ORIGINAL ARTICLE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

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ABSTRACT

BACKGROUND

Although tumor-infiltrating T cells have been documented in ovarian carcinoma, a clear association with clinical outcome has not been established.

METHODS

We performed immunohistochemical analysis of 186 frozen specimens from advanced-stage ovarian carcinomas to assess the distribution of tumor-infiltrating T cells and conducted outcome analyses. Molecular analyses were performed in some tumors by real-time polymerase chain reaction.

RESULTS

CD3+ tumor-infiltrating T cells were detected within tumor-cell islets (intratumoral T cells) in 102 of the 186 tumors (54.8 percent); they were undetectable in 72 tumors (38.7 percent); the remaining 12 tumors (6.5 percent) could not be evaluated. There were significant differences in the distributions of progression-free survival and overall survival according to the presence or absence of intratumoral T cells ($P<0.001$ for both comparisons). The five-year overall survival rate was 38.0 percent among patients whose tumors contained T cells and 4.5 percent among patients whose tumors contained no T cells in islets. Significant differences in the distributions of progression-free survival and overall survival according to the presence or absence of intratumoral T cells ($P<0.001$ for both comparisons) were also seen among 74 patients with a complete clinical response after debulking and platinum-based chemotherapy: the five-year overall survival rate was 73.9 percent among patients whose tumors contained T cells and 11.9 percent among patients whose tumors contained no T cells in islets. The presence of intratumoral T cells independently correlated with delayed recurrence or delayed death in multivariate analysis and was associated with increased expression of interferon-γ, interleukin-2, and lymphocyte-attracting chemokines within the tumor. The absence of intratumoral T cells was associated with increased levels of vascular endothelial growth factor.

CONCLUSIONS

The presence of intratumoral T cells correlates with improved clinical outcome in advanced ovarian carcinoma.

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Epithelial ovarian cancer, which causes more deaths than any other gynecologic cancer, accounts for approximately 14,000 deaths in the United States yearly.[1] Most such cancers are diagnosed at an advanced stage; more than half of patients have remission after surgical debulking and primary chemotherapy, but overall five-year survival remains lower than 25 percent.[1] Although tumor stage, residual disease after surgical debulking, and the response to chemotherapy affect the outcome of ovarian carcinoma,[2,3] the variability in progression-free and overall survival among patients with similar clinical and pathological characteristics[4,5] makes it difficult to predict the outcome reliably.

Ovarian carcinoma may be recognized and attacked by the immune system. The tumor may contain a lymphocytic infiltrate,[2,6,7] and these tumor-associated lymphocytes exhibit oligoclonal expansion,[8,9] recognize tumor antigens[6,10-12] and display tumor-specific cytolytic activity in vitro.[13] With the use of an automated enzyme-linked immunospot assay (Elispot), which can measure antigen-specific T cells at a resolution of a single cell, tumor-specific T cells were recently detected in peripheral blood of 50 percent of patients with advanced ovarian carcinoma.[14] Moreover, promising clinical results have been seen with systemic or intraperitoneal interferons[15-17] or adoptive transfer of T cells.[18] Nevertheless, no clear association between antitumor immunity and clinical outcome has been established. The presence of tumor-infiltrating T lymphocytes has been shown to correlate with clinical outcome in vertical-growth-phase melanoma and in breast, prostate, renal-cell, esophageal, and colorectal carcinomas.[19-24] In the present study, we investigated whether there is an association between the presence of tumor-infiltrating T cells and clinical outcome in ovarian carcinoma.

METHODS

STUDY PATIENTS

We evaluated 186 snap-frozen specimens of ovarian cancers of International Federation of Gynecology and Obstetrics stage III or IV. Tumors were collected between October 1991 and July 1999 at the University of Turin, Turin, Italy, from previously untreated patients undergoing debulking surgery. All patients gave oral informed consent before enrollment in the study.[25,26] Optimal surgical debulking was defined by residual individual tumor nodules measuring 1 cm or less in diameter. Patients received chemotherapy with platinum, platinum plus cyclophosphamide, or (after 1995) platinum plus paclitaxel. A complete response was defined by a normal physical examination, a normal computed tomographic (CT) scan of the abdomen and pelvis, and a normal serum CA-125 level. A partial response was defined by a decrease of at least 50 percent in the sum of the largest dimensions of tumors as measured on CT scanning. A smaller decrease or any increase in tumor size was considered to indicate the lack of a response. The duration of progression-free survival was the time between the completion of chemotherapy and the first recurrence (if a complete response had been achieved) or progression, defined as an increase in tumor size of at least 50 percent as measured on CT scanning or two increasing CA-125 values. The duration of overall survival was the interval between diagnosis and death. Observation time was the interval between diagnosis and last contact (death or last follow-up). Data were censored at the last follow-up for patients without recurrence, progression, or death. Histopathological findings were independently confirmed at the University of Pennsylvania, Philadelphia.

IMMUNOSTAINING

Cryosections 6 μm in thickness were fixed in acetone and stained with hematoxylin and eosin or immunostained by an immunoperoxidase procedure (Vectastain ABC kit, Vector Laboratories), as recommended by the manufacturer. Monoclonal antibodies against CD3, CD4, CD8, CD83, CD45, CD45RO, CD19, CD57, CD11c, monokine induced by interferon-γ, vascular endothelial growth factor, and cytokeratins 8 and 18 and polyclonal antibodies against CD3, Ki-67, cytokeratin, secondary lymphoid-tissue chemokine (also called exodus-2), and macrophage-derived chemokine are described in Supplementary Appendix 1 (available with the full text of this article at http://www.nejm.org). The numbers of tumors examined are listed in Supplementary Appendix 2 (at http://www.nejm.org). For each patient's specimen, at least five sections were examined blindly by two investigators trained in the pathology of ovarian cancer, who analyzed them for each of the following: vascular endothelial growth factor, monokine induced by interferon-γ, macrophage-derived chemokine, and secondary lymphoid-tissue chemokine. T cells were counted manually or by image analysis with the use of a CoolSnap-Pro color digital camera and Image-Pro Plus 4.1 software (Media Cybernetics) in

15 to 20 high-power fields. Intratumoral T cells were graded as 1+, 2+, or 3+ (≤5, 6 to 19, or ≥20 T cells per high-power field, respectively). Indirect immunofluorescence was used for double immunodetection of CD3 and cytokeratin, CD3 and Ki-67, CD3 and CD83, and CD3 and CD45RO (see Supplementary Appendix 2).

FLOW CYTOMETRY

Cells from 10 fresh tumor samples were prepared and subjected to four-color flow cytometry, as described elsewhere,[27] with the use of a FACSCalibur flow cytometer with CellQuest 3.2.1fl software (Becton Dickinson). These studies used monoclonal antibodies against HLA-DR (G46-6), CD3 (UCHT-1), CD4 (RPA-T4), CD8 (RPA-T8), CD16 (3G8), CD19 (HIB19), CD45 (HI30), IgG1, and IgG2a (BD Pharmingen).

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION

Total RNA was isolated by homogenization with the use of the Trizol method (Invitrogen), followed by treatment with DNase I (Invitrogen) and further purification with the RNeasy kit (Qiagen). The quality of the RNA was confirmed by electrophoresis on formaldehyde and agarose gel. Experiments with real-time quantitative polymerase chain reaction (PCR) were performed with the use of the ABI Prism 7700 Analyzer and SYBR Green I PCR kits (Applied Biosystems). Complementary DNA was normalized against housekeeping glyceraldehyde-3-phosphate dehydrogenase. Amplification primers are listed in Supplementary Appendix 3 (available with the full text of this article at http://www.nejm.org).

STATISTICAL ANALYSIS

Kaplan–Meier curves were used to estimate five-year rates and were compared with the use of the log-rank statistic. A multivariate Cox proportional-hazards model was used to estimate adjusted hazard ratios. Descriptive statistical analyses were performed with SPSS software[28]; logistic-regression analyses, survival analyses, and analysis of the area under the receiver-operating-characteristic curve were performed with SAS software.[29]

RESULTS

PATTERNS OF T-CELL INFILTRATION IN OVARIAN CARCINOMA

CD3+ tumor-infiltrating T cells were detected within tumor-cell islets, in peritumoral stroma (Fig. 1A, 1B, and 1C), or both. Intratumoral T cells were detected in 102 of the 186 tumors (54.8 percent); they were undetectable in 72 tumors (38.7 percent); 12 tumors (6.5 percent) could not be evaluated. The number of stroma T cells ranged from 10 to 25 per high-power field (mean [±SD], 13±4). Among 45 randomly selected tumors with intratumoral T cells, 5 (11 percent) were graded 1+, 24 (53 percent) were graded 2+, and 16 (36 percent) were graded 3+ by manual counting; by image analysis, 5 (11 percent) were graded 1+, 27 (60 percent) were graded 2+, and 13 (29 percent) were graded 3+. Using real-time PCR, we found that expression of the CD3ϵ chain, a constitutive subunit of the T-cell receptor, was three times as high in 16 tumors with intratumoral T cells than in 10 tumors without intratumoral T cells (Fig. 1D). The numbers of CD4+ and CD8+ T cells in the 30 tumors we studied were closely correlated ($R^2=0.66$, $P<0.001$), and intratumoral CD4+ and CD8+ cells were either both present or both absent according to immunohistochemical analysis, as were total CD3+ cells (Fig. 1G, 1H, and 1I). Tumors containing T cells and tumors not containing T cells had similar numbers of CD45+ cells (leukocytes), CD11c+ cells (monocytes and granulocytes), CD19+ cells (B lymphocytes), and CD57+ cells (natural killer cells) within tumor islets, indicating that only T cells were absent from tumor islets. In 10 fresh tumors analyzed by flow cytometry, CD3+ T cells composed 30 to 55 percent of all tumor-infiltrating CD45+ leukocytes (Fig. 1E and 1F).

INTRATUMORAL T CELLS AND CLINICAL OUTCOME

A complete response to therapy was achieved in 81 of the 186 patients (43.5 percent); a partial response was achieved in 74 patients (39.8 percent); the remaining 31 patients (16.7 percent) had no response. Five-year progression-free survival and overall survival for all 174 patients whose tumors could be evaluated were 20.9 percent and 25.3 percent, respectively. There were significant differences in the distributions of progression-free survival and overall survival according to the presence or absence of intratumoral T cells ($P<0.001$ for both comparisons) (Fig. 2). Patients whose tumors contained T cells had a median duration of progression-free survival of 22.4 months and a median duration of overall survival of 50.3 months, as compared with 5.8 and 18.0 months, respectively, among patients whose tumors did not contain T cells (Table 1). The five-year overall survival rate was 38.0 percent among the 102 patients whose tumors contained T cells but only 4.5 percent among the 72 patients

whose tumors did not contain T cells. The progression-free survival rate at four years was 31.0 percent among patients whose tumors contained T cells and 8.7 percent among patients whose tumors lacked T cells. In the subgroup of 74 patients with tumors that could be evaluated and a complete response to therapy, there were also significant differences in the distributions of progression-free

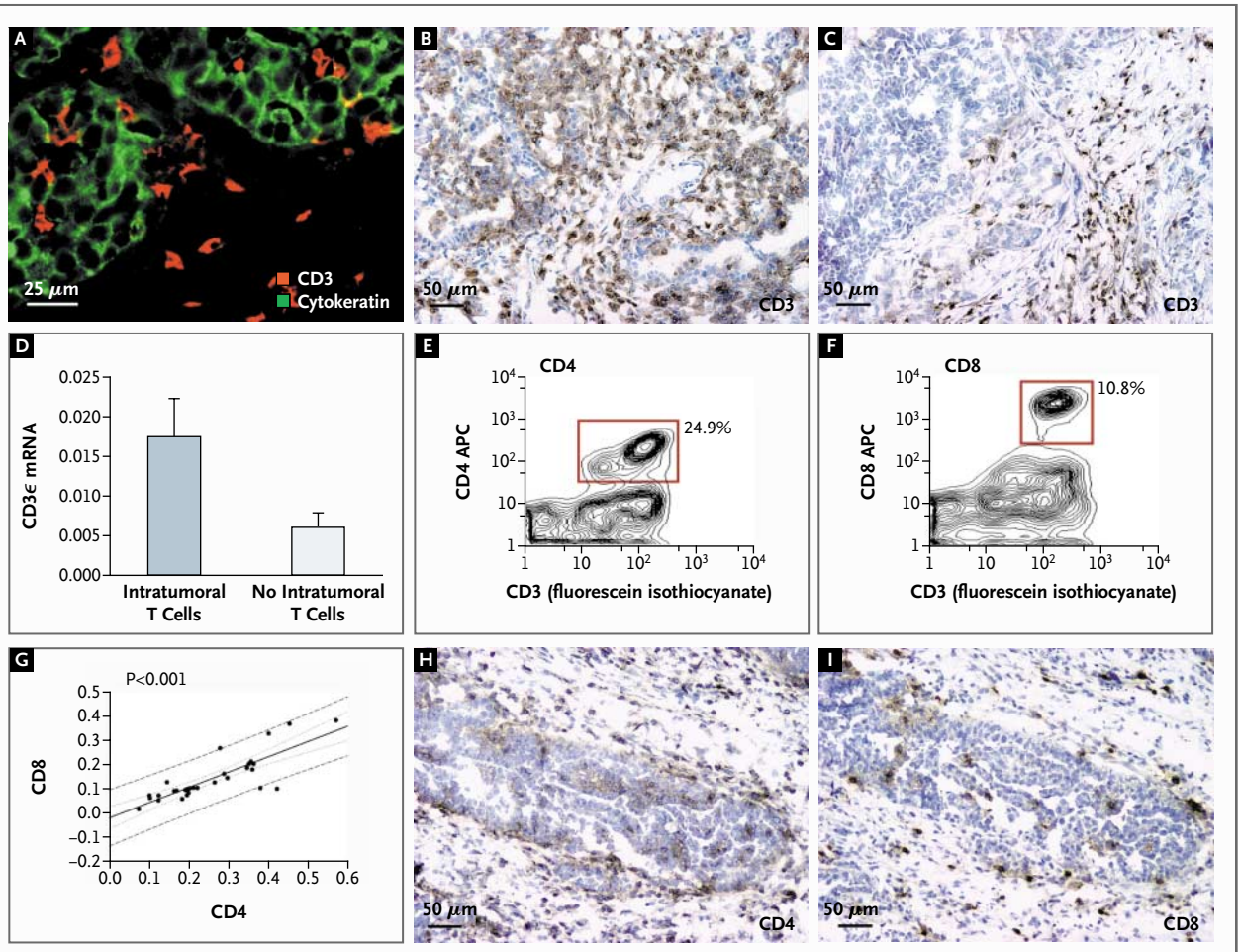

Figure 1. Patterns of T-Cell Infiltration in Ovarian Carcinoma.

Panel A shows double immunofluorescent detection of cytokeratin (fluorescein; green) and CD3 (Texas red; red) demonstrating the organization of cytokeratin-positive tumor cells in well-defined tumor islets and the presence of CD3+ tumor-infiltrating T lymphocytes both within tumor islets and in peritumoral stroma. Panel B shows that T cells appear in tumor islets as well as in tumor stroma; there is an abundance of intratumoral T cells. In Panel C, no intratumoral T cells were detected: T cells are restricted exclusively to the peritumoral stroma in this specimen. In Panel D, real-time quantitative polymerase-chain-reaction analysis of the constitutive CD3ε chain of the T-cell receptor in 16 tumors containing T cells and 10 tumors without T cells reveals overexpression of CD3ε in the former, reflecting a greater number of T cells. The y axis represents the expression of CD3ε in relation to glyceraldehyde-3-phosphate dehydrogenase messenger RNA (mRNA). Panels E and F show four-color flow-cytometric analysis of T cells derived from a fresh stage III ovarian carcinoma with use of monoclonal antibodies against CD3, CD4, and CD8. Gating was carried out on viable CD45+ leukocytes, which comprised up to 35 percent of all cells harvested after mechanical dispersion and enzymatic digestion of solid tumor nodules. T cells represent the most prevalent tumor-infiltrating immune cells. The quantification of CD3+CD4+ T cells is shown in Panel E, and the quantification of CD3+CD8+ T cells is shown in Panel F. Panels G, H, and I show an immunohistochemical analysis of CD4+ and CD8+ T cells in ovarian carcinoma. Panel G shows the correlation of the number of CD4+ T cells with that of CD8+ T cells according to the immunohistochemical analysis of tumors containing T cells and those without T cells. Both intratumoral and peritumoral T cells were counted. A close correlation was noted (R^2=0.66). In the specimen shown in Panel H, both intratumoral and peritumoral CD4+ T cells are present. Intratumoral and peritumoral CD8+ T cells are present in the adjacent section shown in Panel I. APC denotes allophycocyanin.

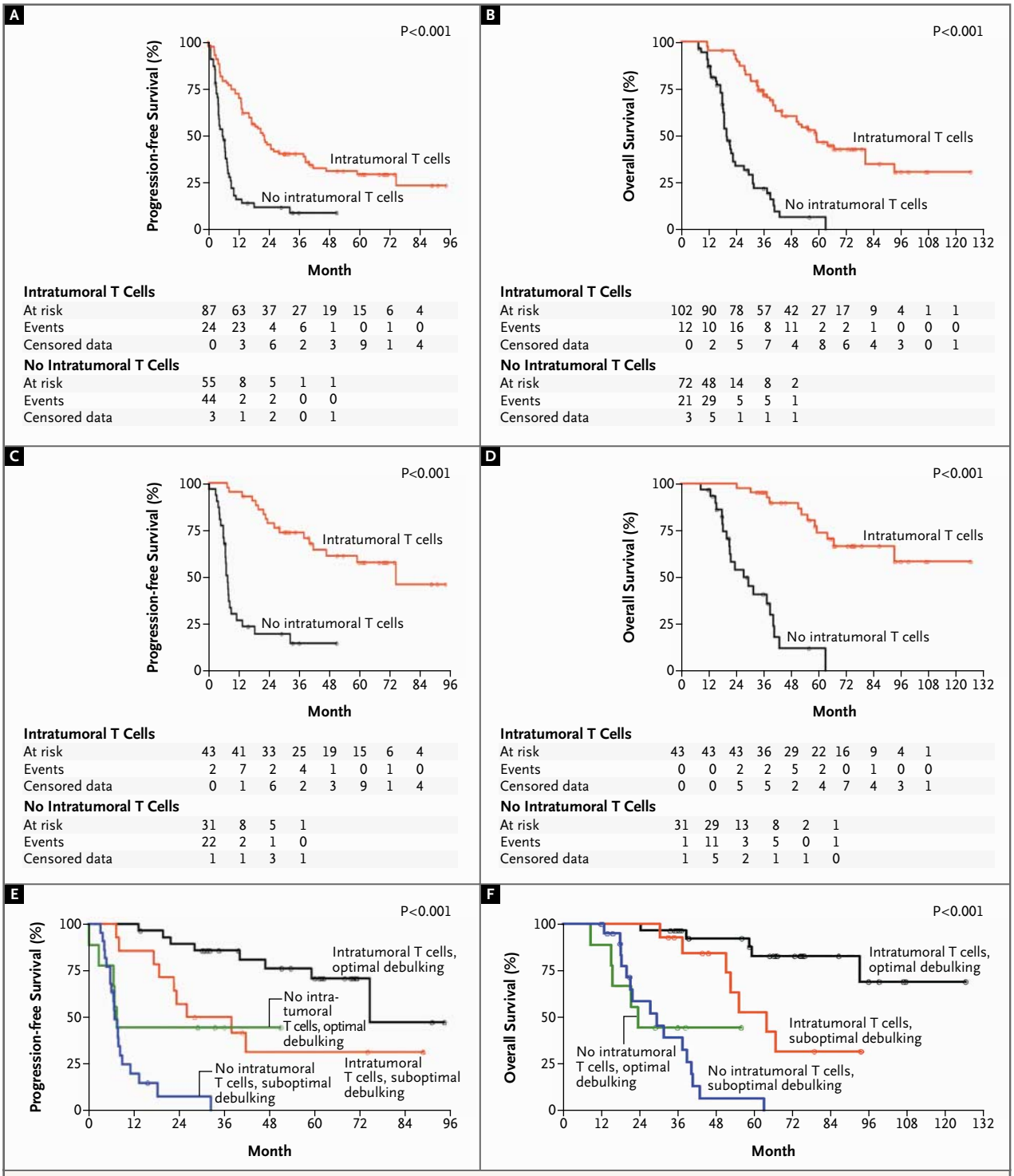

Figure 2. Survival Analyses of Patients with Ovarian Carcinoma, According to the Presence or Absence of Intratumoral T Cells.

Panels A and B show Kaplan–Meier curves for the duration of progression-free survival and overall survival, respectively, according to the presence or absence of intratumoral T cells in 174 patients with stage III or IV epithelial ovarian cancer and complete, partial, or no response to therapy. Panels C and D show Kaplan–Meier curves for the duration of progression-free survival and overall survival, respectively, according to the presence or absence of intratumoral T cells in 74 patients with stage III or IV epithelial ovarian cancer and a complete response to therapy. Panels E and F show survival curves stratified according to the extent of residual disease for the 74 patients with a complete response to therapy, according to the presence or absence of intratumoral T cells. Optimal debulking was defined by residual tumor of less than 1 cm, and suboptimal debulking by residual tumor of 1 cm or more. P values were derived with the use of the log-rank statistic.

Table 1. Progression-free and Overall Survival.*

Group	Progression-free Survival				Overall Survival			
	No. of Patients	Rate at 4 yr	Median Duration	P Value	No. of Patients	Rate at 5 yr	Median Duration	P Value
		%	*mo*			%	*mo*	
Complete or partial response								
All patients	142†	22.2	12.3		174	25.3	30.6	
Intratumoral T cells				<0.001				<0.001
Absent	55	8.7	5.8		72	4.5	18.0	
Present	87	31.0	22.4		102	38.0	50.3	
Complete response								
All patients	74	41.8	32.4		74	51.9	63.1	
Intratumoral T cells				<0.001				<0.001
Absent	31	14.8	7.6		31	11.9	27.5	
Present	43	61.4	74.5		43	73.9	NA	
Suboptimal debulking				<0.001				<0.001
No intratumoral T cells	22	0.0	7.0		22	6.5	29.5	
Intratumoral T cells	14	31.3	32.0		14	52.8	63.8	
Optimal debulking				0.003				<0.001
No intratumoral T cells	9	44.4	7.6		9	44.4‡	23.7	
Intratumoral T cells	29	76.3	74.5		29	82.9	NA	

* P values for comparisons between subgroups were derived by the log-rank test. NA denotes not applicable (and indicates that fewer than 50 percent of patients have died).
† Thirty-two patients with no response were excluded from this analysis.
‡ This was the rate at 48 months.

survival and overall survival according to the presence or absence of intratumoral T cells (P<0.001 for both comparisons) (Table 1 and Fig. 2). These differences were seen both among patients with suboptimal debulking and among those with optimal debulking (P<0.001 for all comparisons) (Table 1). Only 29.0 percent of tumors without T cells were optimally debulked, whereas 67.4 percent of tumors containing T cells were optimally debulked (P=0.001) (Table 2).

No association was found between the presence or absence of T cells and age, histologic type, or tumor grade (Table 2). Univariate analysis revealed that the presence or absence of T cells (P<0.001) and the extent of residual tumor (P<0.001) correlated with overall survival but that tumor grade (P=0.06 for the comparison of grade 1 with grade 3; P=0.30 for the comparison of grade 2 with grade 3), histologic type of tumor (P=0.41), inclusion or noninclusion of paclitaxel in the chemotherapeutic regimen (P=0.74), and age (<55 years vs. ≥55 years, P=0.25) did not. The results were similar for progression-free survival, with the exception of tumor grade (P=0.05 for the comparison of grade 1 with grade 3; P=0.76 for the comparison of grade 2 with grade 3). The presence or absence of intratumoral T cells and the extent of residual tumor but not age, tumor grade, or type of first-line chemotherapy were independent prognosticators of progression-free survival and overall survival in a multivariate analysis (Table 3). The histologic type of the tumor predicted overall survival but not progression-free survival.

CHEMOATTRACTANTS FOR T CELLS

We analyzed whether the presence of intratumoral T cells was associated with factors implicated in the circulation and extravasation of T cells (Fig. 3). We examined 26 tumors from patients with a complete response to chemotherapy and progression-free survival of either 6 months or less or 30 months or more. There were 10 patients with progression-free survival of 6 months or less and no intratumoral T cells, 6 patients with progression-free survival of

6 months or less and intratumoral T cells, and 10 patients with progression-free survival of 30 months or more and intratumoral T cells. The mean observation time in these groups was 20.8, 28.6, and 83.6 months, respectively. Monokine induced by interferon-γ attracts primarily activated T cells.[30,31] In the 16 tumors with intratumoral T cells, the mean level of monokine induced by interferon-γ messenger RNA (mRNA) was 50 times as high as that in the 10 tumors lacking T cells (P=0.05). Strong expression of monokine induced by interferon-γ in and around tumor islets was confirmed by immunohistochemical analysis only in tumors containing T cells (Fig. 3). Secondary lymphoid-tissue chemokine (exodus-2) and macrophage-derived chemokine attract naive or memory noneffector T cells.[32,33] Tumors with T cells had levels of secondary lymphoid-tissue chemokine mRNA that were 43 times as high as those in tumors without T cells (P=0.05) and levels of macrophage-derived chemokine mRNA that were 14 times as high as those in tumors without T cells (P=0.03). No mRNA of either chemokine was detectable in 5 of the 10 tumors without T cells. Expression of these chemokines was confirmed by immunohistochemical analysis at the protein level.

The expression of chemokines implicated in the chemoattraction of other types of immune cells, such as stroma-derived factor 1, monocyte chemoattractant protein 1, or I-309 was similar in tumors with T cells and those without T cells (data not shown). The level of vascular endothelial growth factor, an angiogenic and immunosuppressive factor,[34] was three times as high in tumors without T cells as in tumors with T cells (P=0.007) (Fig. 3B). The expression of vascular-cell adhesion molecule 1 and intercellular adhesion molecule 1 was similar in the two groups of tumors (data not shown). The mRNA levels for interferon-γ and interleukin-2, two cytokines secreted by activated T cells, were 10 (P=0.02) and 26 (P=0.09) times as high in tumors with T cells as in tumors without T cells and were undetectable in 7 of 10 and 9 of 10 tumors without T cells, respectively. Expression of tumor necrosis factor α, a cytokine secreted by T cells, monocytes, macrophages, and natural killer cells, was similar in tumors with and without T cells (P= 0.15, data not shown). Additional evidence of immune activation is presented in Supplementary Appendix 4 (available with the full text of this article at http://www.nejm.org).

Table 2. Clinical Characteristics of Patients with a Complete Clinical Response to Therapy.

Characteristic	No Intratumoral T Cells (N=31)	Intratumoral T Cells (N=43)	P Value
Age — yr			0.34
Mean	59.5	57.1	
Range	26–76	37–80	
Histologic type — no. (%)			0.24
Serous, mucinous, or endometrioid	26 (84)	31 (72)	
Clear-cell or undifferentiated	5 (16)	12 (28)	
Grade — no. (%)			0.59
1	2 (6)	6 (14)	
2	7 (23)	9 (21)	
3	22 (71)	28 (65)	
Residual disease — no. (%)			0.001
Optimal (≤1 cm)	9 (29)	29 (67)	
Suboptimal (>1 cm)	22 (71)	14 (33)	
First-line chemotherapy — no. (%)			0.21
Platinum	5 (16)	14 (33)	
Platinum plus cyclophosphamide	5 (16)	8 (19)	
Platinum plus paclitaxel	21 (68)	21 (49)	

Table 3. Multivariate Cox Proportional-Hazards Analysis of Progression-free and Overall Survival.

Variable	No. of Patients	Progression-free Survival	Overall Survival
		*hazard ratio (95% CI)**	
Intratumoral T cells			
Present	43	0.17 (0.08–0.36)	
Absent	31	1.00	
Residual disease			
Optimal (≤1 cm)	38	0.31 (0.15–0.67)	0.40 (0.17–0.95)
Suboptimal (>1 cm)	36	1.00	1.00
Histologic type			
Clear-cell or undifferentiated	17	1.00	1.00
Serous or mucinous or endometrioid	57	0.79 (0.35–1.75)	0.36 (0.15–0.91)
Tumor grade			
1	8	0.27 (0.04–2.17)	0.37 (0.40–3.14)
2	16	0.84 (0.40–1.80)	0.51 (0.21–1.27)
3	50	1.00	1.00
Paclitaxel therapy			
Received	42	1.16 (0.60–2.25)	1.84 (0.80–4.21)
Not received	32	1.00	1.00
Age			
<55 yr	27	1.00	1.00
≥55 yr	47	0.83 (0.42–1.65)	0.83 (0.38–1.81)

* CI denotes confidence interval.

PREDICTIVE VALUE OF INTRATUMORAL T CELLS AND FACTORS FROM THE TUMOR MICROENVIRONMENT

Among 174 patients whose tumors could be evaluated for T-cell infiltration, 159 were observed for at least three years. The absence of intratumoral T cells (in 66 patients) was associated with a 1.5 percent likelihood of progression-free survival at three years and a 7.6 percent likelihood of overall survival at three years. Among 74 patients with a complete response, 61 were observed for at least three years. The absence of intratumoral T cells (in 26 patients) was associated with a 3.9 percent likelihood of progression-free survival at three years and a 19.2 percent likelihood of overall survival at three years. In the 26 patients whose tumors were analyzed for vascular endothelial growth factor and chemokines, a logistic-regression analysis and associated receiver-operating-characteristic curve showed that overexpression of vascular endothelial growth factor was associated with early recurrence (within 6 months; odds ratio, 0.34; area under the curve, 0.80; P=0.05), whereas overexpression of macrophage-derived chemokine was strongly associated with late recur-

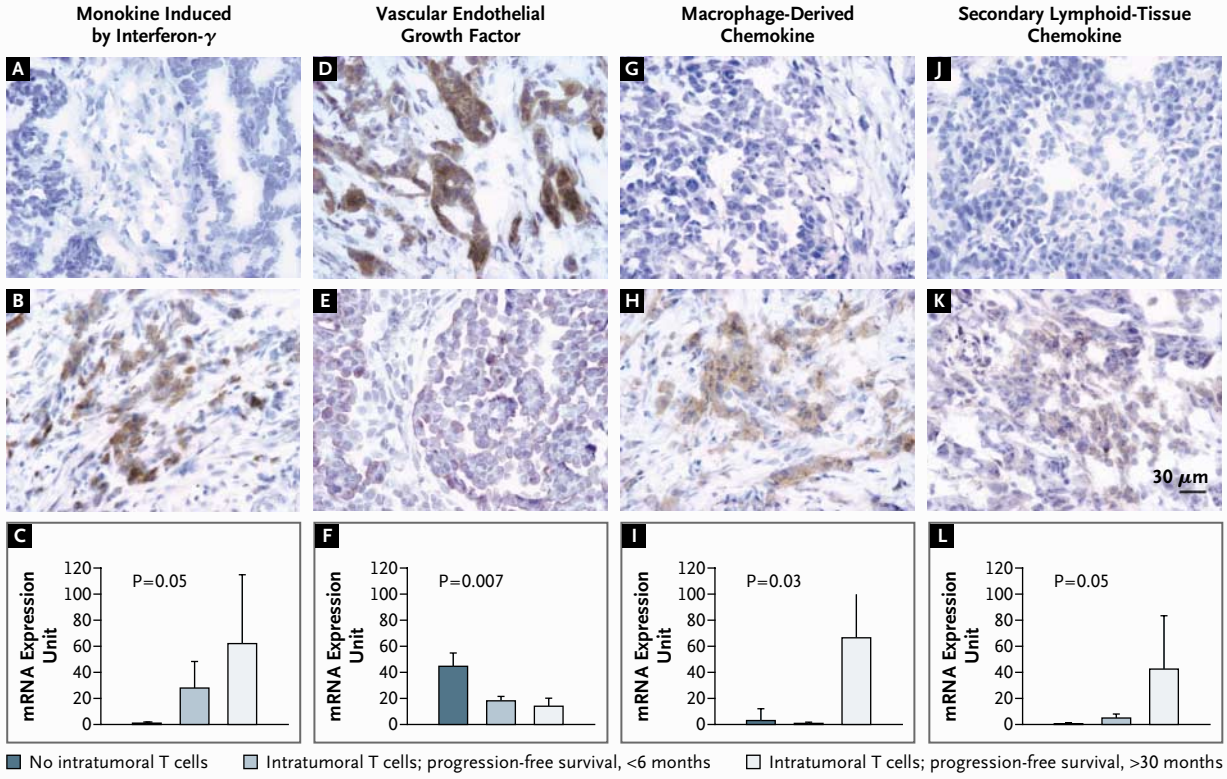

Figure 3. Expression of Lymphocyte-Attracting Chemokines and Vascular Endothelial Growth Factor in Tumors with and without T Cells.

No monokine induced by interferon-γ (Panel A), macrophage-derived chemokine (Panel G), or secondary lymphoid-tissue chemokine protein (Panel J) was detected by immunohistochemical analysis of specimens of ovarian carcinoma in which there were no intratumoral T cells. Strong expression of vascular endothelial growth factor protein was detected by immunohistochemical analysis in a specimen of ovarian carcinoma without T cells (Panel D). Strong expression of monokine induced by interferon-γ (Panel B), macrophage-derived chemokine (Panel H), or secondary lymphoid-tissue chemokine protein (Panel K) was detected by immunohistochemical analysis in specimens of ovarian carcinoma exhibiting intratumoral T cells. Low expression of vascular endothelial growth factor was detected in a specimen of ovarian carcinoma exhibiting intratumoral T cells (Panel E). Real-time quantitative polymerase-chain-reaction (PCR) analysis revealed significantly higher levels of monokine induced by interferon-γ (Panel C), macrophage-derived chemokine (Panel I), or secondary lymphoid-tissue chemokine (Panel L) in tumors containing T cells than in tumors without T cells. Real-time quantitative PCR analysis revealed greater expression of vascular endothelial growth factor in tumors without T cells than in tumors with T cells (Panel F). P values are for the comparison between the group with no intratumoral T cells and the two groups with intratumoral T cells.

rence (after 40 months; odds ratio, 1.57; area under the curve, 0.73; P=0.08).

DISCUSSION

Our study indicates that the presence or absence of intratumoral T cells correlates with the clinical outcome of advanced ovarian carcinoma after surgical debulking and adjuvant chemotherapy. In a cohort of 174 consecutive patients with complete, partial, or no response to surgical debulking and adjuvant chemotherapy, the presence of intratumoral T cells was associated with a median duration of progression-free survival that was 3.9 times as long and a median duration of overall survival that was 2.8 times as long as that among patients whose tumors contained no T cells. Moreover, in patients with a complete response to therapy, the duration of progression-free survival was increased by a factor of approximately 10 if intratumoral T cells were present. The association between optimal surgical debulking and improved clinical outcome in ovarian cancer has been established by numerous retrospective studies.[2,3] We found a significant association between optimal debulking and the presence of intratumoral T cells among patients with a complete response to therapy, suggesting that intratumoral T cells may be associated with an increased likelihood of optimal tumor debulking. However, T cells emerged as an independent prognostic factor in multivariate analysis, suggesting that both T cells and residual disease contribute to the outcome.

Only patients whose tumors had intratumoral T cells survived beyond 63 months. In fact, there was a five-year survival rate of 73.9 percent among the 43 patients who had optimal debulking, a complete response to therapy, and intratumoral T cells. The rate of overall long-term survival among patients with advanced ovarian carcinoma in the United States is approximately 20 to 25 percent,[1] which is similar to that seen in this study; patients who are free of disease beyond 5 years are likely to be free of disease at 10 years.[5] Our findings indicate that ovarian carcinoma belongs on the list of tumors in which tumor–host interactions correlate with clinical outcome; this list now includes vertical-growth-phase melanoma and breast, prostate, renal-cell, esophageal, and colorectal carcinomas.[19-24] Our work underscores the fact that improved clinical outcome is strictly associated with the presence of intratumoral T cells in ovarian carcinoma; these findings are similar to those for esophageal and colorectal tumors.[19,23]

Interferon-γ and interleukin-2, which T cells release when activated by antigens,[35] were undetectable in most tumors that did not contain T cells in islets but were readily detected in tumors with T cells in islets, indicating the presence of activated T cells. Several studies have indicated that despite various mechanisms whereby tumors can escape immune surveillance,[27,36-39] immune mechanisms can attack ovarian carcinoma in some patients.[6-13] The detection of tumor-specific circulating T cells in 50 percent of patients with ovarian carcinoma was recently reported.[14] There is also evidence that tumor-specific T-cell lines can be generated from tumor-infiltrating T cells in approximately 50 percent of patients.[40] Taken together, these findings suggest that intratumoral T cells are a marker of antitumor response mechanisms, and they occur in approximately half of patients with advanced ovarian carcinoma.

Levels of the lymphocyte-attracting chemokines monokine induced by interferon-γ, secondary lymphoid-tissue chemokine, and macrophage-derived chemokine were high in tumors from patients with prolonged remission and survival. These chemokines induce T-cell–mediated rejection of tumors in experimental models of syngeneic tumors transplanted into immunocompetent animals,[41,42] but their role in tumors in humans is unknown. Our data indicate that they may be implicated in mechanisms affecting clinical outcome. Given the role of monokine induced by interferon-γ in attracting activated T cells[31,43] and inhibiting angiogenesis,[44] and the ability of secondary lymphoid-tissue chemokine and macrophage-derived chemokine to attract memory T cells as well as mature dendritic cells and to promote antigen presentation,[32,33] these chemokines may be involved in antitumor mechanisms. By contrast, increased expression of vascular endothelial growth factor was associated with early recurrence and short survival, as it has been in other studies of ovarian cancer.[45-47] Although vascular endothelial growth factor renders blood vessels leaky,[48] its increased expression was associated with the absence of intratumoral T cells. Vascular endothelial growth factor may therefore affect the behavior of ovarian carcinoma not only by promoting angiogenesis, but also by reducing the numbers of T cells.

In summary, the presence of intratumoral T cells correlates with improved progression-free surviv-

al and overall survival among patients with ovarian carcinoma and is associated with molecular evidence of activation of antitumor mechanisms. Prospective studies are warranted to validate the use of detection of intratumoral T cells in the classification and treatment of patients with ovarian carcinoma.

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