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ORIGINAL REPORT

Long-Term Survival for Patients With Non–Small-Cell Lung Cancer With Intratumoral Lymphoid Structures

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ABSTRACT

Purpose
It has been established that the immune system plays an important role in tumor rejection. There is also compelling evidence that immune responses can develop independently of secondary lymphoid organs in tertiary lymphoid structures. We studied the presence and the correlation of tertiary lymphoid structures with clinical outcome in non–small-cell lung cancer (NSCLC), as the prognostic value of these structures in patients with cancer had not yet been established.

Patients and Methods
This retrospective study was performed by immunohistochemistry on paraffin-embedded tissue specimens from 74 patients with early-stage NSCLC.

Results
Tertiary lymphoid structures were detected in some tumors but not in nontumoral lungs. Thus we called these structures tumor-induced bronchus-associated lymphoid tissue (Ti-BALT). As in lymph nodes, Ti-BALTs were composed of mature dendritic cell (DC)/T-cell clusters adjacent to B-cell follicles and had features of an ongoing immune response. Because the quantitative counting of Ti-BALT was difficult to achieve, we used mature DCs that homed exclusively in Ti-BALT as a specific marker of these structures. Univariate analysis showed that the density of mature DCs was highly associated with a favorable clinical outcome (overall, disease-specific, and disease-free survival), suggesting that Ti-BALT may participate in antitumoral immunity. The density of tumor-infiltrating lymphocytes, in particular, $CD4^+$ and $T\text{-}bet^+$ Th1 T cells, was profoundly decreased in tumors weakly infiltrated by mature DCs.

Conclusion
The density of mature DCs was found to be a better predictor of clinical outcome than the other parameters tested. The number of tumor-infiltrating mature DCs may identify patients with early-stage NSCLC who have a high risk of relapse.

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INTRODUCTION

Lung cancer is the most common cause of cancer-related death in the world. Approximately 80% to 90% of cases involve non–small-cell lung cancer (NSCLC), which includes adenocarcinoma and squamous cell carcinoma. Only patients whose tumors can be completely resected have a significant chance of increased survival. However, as many as 30% of patients with stage I disease experience recurrence after surgery. The correlation between tumor-infiltrating immune cells and the prognosis of patients with lung cancer is controversial.

Accumulating evidence indicates that adaptive immunity can be initiated independent of secondary lymphoid organs. For instance, splenectomized alymphoplastic mice can reject xenografts,[1] clear viral infection,[2] or mount an allergic airway inflammation after inhalation of allergens.[3] The de novo formation of ectopic lymphoid structures (also called tertiary lymphoid structures) at the site of inflammation can arise in potentially all inflamed noncanonical lymphoid organs.[4] Bronchus-associated lymphoid tissues (BALTs) have been described in the human fetus and infant lung.[5] They disappear in the normal adult lung,[6] in contrast with other organs such as the gut, where they are constitutively present (gut-associated lymphoid tissue). BALTs have been observed in several inflammatory lung diseases in humans (fibrosis, pneumonia, hypersensitivity pneumonitis, diffuse panbronchiolitis, and tobacco-induced inflammation),

although it is still unclear whether these structures are simple lymphoid aggregates or functional immune structures.

A tumor is composed of malignant, stromal, endothelial, and immune cells that form a heterogeneous network and exhibit complex interactions. Although tumor eradication by the immune system is often inefficient, there is evidence that many developing cancers are not ignored by the immune system.[7] Spontaneous tumor regressions occurring concomitantly with autoimmune manifestations and the higher incidence of tumors in immunosuppressed patients are indications of the involvement of the immune system in tumor rejection. Mice deficient in immune functions spontaneously develop tumors. The density of tumor-infiltrating lymphocytes (TILs) with cytotoxic and memory phenotypes is highly predictive of good clinical outcome.[8-14] However, although prognosis is related to the homing of effector immune cells, it is still unclear where the activation of the specific immune response takes place: in the tumor, the draining lymph node, or both.

The presence of tertiary lymphoid structures has never been reported in lung cancer, and thus far, no studies have been performed investigating putative correlation with clinical outcome. Here we provide evidence that in certain tumors, immune cells are organized in tertiary lymphoid structures and that the density of mature dendritic cells (DCs), a cell population that is exclusively detected in BALT, is correlated with prolonged survival.

PATIENTS AND METHODS

Patients

Paraffin-embedded tumor biopsies with representative areas of tumor and adjacent lung parenchyma were retrieved retrospectively from 74 successive patients diagnosed between 1998 and 2002 with early-stage NSCLC.[15] Lung biopsies from five patients taken either at a distance from the primary tumor or from a different lesion-free lobe were used as tissue references. Preoperative evaluation of patients included lung, brain, and adrenal computed tomography scan and liver ultrasound echography. All patients underwent complete surgical resection of their tumors, including multilevel lymph node sampling or lymphadenectomy, but none received preoperative chemotherapy or radiotherapy. Patients with an Eastern Cooperative Oncology Group performance status[16] ≤ 1 were eligible. The main clinical and pathologic features of the patients are presented in Table 1. Patients with mixed histologic features, a T3 tumor, or pleural invasion were ineligible. At the completion of the study, the minimal clinical follow-up was 48 months for the last patient included in the cohort. The consent of all patients was obtained, even though French law does not require specific approval of an institutional review board or the consent of patients for retrospective observational, noninterventional analysis of medical records.

Immunohistochemistry

Antibodies used are listed in Appendix Table A1 (online only). Paraffin-embedded lung tumors were sectioned for immunohistochemistry as described.[19] Briefly, serial 5-μm tissue sections were deparaffinized, rehydrated, and pretreated in 10 mmol/L of citrate buffer pH6 for antigen retrieval. Sections were incubated with 5% human serum before adding primary antibodies followed by secondary antibodies. The binding of biotinylated antibodies was revealed by streptavidin-peroxidase, whereas alkaline phosphatase activity was revealed using alkaline phosphatase substrate III (SK-5300; Vector Laboratories Inc, Burlingame, CA), and peroxidase activity was revealed using either 3-amino-9-ethylcarbazole substrate (SK-4200; Vector) or diaminobenzoate substrate (IM2394; Immunotech, Marseille, France). Negative controls were established by adding nonspecific isotype controls as primary antibodies.

Table 1. Baseline Characteristics of Patients With Early-Stage NSCLC (n = 74)

Characteristic	No.	%
Sex		
Male	60	81
Female	14	19
Age, years		
Mean	64	
SEM	1	
Range	41-79	
Smoking history		
Current smoker	67	91
Never smoker	7	9
Pack-years		
Median	45	
SEM	3	
Range	0-100	
Histologic type		
ADC	46	62
SCC	28	38
Tumor differentiation		
Well	28	38
Intermediate	22	30
Poor	24	32
pTNM stage		
pT1N0M0	48	65
pT2N0M0	14	19
pT1N1M0	12	16
Fibrosis, %		
Mean	25	
SEM	2	
Range	0-85	
Necrosis, %		
Mean	12	
SEM	2	
Range	1-75	
Ki67$^+$ tumor cells, %		
Mean	36	
SEM	3	
Range	1-80	
Vital status of patients		
Alive	54	73
Disease free	51	
In relapse	3	
Dead	20	27
From metastasis of NSCLC	9	
From other causes	11	

NOTE. Pathologic staging and histologic types of lung cancer were determined according to the TNM staging system[17] and to the histologic classification of the WHO,[18] respectively.
Abbreviations: NSCLC, non–small-cell lung cancer; ADC, adenocarcinoma; SCC, squamous cell carcinoma; pTNM, pathologic TNM.

Method for Cell Quantification

Stained cells were counted semi-quantitatively (score 0, 1, 2, 3, and 4 for none, very low, weak, intermediate, and high density of positive cells, respectively) in each intermediate-power field (IPF) in the tumoral areas of the entire tissue section (from 19 to 76 fields, original magnification $\times$100), and expressed as mean score per IPF, with SEMs calculated. The numbers of DC-Lamp$^+$ mature DCs and Granzyme-B$^+$ CD8$^+$ T cells were lower than the number of cells described above, allowing us to realize a quantitative counting. Those stained cells were expressed as mean cells per IPF, with SEMs calculated. CD4, CD8, CD45ra, CD45ro, TIA-1 stainings

were counted as a percentage of positive cells among $CD3^+$ T cells. The necrosis and fibrosis were counted as the percentage of the positive areas among the whole tumor mass section. Both immunostaining and scoring were evaluated according to the criteria described above by four independent observers (M.-C.D.-N., M.A., V.P., and L.D.C.) who were blinded to clinical outcome. The investigators reviewed the few cases with conflicting results to reach a consensus.

Statistical Analysis

The variables taken into account for statistical analysis included clinical (age, sex, smoking, tumor relapse, and vital status), histopathologic (histology, pathologic TNM [pTNM], tumor differentiation, localization of the primary tumor, necrosis, fibrosis, and proliferation of the tumor), and immunologic parameters (markers described above). To perform univariate analysis, groups of patients were defined according to the bimodal distribution of the density of positive cells, appointed using the following cut-offs: CD3, 1.5 mean score/tumor IPF; CD20, 1.5 mean score/tumor IPF; DC-Lamp, 1.65 mean cells/tumor IPF.

χ^2 test with Yates correction and analysis of variance test (posthoc tests with Fisher's and Bonferroni methods) were used for univariate analysis. Overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) curves were estimated by Kaplan-Meier method, and significant differences between the groups of patients were evaluated using the log-rank test. An event affecting OS was defined as death from any cause, DSS as death from NSCLC, and DFS as relapse of the primary tumor. Statistical analysis was performed using StatView and JMP softwares (SAS Institute, Cary, NC). A P value less than .05 was considered statistically significant.

RESULTS

Presence of Tumor-Induced BALT in Some Early-Stage Lung Cancers

We first searched for the presence of tertiary lymphoid structures on paraffin-embedded sections of 74 early-stage NSCLCs (46 adenocarcinomas and 28 squamous cell carcinomas) and five non-pathologic lung biopsies. These lymphoid organizations have been found in many tumors (Fig 1A), whereas they had not yet been observed in nontumoral lung. Therefore, we called these lymphoid structures tumor-induced BALT (Ti-BALT). To evaluate the prognostic value of Ti-BALT, we tried to quantify these structures;

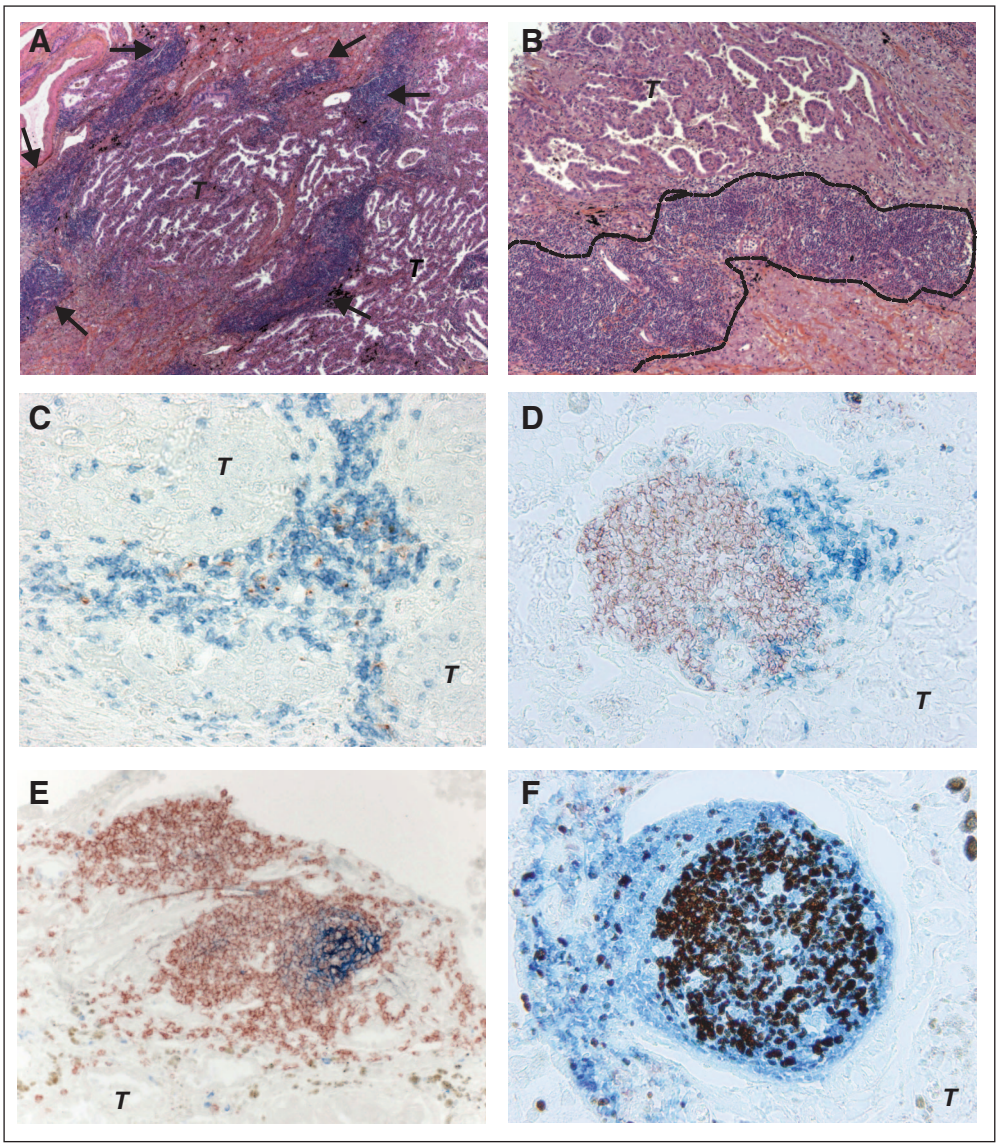

Fig 1. Characterization of tumor-induced bronchus-associated lymphoid tissue (Ti-BALT) in non–small-cell lung cancer via staining on paraffin-embedded lung tumor biopsies. (A, B) Presence of Ti-BALT (arrow in A or as limited by dashed line in B) in lung tumor section counterstained with hematoxylin and eosin. (C) DC-$Lamp^+$ mature dendritic cells (red) home exclusively into $CD3^+$ T-cell clusters (blue). (D) Presence of adjacent $CD20^+$ B (red) and $CD3^+$ T (blue) cell rich areas of Ti-BALT. (E) $CD20^+$ B-cell follicles (red) are characterized by the presence of a $CD21^+$ follicular dendritic cell network (blue). (F) Some $CD20^+$ B-cell follicles (blue) contained $Ki67^+$ proliferating germinal center B cells (brown). Original magnification: A, ×50; B, ×100; C, D, F, ×400; E, ×200. T, tumor nest.

unfortunately, their size was extremely variable and several Ti-BALT can colocalize, which makes quantification by counting uncertain (Fig 1B). Thus we decided to characterize the cellular composition of these lymphoid structures to identify a specific marker of Ti-BALT. We thus used immunohistochemistry to examine the localization of $CD3^+$ T cells, $CD20^+$ B cells, and DC-$Lamp^+$ mature DCs. The density of adaptive immune cells was heterogeneous between tumors, as well as within a given tumor. In some biopsies, $CD3^+$ T lymphocytes were detected in clusters where DC-$Lamp^+$ mature DCs exclusively homed (Fig 1C). The presence of mature DCs was confirmed by the expression of CD83, another marker of mature DCs (data not shown). These mature DC/T-cell clusters were often surrounded by $CD20^+$ B-cell follicles (Fig 1D) characterized by the presence of both a $CD21^+$ follicular dendritic cell network (Fig 1E) and $Ki67^+$ proliferating germinal center (GC) B cells (Fig 1F).

By statistical analysis, we confirmed that the density of mature DCs, T cells, and B cells were strongly correlated to each other in the tumor (DC-Lamp/CD3, $P < .0001$, $r = 0.53$; CD3/CD20, $P < .0001$, $r = 0.55$; CD20/DC-Lamp, $P < .0010$, $r = 0.32$).

Taken together, the presence of Ti-BALT was very heterogeneous between tumors. These structures were composed of mature DCs, T cells, and B cells organized with a distribution reminiscent of the secondary lymphoid organs where mature DCs exclusively home.

Prognostic Value of Ti-BALT

At the completion of the study, 54 patients were alive (73%) and 20 patients had died (27%; Table 1). Seven patients died as a result of postoperative complications at the hospital or within 1 month of leaving the hospital and were therefore considered as having died from cancer progression-unrelated causes. Nine deaths were NSCLC-related and four deaths were NSCLC-unrelated (myeloma, n = 1; sepsis, n = 1; vascular brain damage, n = 1; unknown cause, n = 1).

We analyzed the prognostic value of Ti-BALT. As mentioned previously, as a result of the uncertainty associated with quantification by counting, this question was difficult to answer. As it had already been observed that DC-$Lamp^+$ mature DCs are selectively detected in Ti-BALT, DC-Lamp was chosen as a specific marker of Ti-BALT. Except for five tumors, a trend was observed by statistical analysis between these two parameters (Fig 2A; $R^2 = 0.317$ for 74 tumors and $R^2 = 0.488$ for 69 tumors).

We used univariate analysis to investigate the prognostic value of mature DCs. This was compared with the prognostic values of T and B cells, which were observed in (but not specific to) Ti-BALT, histologic and clinical features of the tumors (Table 2). The density of DC-$Lamp^+$ mature DCs was the only parameter that was correlated with the three survival parameters (OS, DSS, and DFS). The hazard ratios of 4-year OS, DSS, and DFS rates for patients with DC-Lamp–low versus DC-Lamp–high tumors were 1.88 ($P = .0058$), 3.34 ($P = .0007$), and 2.11 ($P = .0114$), respectively. The median DFS was not reached for the patients characterized as having DC-Lamp–high tumors; however, median DFS was 44.2 months for the patients with DC-Lamp–low tumors (Fig 2B; $P = .0056$). There were no distinguishable clinical (sex and smoking history), tumor (tumor differentiation, pTNM staging, fibrosis, necrosis, and proliferating tumor cells), or histologic characteristics between the patients with DC-Lamp–high versus DC-Lamp–low tumors (Appendix Table A2, online only).

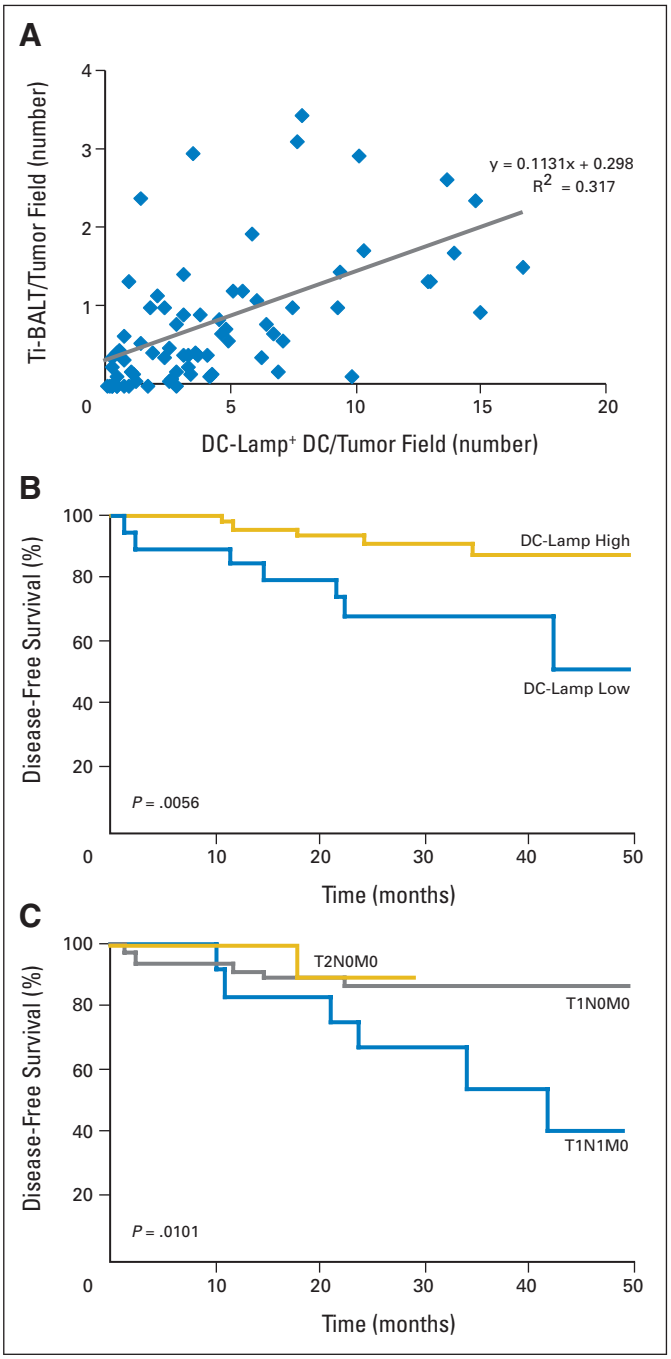

Fig 2. Evaluation of DC-Lamp as a marker of tumor-induced bronchus-associated lymphoid tissue (Ti-BALT) and its prognostic value. (A) Correlation between the density of Ti-BALT and the density of tumor-infiltrating DC-$Lamp^+$ mature dendritic cells (DCs). Kaplan-Meier curves of disease-free survival for 74 patients with non–small-cell lung cancer depending on (B) the density of tumor-infiltrating DC-$Lamp^+$ mature DCs and (C) the pathologic TNM stage.

T and B cells were also not associated with patient outcome. As expected, pTNM staging was predictive of DFS (Table 2 and Fig 2C), whereas age, sex, smoking history, and tumor differentiation were not associated with survival (data not shown).

Table 2. Prognostic Parameters for Survival in Univariate Analysis

Variable	No.	%	OS 4-Year Survival (%)	OS HR	OS 95% CI	OS P	DSS 4-Year Survival (%)	DSS HR	DSS 95% CI	DSS P	DFS 4-Year Survival (%)	DFS HR	DFS 95% CI	DFS P
CD3														
Low	33	45	69.6	1.14	0.73 to 1.77	NS	86.2	1.01	0.50 to 1.96	NS	82.6	0.95	0.52 to 1.69	NS
High	41	55	67.5	1			79.0	1			75.2	1		
CD20														
Low	23	31	60.3	2.18	0.90 to 5.32	NS	74.3	3.62	0.96 to 13.68	.058	75.0	1.95	0.61 to 6.17	NS
High	51	69	73.9	1			87.0	1			81.2	1		
DC-Lamp														
Low	22	30	35.4	1.88	1.21 to 2.96	.0058	44.7	3.34	1.64 to 8.68	.0007	51.2	2.11	1.19 to 3.89	.0114
High	52	70	81.3	1			95.5	1			87.7	1		
Histologic type														
ADC	46	62	79.5	0.58	0.36 to 0.90	.0155	81.2	1.28	0.63 to 3.31	NS	73.7	1.52	0.78 to 3.88	NS
SCC	28	38	51.9	1			84.7	1			91.1	1		
pTNM stage														
pT1N0M0	48	65	71.2	1			87.0	1			88.3	1		
pT2N0M0	14	19	67.3*	1.09	0.56 to 2.00	NS	85.7*	1.38	0.45 to 3.58	NS	90.9*	1.66	0.55 to 4.11	NS
pT1N1M0	12	16	61.9	0.88	0.60 to 0.97	NS	61.9	2.26	1.94 to 2.33	NS	40.0	4.81	4.74 to 5.07	< .05

NOTE. All parameters were evaluated among 74 patients with early-stage non–small-cell lung cancer. Survival was measured from the time of surgery. The median OS of the population was not reached (25% of patients died after 32.8 ± 11.8 months after surgery). The 75% DSS and DFS of the cohort were not reached in this study (12% of patients died from lung cancer and 16% of patients experienced relapse from their lung cancer after surgery). *P* values were determined using the Wald test.

Abbreviations: OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival; HR, hazard ratio; NS, not significant; ADC, adenocarcinoma; SCC, squamous cell carcinoma; pTNM, pathologic TNM.

*The follow-up evaluation was not done at 4 years, but was instead done at 29 months.

These results indicate that DC-Lamp, which is a specific marker of Ti-BALT, is the highest predictive marker for survival. Increased density of mature DCs is associated with a favorable clinical outcome for patients with early-stage NSCLC.

Density and the Phenotype of TILs Are Distinct Among DC-Lamp Low Versus DC-Lamp High Tumors

TILs were further compared in DC-Lamp–low versus DC-Lamp–high tumors using immunohistochemistry. A combination of T-cell markers let us identify the following subsets: $CD4^+$ T cells, $CD8^+$ T cells, $CD45ra^+CD45ro^-$-naïve T cells, $CD45ro^+CD45ra^-$ memory T cells, T-bet [T-box transcription factor 21]$^+$ Th1 T cells, and T cytotoxic cells (granzyme-B). As presented in Table 3, the density of T cells was closely correlated with the density of mature DCs ($P = .0018$), indicating that DC-Lamp–low tumors have fewer TILs than DC-Lamp–high tumors. The main difference between the two groups of DC-Lamp tumors was the $CD4^+$ T-cell compartment, which was dramatically collapsed in DC-Lamp–low tumors, whereas the $CD8^+$ T-cell subset was less affected (as observed by immunohistochemistry). Thus the ratio of CD4/CD8 in DC-Lamp–low tumors was inverse to the ratio seen in DC-Lamp–high tumors ($P = .0056$). Most $CD4^+$ T cells formed clusters with mature DCs (Fig 3A and inset), in contrast with $CD8^+$ T cells, which infiltrated all the areas of the tumor (Fig 3B and inset). $CD45ra^+$–naïve T cells were rare in both groups of tumors (< 10% of total $CD3^+$ T cells), which is in agreement with the expression of CD45ro by most T cells (Fig 3C). Thus the ratio of CD45ra/CD45ro on T-cell subsets was relatively constant in both tumor groups (Table 3; $P = .3693$). However, the density of T-bet$^+$ cells was highly enhanced in DC-Lamp–high tumors as compared with DC-Lamp–low tumors ($P = .0037$). Cells were detected in tumor nests (Fig 3D) and the stroma reaction, but never in Ti-BALT. Finally, a modest increase of granzyme-B$^+$ cytotoxic T lymphocyte was also seen in DC-Lamp–high tumors, but the difference between the two groups of tumors did not reach significance.

Similar to T cells, the density of tumor-infiltrating B cells was also strongly increased in the group of DC-Lamp–high tumors ($P < .0001$). These data indicate that the main difference between DC-Lamp–low versus DC-Lamp–high tumors is the density of TILs, in particular, that of the $CD4^+$ and T-bet$^+$ T-cell subsets.

DISCUSSION

In human lung tumors, we report the presence of tertiary lymphoid structures composed of mature DC/T-cell clusters and B-cell follicles. The B-cell areas include proliferating GC B cells and a follicular DC network, features of an ongoing immune response as observed in canonical lymphoid organs. We did not observe these structures in sites distant from the tumor, suggesting that they are induced in response to the tumor microenvironment. Therefore, we have called these structures Ti-BALT. Nonproliferating GC B cells have also been described in ectopic lymphoid structures of idiopathic lung fibrosis,[20] indicating that BALT might not have the same maturation status in different lung diseases and that the local microenvironment influences BALT development and function.

We have shown through univariate analysis that the density of mature DCs, a cell population that homes exclusively to Ti-BALT, is highly predictive of survival (OS, DSS, and DFS) in early-stage NSCLC. In this study, smoking history (never/current smokers and pack-years) was identical in both groups of DC-Lamp patients, which argues again for a major role of the tumor microenvironment itself in BALT neogenesis and immune function. We have shown that $CD4^+$ T

Table 3. Quantification of Immune Cell Infiltration in Patients With DC-Lamp–Low Versus DC-Lamp–High Tumors

Variable	Total	DC-Lamp Low	DC-Lamp High	*P*
$CD3^+$ T cells*				
Mean	1.8	1.3	2.0	.0018
SEM	0.1	0.1	0.1	
Range	0.5-4.0	0.5-2.5	0.5-4.0	
$CD20^+$ B cells*				
Mean	1.9	1.0	2.3	< .0001
SEM	0.1	0.2	0.1	
Range	0-4.0	0-2.5	0.5-4.0	
Ratio $CD4^+/CD8^+$ T cells†				
Mean	2.7	0.4	3.7	.0056
SEM	0.6	0.1	0.7	
Range	0-9.0	0-1.0	1.5-9.0	
Ratio $CD45ra^+/CD45ro^+$ T cells†				
Mean	0.1	0.2	0.1	.3693
SEM	0.0	0.1	0.0	
Range	0.1-0.7	0.1-0.7	0.1-0.4	
T-bet^+ cells†				
Mean	1.7	0.7	2.3	.0037
SEM	0.3	0.4	0.3	
Range		0-3.0	1.0-4.0	
Granzyme-B^+ $CD8^+$ T cells†				
Mean	1.0	0.2	1.6	.3151
SEM	0.6	0.2	1.1	
Range	0-5.9	0-0.8	0-5.9	

NOTE. *P* values were determined using the Fisher's and the Bonferroni-Dunn exact tests.
Abbreviation: DC, dendritic cells.
*Quantification evaluated in 74 patients with early-stage non–small-cell lung cancer.
†Quantification evaluated in 24 of 74 tumors.

cells colocalized preferentially with mature DCs and that their density was profoundly affected in DC-Lamp–low tumors. These observations suggest a direct link between low densities of mature DCs and $CD4^+$ T cells, rare Ti-BALT, and poor clinical outcome. This conclusion is in agreement with previous data[21] showing that $CD4^+$ TILs are associated with a favorable prognosis in NSCLC and that the ratio of CD4/CD8 T cells is increased in regressing melanoma and basal cell carcinoma.[22-24] However, the prognostic value of DC-Lamp was higher than that of CD4, most likely because $CD4^+$ T cells contain several cell subsets (naïve, memory/effector, anergic, and regulatory T cells) with opposite functional activities. In particular, the densities of T and B cells, although correlated with the density of mature DCs, were not associated with clinical outcome. In contrast with mature DCs, lymphocytes do not home exclusively to Ti-BALT. Our data suggest that the more tumors are infiltrated by mature DCs, the more T and B cells are organized and proliferated in Ti-BALT. However in the absence of mature DCs, infiltrating lymphocytes neither cluster nor proliferate because of the absence of lymphoid structures. Moreover, lymphocytes, and especially T cells, contain different cell subsets with opposite functional activities, indicating that the quantification of total T and B cells may not be the only method for evaluating lymphocyte implication in clinical outcome.

We have shown that the T-cell composition of Ti-BALT mimics that of gut-associated lymphoid tissue[25] and is completely different from conventional lymphoid organs, where naïve and memory T cells are present in equal proportion, always with more $CD4^+$ than $CD8^+$ T cells.

We have shown that DC-Lamp–high tumors are highly infiltrated by cells positive for T-bet, the interferon gamma–specific transcription factor. Interferon gamma is known to induce CXCR3 ligands, among which the chemokine CXCL10 has been reported to inhibit NSCLC tumorigenesis and spontaneous metastases.[26]

Cumulative data report the participation of extranodal lymphoid structures in the development of specific immune responses.[1,27] In mice deficient in secondary lymphoid organs, Moyron-Quiroz et al[2,28] demonstrated that robust primary T- and B-cell responses to influenza virus and the maintenance of immunologic memory are developed in inducible BALT, which argues for a crucial role of BALT in protective immunity. Thus the high density of mature DCs (a population that homes selectively to Ti-BALT) in patients with a better survival outcome suggests that the first step of specific immune responses might be initiated in the tumor itself. Tertiary lymphoid structures have been reported to be harmful in autoimmunity, because they are involved in the exacerbation of the local immune response and thus participate in the aggravation of pathogenesis. In the present study, no patient with NSCLC had clinical manifestations of autoimmune or infectious diseases, indicating that lymphoid structures were instead induced in response to the tumor microenvironment. In lung cancer, we propose that the absence of Ti-BALT leads to poor T cell priming, with improper T cell localization and/or activity, and thereby resulting in inefficient antitumor immunity. Tumor-associated antigens, which are continuously sampled and processed by DCs, would potentially be in direct contact with specific T cells, thereby increasing the efficiency

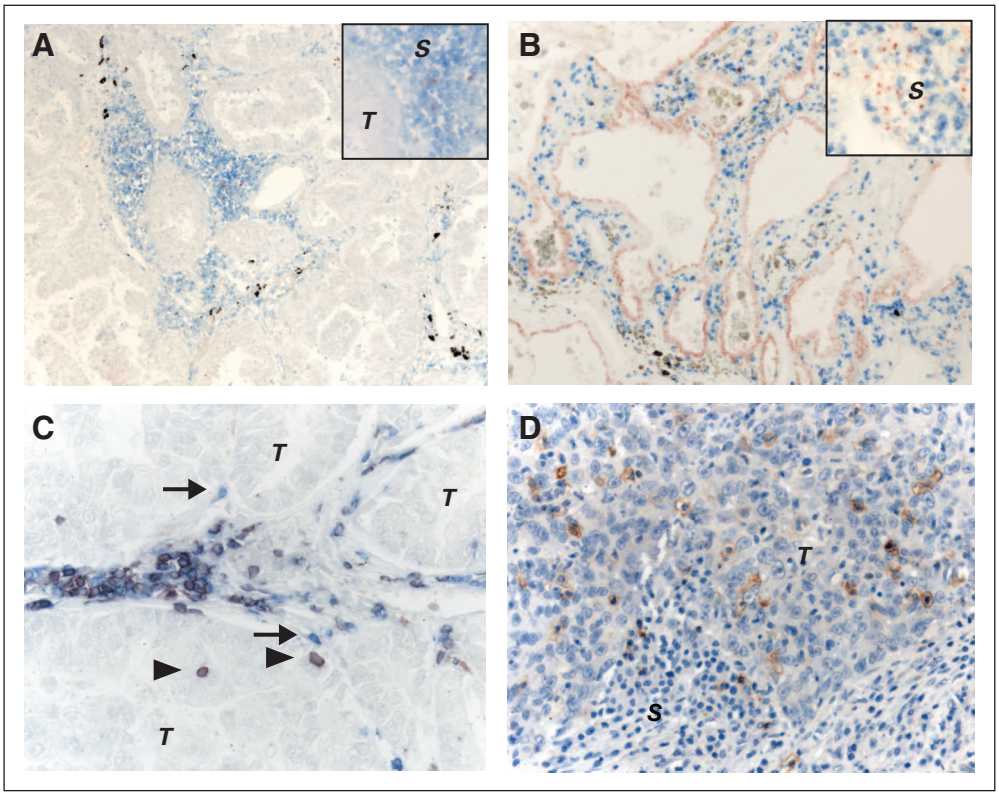

Fig 3. Characterization of T-cell subsets and their activities in DC-Lamp–high tumors. (A, B, C) Double immunostainings and (D) single immunostaining followed by hematoxylin counterstaining on tumors DC-Lamp–high. (A, B) DC-Lamp$^+$ mature dendritic cells (DCs; red) are preferentially in contact with CD4$^+$ T helper cells (blue, A) compared with cytotoxic CD8$^+$ T cells (blue, B). A and B insets represent higher magnification of A and B, respectively. (C) Double staining with anti-CD45ro (red) and anti-CD3 (blue) reveals the presence of a vast majority of memory T cells (gray, arrowhead) versus naïve T cells (blue, arrow) in the stroma reaction and tumor nests. (D) Presence of numerous T-bet$^+$ cells (brown) in both the stroma reaction and tumor nests. Original magnification: A, B, ×100; A and B insets, ×200; C, D, ×400. Abbreviation: T, tumor nest; S, stroma reaction.

and specificity of the priming. During tumor progression, Ti-BALT may thus allow T cells to react more rapidly to the shifting expression profile of tumor antigens in situ.

The presence of proliferating GC B cells in some Ti-BALTs suggests that they may be an active partner in the local initiation of potential antitumoral immunity. As previously described in chronic inflammatory diseases,[29-31] Ti-BALT GCs may support antigen-driven clonal expansion and extensive diversification. Ti-BALT B cells may also play an important role in T-cell–mediated immunity against the tumor.

Because clinical outcome is obviously established after the resection of Ti-BALT–containing tumor, these structures are likely not directly involved in survival. We speculate that Ti-BALT–derived memory cells migrate into the draining lymph nodes to develop a central memory and robust systemic response against micro-metastasis. Finally, DC-Lamp, a marker of Ti-BALT, may have a direct application in the clinical setting, as it could be used to identify patients with early-stage NSCLC who have a high risk of relapse.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

