shown in Fig. 1 is a representative case of endometrioid endometrial carcinoma that illustrates the distribution pattern of $CD8^+$ T lymphocytes in the four compartments evaluated: intraepithelial lymphocytes within superficial tumor epithelium, within the tumor epithelium at the invasive border, within the underlying stroma, and in the perivascular areas of the myometrium. The numbers of $CD8^+$ T lymphocytes in various compartments of the endometrioid carcinoma group are shown in Table 2. In the papillary serous carcinoma group, the numbers of $CD8^+$ T lymphocytes were 9.9 ± 2.3, 13.2 ± 3.0, and 21.1 ± 4.0 within the superficial tumor epithelium, within the tumor epithelium at the invasive border, and within the underlying stroma, respectively. The number of $CD8^+$ lymphocytes in different compartments did not differ significantly between the high-grade (grade 3) endometrioid and papillary serous carcinoma groups.

The relationship between the number of $CD8^+$ lympho-

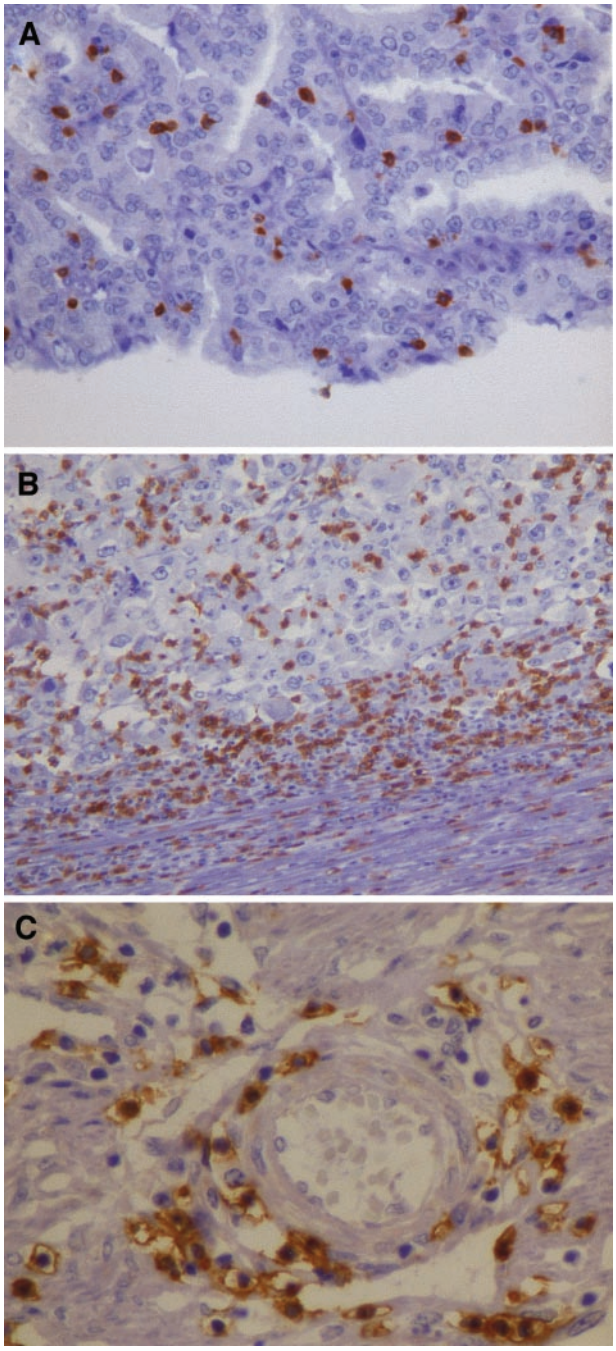

Fig. 1 Immunohistochemistry for cytotoxic markers. Distribution of CD8$^+$ lymphocytes in superficial tumor epithelium (*A*), tumor epithelium at the invasive border (*B*; *upper*) and underlying stroma (*B*; *lower*), and perivascular areas in the myometrium (*C*).

cytes and other clinicopathological variables was assessed by univariate analysis. As shown in the Table 2, tumor grade and stage were not significantly associated with the number of CD8$^+$ lymphocytes present in the compartments evaluated. The number of CD8$^+$ lymphocytes in the underlying tumor stroma significantly correlated with the presence of vascular invasion. In tumors with vascular invasion, the number of CD8$^+$ lymphocytes was higher (32.9 $\pm$ 5.0) than in tumors without vascular invasion (23.4 $\pm$ 2.0; $P = 0.05$). The numbers of CD8$^+$ lymphocytes in other areas did not correlate with vascular invasion.

Immunohistochemistry revealed that the majority (70.3 $\pm$ 11.5%) of the lymphocytes within the perivascular infiltrates were of the CD8$^+$ cytotoxic phenotype. The number of perivascular CD8$^+$ lymphocytes was significantly associated with vascular invasion. As demonstrated in the Table 2, tumors with $>$20 perivascular CD8$^+$ lymphocytes showed an increased risk for vascular invasion. Seven of nine (77%) cases with vascular invasion exhibited $>$20 perivascular CD8$^+$ lymphocytes compared with 11 of 66 (16.6%) cases without evidence of vascular invasion ($P = 0.0004$; odds ratio = 17.5).

Survival and Classical Variables. By univariate analysis stage, grade, and vascular invasion all correlated significantly with patient survival in the endometrioid carcinoma group (stage, $P = 0.0054$ for I *versus* II; grade, $P = 0.042$ for I *versus* II and 0.022 for I *versus* III; vascular invasion, $P < 0.0001$; Fig. 2, *A–C*).

Survival and CD8$^+$ Lymphocytes. A significant correlation was found between the number of intraepithelial CD8$^+$ T lymphocytes at the invasive border and patient outcome in the endometrioid carcinoma group (Fig. 3). Greater overall survival was seen in patients with tumors exhibiting $\geq$10 intraepithelial lymphocytes/field ($\times$200) at the invasive border ($P = 0.027$). At the end of the study, 87% of the patients with $>$10 lymphocytes/field were alive compared with 50% of patients with $<$10 lymphocytes/field. The number of lymphocytes present within the underlying stroma and in the superficial tumor epithelium did not show a significant correlation with prognosis. The number of perivascular lymphocytes (total number or CD8$^+$ subsets) also did not correlate with survival.

Survival in the Papillary Serous Carcinoma Group. Histological tumor type correlated significantly with patient survival by univariate analysis (Fig. 2*D*). At the end of the follow-up, 71% of the patients with endometrioid carcinoma were alive compared with 22% patients within the papillary serous carcinoma group ($P < 0.0001$). Within the papillary serous carcinoma group, stage was the only significant predictor of patient survival ($P = 0.009$). Univariate analysis did not show significant association between the number of CD8$^+$ lymphocytes and other clinicopathological variables in the papillary serous carcinoma group. In the papillary serous carcinoma group, the number of CD8$^+$ lymphocytes did not correlate significantly with patient survival in any of the compartments ($P > 0.1$).

Evaluation of Granzyme B Immunoreactivity. To evaluate the activation status of the cytotoxic CD8$^+$ lymphocytes, we stained 31 tumors for granzyme B. Granzyme B$^+$ cells stained in a distinctly granular cytoplasmatic pattern. In areas of necrosis, a population of cells exhibiting diffuse rather than granular cytoplasmatic pattern morphologically consistent with neutrophils was detected. These cells were not enumerated. The mean proportion of granzyme B$^+$ to CD8$^+$ lymphocytes at the invasive border was 0.32 $\pm$ 0.07 in the endometrioid carcinoma group and 0.31 $\pm$ 0.09 in the papillary serous carcinoma group.

Multivariate Analysis of Survival. Multivariate analysis included all patients (endometrioid and papillary serous

Table 2 Distribution of $CD8^+$ lymphocytes in the different compartments of the endometrioid carcinoma group
The results are expressed as mean ± SE number of cells per microscopically computerized field (×200).

Variable	Superficial tumor epithelium	*P*	Tumor epithelium at invasive border	*P*	Underlying stroma	*P*	No. (%) of cases with >20 $CD8^+$ PLI[a]	*P*
Grade								
G1 (n = 42)	12.3 ± 1.3		14.4 ± 1.4		21.3 ± 1.7		7 (17)	
G2 (n = 25)	16.1 ± 4.1	0.75	24.8 ± 5.7	0.48	30.2 ± 4.3	0.29	5 (20)	0.80
G3 (n = 8)	12.2 ± 3.4		11.8 ± 3.8		24.8 ± 8.2		2 (25)	
Stage								
I (n = 50)	12.7 ± 1.8		16.1 ± 2.4		22.4 ± 2.2		9 (18)	
II (n = 17)	11.1 ± 2.3	0.96	12.7 ± 2.2	0.73	22.1 ± 3.0	0.54	2 (12)	0.73
III (n = 8)	20.6 ± 7.6		27.0 ± 7.0		35.2 ± 4.8		2 (25)	
Vascular invasion								
Absent (n = 66)	13.0 ± 1.7		17.1 ± 2.3		23.4 ± 2.0		11 (16.6)	
Present (n = 9)	18.4 ± 7.1	0.61	19.3 ± 6.0	0.68	32.9 ± 5.0	0.05	7 (77)	0.0004

[a] PLI, perivascular lymphocytic infiltrates.

carcinomas) to adjust for all variables, including the histological tumor type. The multivariate test revealed that the only independent predictors of survival were the stage ($P < 0.0001$), grade ($P = 0.011$), the presence or absence of vascular invasion ($P = 0.001$) and the number of intraepithelial $CD8^+$ lymphocytes at the invasive border ($P = 0.025$; Table 3). As seen from the odds ratios, stage had the highest impact on survival, followed by vascular invasion and grade (odds ratios of 8.56, 5.33, and 3.04, respectively). Patients with <10 $CD8^+$ lymphocytes/field had a 2.79-fold increased risk for unfavorable outcome (Fig. 4).

In addition, we analyzed the effect of CTLs on survival rates in relation to other prognostic factors, such as grade and stage, by multivariate analyses. In early-stage, low-grade tumors, the number of $CD8^+$ cells did not impact survival rates as significantly as in cases with high-grade, advanced-stage tumors

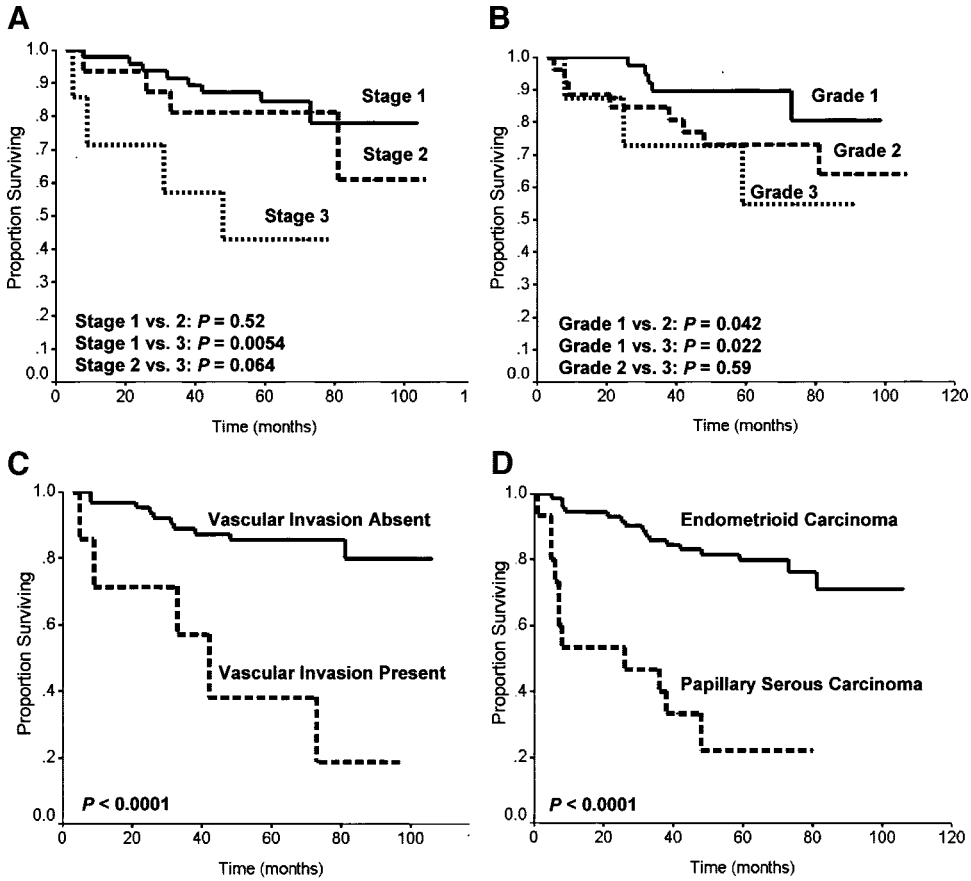

Fig. 2 Univariate analysis of survival in endometrioid endometrial carcinoma according to Fédération Internationale des Gynaecologistes et Obstetristes grade (*A*), Fédération Internationale des Gynaecologistes et Obstetristes stage (*B*), vascular invasion (*C*), and histological type (*D*).

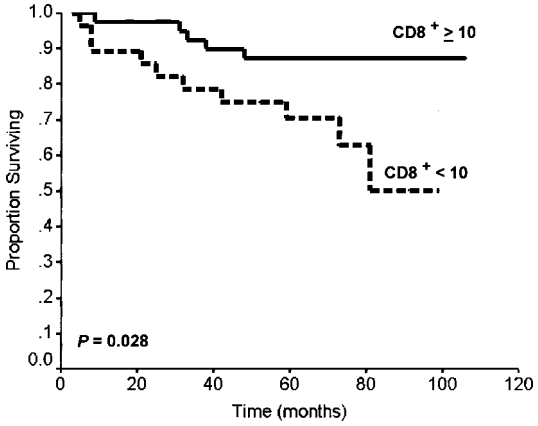

Fig. 3 Kaplan–Meier survival curves for endometrioid endometrial carcinoma patients, with 10 CD8$^+$ lymphocytes/computerized ×200 field used as the cutoff point.

Table 3 Multivariate analysis of survival (Cox regression)

Variable	β	SE	*P*
FIGO[a] stage	2.14	0.47	<0.0001
Vascular invasion	1.67	0.48	0.001
FIGO grade	1.11	0.43	0.011
CD8^{+b}	1.02	0.46	0.025

[a] FIGO, Fédération Internationale des Gynaecologistes et Obstetristes.

[b] CD8$^+$ lymphocytes in tumor epithelium at the invasive border.

(Fig. 5); therefore, the prognostic value of CD8$^+$ T lymphocytes is enhanced when the stage and the grade are increased.

DISCUSSION

CTLs are often the most prominent immune component infiltrating solid tumors; however, their biological and prognostic significance remains a subject of considerable debate. The survival advantage of marked lymphocytic infiltration has been demonstrated in patients with malignant melanoma; colorectal, esophageal, and cervical carcinoma; medullary carcinoma of the breast; and epithelial ovarian cancer (12, 14, 15, 17, 21, 22). In contrast, the presence of activated CTLs correlated positively with aggressive disease and with reduced overall survival in patients with Hodgkin's or non-Hodgkin's lymphoma (23, 24), and more abundant infiltration of tumor tissue by CTLs was associated with shorter survival in patients with renal cell carcinoma (18).

In the present study we demonstrate for the first time that infiltration of CD8$^+$ T cells in the tumor epithelium at the invasive border is a favorable prognostic factor in endometrial carcinoma patients. Two major methodological aspects differentiate this study from other studies that have characterized TILs in solid tumors. First, we analyzed TILs separately within four compartments: TILs infiltrating tumor epithelium at the invasive border, within the underlying stroma, in the superficial tumor epithelium, and in the perivascular areas within the proximal myometrium. Second, the number of lymphocytes was assessed quantitatively by computer morphometry, compared with previous studies based on semiquantitative analysis (4, 11, 25, 26).

The presence of a host-immune antitumor response in endometrial carcinoma has been reported previously in several studies. In agreement with our results, Silverberg *et al.* (27) reported that lymphocyte infiltrates were localized to the tumor–myometrium junction. This finding was more common in high-grade endometrial carcinoma; however, there was no correlation between the lymphocytic infiltrate and patient survival. According to Ambros and Kurman (2), the presence of TILs at the tumor–myometrium junction in low-grade endometrial carcino-

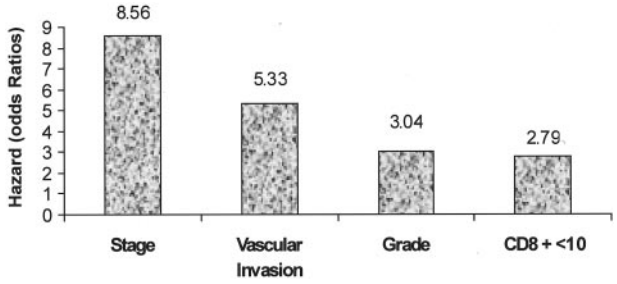

Fig. 4 Risk factors in patients with endometrioid endometrial carcinoma. Stage and grade according to the Fédération Internationale des Gynaecologistes et Obstetristes classification.

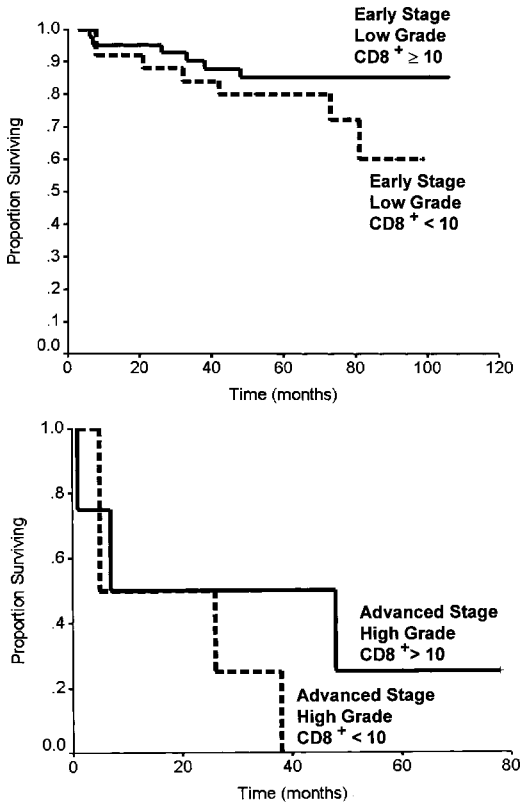

Fig. 5 Effect of CD8$^+$ T lymphocytes on survival rates in relation to other prognostic factors (stage and grade). A cutoff of 10 CD8$^+$ lymphocytes/computerized ×200 field was used.

mas also did not correlate with survival. In contrast, Deligdisch (28) found a higher frequency of TILs in low-grade endometrial carcinoma and suggested that TILs are associated with a more favorable prognosis. In each of these reports the authors described lymphocytic infiltrates at the tumor–myometrium junction, referring to the total number of lymphocytes, including both B and T lymphocytes. In contrast to these studies, we enumerated only $CD8^+$ lymphocytes and determined their prognostic value in three distinct compartments as described above.

Other studies have addressed the significance of perivascular lymphocytic infiltrate (PLI) in endometrial carcinoma (2, 25, 26). This subject remains controversial. Ambros and Kurman (2) found that PLI was associated with decreased survival; however, Lee *et al.* (26) and Yamazawa *et al.*(25) showed that PLI had a favorable impact on prognosis. In our study we also evaluated PLI and found that a significant proportion of lymphocytes surrounding lymphovascular vessels within the myometrium were $CD8^+$ T lymphocytes. Their presence in the perivascular areas was strongly associated with tumor vascular invasion; however, our study did not confirm the usefulness of PLI as a predictor of survival.

A significant proportion (32%) of TILs in this study were granzyme B^+, confirming their activated phenotype. To our knowledge, this is the first description of TIL activation in endometrial carcinoma. Increased proportions of granzyme B^+/$CD8^+$ or granzyme B^+/$CD3^+$ lymphocytes have been detected in colorectal, renal, breast, and cervical carcinomas (range, 15–72%; Refs. 12, 14, 15, 18).

The mechanism of TIL activation and distribution of activated TILs in endometrial carcinoma is not clear. Lymphocyte activation and proliferation may occur after presentation of a tumor-specific antigen by professional antigen-presenting cells or by tumor cells themselves in a HLA-restricted fashion (29). A family of melanoma-associated antigens that encode tumor antigens recognized by CTLs has been demonstrated in certain neoplasms, including germ-cell tumors (30–32). We recently found strong melanoma-associated antigen-A4 and NY-ESO-1 cancer-testis antigen expression in a subset of uterine malignancies (33). It will be interesting to determine whether the presence of $CD8^+$ and/or granzyme B^+ CTLs correlates with cancer-testis antigen expression. MHC class I molecules expression is required by professional antigen-presenting cells or neoplastic cells for specific CTL activation. Ferguson *et al.* (11) reported that MHC class I antigens were detected in four of eight endometrial carcinomas compared with their normal tissue counterparts. Failure to express MHC class I antigens by malignant cells arises from their ability to transform and be selected during tumor progression and is thought to be an advantage of tumor resistance to attack by cytotoxic T cells (11). Interestingly, Ferguson *et al.* (11) demonstrated that some endometrial carcinomas express MHC class II DR antigen on the epithelial cells, suggesting that other antitumor mechanisms also play a role in the immune response. Alternatively, the CTLs in endometrial carcinoma may be nonspecifically activated by a mechanism of a general inflammatory reaction, such as the release of activating cytokines (34).

In conclusion, our results indicate that increased numbers of TILs at the invasive border of endometrial carcinomas may be a reliable independent prognostic factor of improved patient survival. TILs in endometrial carcinoma express immunohistochemical markers of cytotoxic activity, suggesting that CTL-mediated cytotoxicity may be a key mechanism active in host *versus* tumor immune response.

REFERENCES

1. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics. CA Cancer J Clin 1998;48:6.

2. Ambros RA, Kurman RJ. Combined assessment of vascular invasion and myometrial invasion as a model to predict prognosis in stage I endometrioid adenocarcinoma of uterine corpus. Cancer (Phila) 1992; 69:1424–31.

3. Zaino RJ, Kurman RJ, Diana KL, Morrow CP. Pathologic models to predict outcome for women with endometrial adenocarcinoma. Cancer (Phila) 1996;77:1115–21.

4. Ambros RA, Kurman RJ. Identification of patients with stage I uterine endometrioid adenocarcinoma at high risk of recurrence by DNA ploidy, myometrial invasion, and vascular invasion. Gynecol Oncol 1992;45:235–9.

5. Yoshiki I, Koshiro O, Kunio A, et al. The prognostic significance of vascular invasion by endometrial carcinoma. Cancer (Phila) 1996;78: 1447–51.

6. Gehrig PA, Linda Van Le, Olatidoye B, Geradts J. Estrogen receptor status, determined by immunohistochemistry, as a predictor of the recurrence of stage I endometrial carcinoma. Cancer (Phila) 1999;86: 2083–9.

7. Fine BA, Valente PT, Feinstein GI, Dey T. VEGF, flt-1, and KDR/flk-1 as prognostic indicators in endometrial carcinoma. Gynecol Oncol 2000;76:33–9.

8. McCluggage WG, Maxwell P, Hamilton PW, Jasani B. High metallothionein expression is associated with features predictive of aggressive behaviour in endometrial carcinoma. Histopathology 1999;34:51–5.

9. Fanning J, Brown S, Phibbs G, Kramer T, Zaher A. Immunohistochemical evaluation is not prognostic for recurrence in fully staged high-risk endometrial cancer. Int J Gynecol Cancer 2002;12:286–9.

10. Bell JG, Minnick A, Reid GC, Judis J, Brownell M. Relationship of nonstaging pathological risk factors to lymph node metastasis and recurrence in clinical stage I endometrial carcinoma. Gynecol Oncol 1997;66:388–92.

11. Ferguson A, Moore M, Fox H. Expression of MHC products and leukocyte differentiation antigens in gynaecological neoplasms: an immunohistological analysis of the tumor cells and infiltrating leukocytes. Br J Cancer 1995;52:551–63.

12. Yoshitaka N, Kazuya S, Kenichi S, Akio O, Katsunoni S, Hiroshi N, Ohtani H. $CD8^+$ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 1998;58: 3491–4.

13. Saiki Y, Ohtani H, Naito Y, Miyazawa M, Nagura H. Immunophenotypic characterisation of EBV-associated gastric carcinoma: massive infiltration by proliferating CD8+ lymphocytes. Lab Investig 1996;75: 67–76.

14. Bontkes HJ, Walboomers JMM, Gunter AW, et al. Assessment of cytotoxic T-lymphocyte phenotype using the specific markers granzyme B and TIA-1 in cervical neoplastic lesions. Br J Cancer 1997;76:1353–60.

15. Yakirevich E, Ben Izhak O, Rennert G, Kovacs ZG, Resnick MB. Cytotoxic phenotype of tumor infiltrating lymphocytes in medullary carcinoma of the breast. Mod Pathol 1999;12:1050–6.

16. Yakirevich E, Lefel O, Sova Y, et al. Activated status of tumour-infiltrating lymphocytes and apoptosis in testicular seminoma. J Pathol 2002;196:67–75.

17. Schumacher K, Haensch W, Refzaad C, Schlag RM. Prognostic significance of activated $CD8^+$ T cells infiltrations within esophageal carcinomas. Cancer Res 2001;61:3932–6.

18. Nakano O, Sato M, Naito Y, et al. Proliferative activity of intratumoral $CD8^+$ T lymphocytes as a prognostic factor in human renal cell carcinoma. Cancer Res 2001;61:5132–6.

19. Anonymous. Announcements: FIGO stages—1988 revision. Gynecol Oncol 1989;35:125–7.

20. Creasman W. New gynecologic cancer staging. Obstet Gynecol 1990;75:287–8.

21. Clark WH, Elder DE, Guerry DP, et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst (Bethesda) 1989;81:1893–904.

22. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203–13.

23. Pui CH, Ip SH, Dodge RK, et al. Serum levels of CD 8 antigen in childhood lymphoid malignancies: a possible indicator of increased suppressor cell activity in poor-risk patient. Blood 1988;73: 1015–21.

24. Oudejans JJ, Jiwa NM, Kummer JA, et al. Activated cytotoxic T cells as prognostic marker in Hodgkin's disease. Blood 1997;89:1376–82.

25. Yamazawa K, Seki K, Matsui H, Sekiya S. Significance of perivascular lymphocyte infiltrates in endometrial carcinoma. Cancer (Phila) 2001;91:1777–84.

26. Lee KR, Vacek PM, Belinson JL. Traditional and nontraditional histopathologic predictors of recurrence in uterine endometrioid carcinoma. Gynecol Oncol 1994;54:10–8.

27. Silverberg SG, Sasano N, Yajima A. Endometrial carcinoma in Miyagi prefecture, Japan: histopathological analysis of cancer registry-based series and comparison with cases in American women. Cancer (Phila) 1982;449:1504–10.

28. Deligdisch L. Morphologic correlates of host response in endometrial carcinoma. Am J Reprod Immunol 1982;2:54–7.

29. Germain RN, Margulies DM. The biochemistry and cell biology of antigen processing and presentation. Annu Rev Immunol 1993;11:403–50.

30. Boon T, Old LJ. Cancer tumor antigens. Curr Opin Immunol 1997;9:681–3.

31. Van Der Eynde BJ, Van Der Bruggen P. T cells defined tumor antigens. Curr Opin Immunol 1997;9:684–93.

32. Van Der Bruggen P, Traversari C, Knuth T, Boon A. A gene encoding an antigen recognized by cytotoxic T lymphocytes on a human melanoma. Science (Wash DC) 1991;254:1643–7.

33. Resnick MB, Sabo E, Kondratiev S, Kerner H, Spagnoli GC, Yakirevich E. Cancer-testis antigen expression in uterine malignancies with an emphasis on carcinosarcomas and papillary serous carcinomas. Int J Cancer 2002;101:190–5.

34. Kummer JA, Kamp AM, Tadema TM, Vos W, Meijer CJLM, Mack CE. Localization and identification of granzyme A and B-expressing in normal human lymphoid tissue and peripheral blood. Clin Exp Immunol 1995;100:164–72.