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ORIGINAL ARTICLE

High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-α in lung cancer

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Abstract
Introduction Antigen-presenting cells, like dendritic cells (DCs) and macrophages, play a significant role in the induction of an immune response and an imbalance in the proportion of macrophages, immature and mature DCs within the tumor could affect significantly the immune response to cancer. DCs and macrophages can differentiate from monocytes, depending on the milieu, where cytokines, like interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) induce DC differentiation and tumor necrosis factor (TNF)-α induce DC maturation. Thus, the aim of this work was to analyze by immunohistochemistry the presence of DCs (S100+ or CD1a+), macrophages (CD68+), IL-4 and TNF-α within the microenvironment of primary lung carcinomas.
Results Higher frequencies of both immature DCs and macrophages were detected in the tumor-affected lung, when compared to the non-affected lung. Also, TNF-α-positive cells were more frequent, while IL-4-positive cells were less frequent in neoplastic tissues. This decreased frequency of mature DCs within the tumor was further confirmed by the lower frequency of CD14-CD80+ cells in cell suspensions obtained from the same lung tissues analyzed by flow cytometry.
Conclusion These data are discussed and interpreted as the result of an environment that does not oppose monocyte differentiation into DCs, but that could impair DC maturation, thus affecting the induction of effective immune responses against the tumor.

Keywords Lung cancer · Dendritic cells · Macrophages · IL-4 · TNF · Immunohistochemistry

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Introduction

Lung cancer is the leading cause of cancer mortality in the United States, and globally there are more than 1 million new cases diagnosed each year [1]. In spite of aggressive treatment with surgery, radiation, and chemotherapy, the long-term survival for lung cancer patients still remains low. Even patients with "early stage" disease frequently succumb to lung cancer due to development of metastases, pointing to the need of effective approaches for systemic therapy of this condition. In this context, immunotherapeutic strategies, which would be advantageous due to the specificity of the immune response, are continuously considered as an option for the management of lung cancer patients [2]. However, the frequent failure of these approaches indicates that our understanding of the immune system–lung cancer interactions needs to be deepened in order to achieve the full potential of immune intervention for lung cancer treatment. In this setting, the evaluation of the distribution, frequency and function of monocytes, macrophages and dendritic cells (DCs) on blood and lung of patients bearing lung carcinomas has a definite role, and

has been one of our group's interests [3, 4], since these cells are involved in innate immunity and play a central role in the adaptive immune response.

Dendritic cells are antigen-presenting cells (APC) that play a crucial role in the initiation and modulation of an appropriate response of the immune system to danger signals (bacteria, viruses, for instance) by linking innate to adaptive immune responses [5]. These cells have unique functional and morphologic properties and compared to other APC such as monocytes/macrophages and B cells, are much stronger T cell stimulators [5, 6]. Immature DCs, thought to differentiate from blood monocytes upon their exposure to an appropriate tissue environment [5], are present in peripheral organs, where they continuously sample the environment, taking up and processing antigens [7]. Exposure of DCs to microbial agents or inflammatory mediators induce their maturation, characterized by an increased expression of class I and II molecules of the major histocompatibility complex (MHC-I and II), and of costimulatory molecules, such as CD80 and CD86, and by the expression of maturation markers, such as CD83 [8, 9]. Such maturation process enables DCs for optimal T cell activation [7], which is supposed to occur in the T cell zone of secondary lymphoid organs, to where mature DCs migrate due the maturation-induced expression of specific chemokine receptors (CCR7) and adhesion molecules [10].

Not surprisingly, DC dysfunction is frequently observed in malignancy [11–16], but the underlying mechanisms of these functional alterations are poorly defined and probably heterogeneous [17]. Not only tissue DCs have been reported as affected by the tumor microenvironment, but also the differentiation of blood monocytes seems to be affected in cancer patients [18]. Considering the role of interleukin-4 (IL-4) in the differentiation of recently egressed blood monocytes into dendritic cells within tissues [19], and of tumor necrosis factor-alpha (TNF-α) in the induction of DC maturation [20], in the present study we describe the immunohistochemical detection of these two cytokines within lung tissues. This was performed both in tumor and non-tumor areas from the same patients, where the presence of macrophages (CD68+ cells) and immature DCs (S-100+ or CD1a+ cells) was also evaluated. The results show a higher frequency of DCs and macrophages in lung cancer, when compared to non-affected lung in the same patient, as well as an altered expression of both IL-4 and TNF-α in the same tissues.

Material and methods

Patients and tissue specimens

Biopsies of non-small cell lung cancers (NSCLC) and non-affected lung tissue were obtained during surgery for primary tumor resection from 15 patients, whose demographic characteristics are summarized in Table 1 in the "Results". No patient had received chemotherapy or radiotherapy at the time of surgery. Written informed consent was obtained from all subjects, and the protocol was approved by the Ethical Committee of the Centro de Tratamento e Pesquisa Hospital do Câncer A C Camargo (HCACC) (protocol n°742/05) and of the Institute of Biomedical Sciences (protocol n°676/CEP).

Immunohistochemical staining

One paraffin block per tumor/non-affected tissue was used. Five serial sections were cut from formalin fixed and paraffin embedded tissues representing each tissue for subsequent analysis of immature DCs (CD1a+ and S-100+), macrophages (CD68+), IL-4, and TNF-α-secreting cells. Expression of the markers was evaluated using a streptoavidin-biotinylated horseradish peroxidase detection system (LSAB+kit/HRP; DAKO, Kyoto, Japan). After retrieval of the antigen by heating in a microwave oven for 15 min, sections were incubated overnight at 4°C with a primary antibody for each marker (CD1a clone CTB6, Santa Cruz; CD68 clone PG-M1, Dako; polyclonal antibodies against S-100 protein, Dako; and IL-4 and TNF-α polyclonal from R&D Systems). For the negative control, the primary antibodies were omitted. As chromogen, a solution of diaminobenzidine–tetrahydrochloride (0.03%) containing 0.1%

Table 1 Demographic and clinical characteristics of the patients

Patient	Age	Sex	Smoker	Diagnosis	PTNM[a]-CS[b]
1	69	M	Ex	LCC	T2N2M0-3A
2	58	F	N	SCC	T2N0M0-1B
3	66	M	N	SCC	T1N2M0-3A
4	47	F	N	ADC	T2N1M0-2B
5	81	M	Ex	SCC	T4N1M0-3B
6	74	M	Ex	SCC	T2N0M0-1B
7	73	M	S	LCC	T2N0M0-1B
8	75	M	S	LCC	T4N1M0-3B
9	45	F	Ex	ADC	T2N0M0-1B
10	70	M	Ex	ADC	T1N0M0-1A
11	74	M	Ex	ADC	T2N0M0-1B
12	44	M	S	SCC	T3N0M0-2B
13	77	M	S	SCC	T1N0M0-1A
14	65	F	Ex	ADC	T1N0M0-1A
15	64	M	S	ADC	T2N0M0-1B

F Female, *M* male, *Ex* ex-smoker, *N* non-smoker, *S* smoker, *Adc* adenocarcinoma, LCC large cell carcinoma, SCC squamous cell carcinoma

[a] Pathological tumor-node metastasis classification

[b] Clinical stage

Table 2 Average number of positive cells for CD1a, S-100 and CD68 in lung tissues by immunohistochemistry (*P* in paired Student's *t* test)

Patient	CD1a		S-100		CD68	
	Non-affected lung	Tumor-affected lung	Non-affected lung	Tumor-affected lung	Non-affected lung	Tumor-affected lung
1	0.6	77.4	46.8	117.6	151.0	400.0
2	19.2	32.4	97.4	101.8	204.6	538.6
3	0.0	92.8	0.0	152.0	128.6	152.0
4	–	9.6	–	15.2	–	460.4
5	52.6	–	144.8	–	217.2	–
6	1.0	0.2	6.2	12.0	145.8	188.6
7	–	24.6	–	6.8	–	124.2
8	–	0.0	–	34.6	–	145.4
9	1.0	3.0	12.6	137.2	223.2	228.0
10	0.0	120.0	0.0	128.8	96.4	227.4
11	0.0	0.0	0.2	5.6	198.4	495.4
12	0.0	0.0	1.0	33.4	50.0	53.4
13	0.2	151.0	4.8	20.0	185.2	471.4
14	0.0	–	15.6	–	88.6	–
15	0.0	0.0	0.0	6.0	380.0	400.0
Mean	6.2	39.3	27.4	59.3	172.4	298.8
P value	0.0371		0.0177		0.0108	

Each marker was analyzed in five fields of one slide/patient

– Not done

hydrogen peroxide was used. Sections were counterstained with hematoxylin. The number of immature DCs and macrophages was counted in five fields, at a magnification of 100×, under light microscopy. The average count was used as the final score. The level of IL-4 and TNF-α expression was scored according to Tazi et al. [21] and Ogawa et al. [22], based on the staining intensity of the cells: score 0, if no staining was detected; score 1, if the staining intensity was weak; score 2, if the intensity was moderate; score 3 if the intensity was high.

Preparation of single-cell suspension from lung tissues and labeling for flow cytometry

Lung tissues (tumor affected and non-affected), aseptically obtained from surgical resection, were minced and single-cell suspensions were generated by digestion with collagenase type VIII (0.56 mg/ml; Sigma, St Louis, MO, USA) during 120 min incubation at 37°C. After this, the cell suspensions derived from tumor-affected lung or non-affected lung were stained with fluorescent monoclonal antibodies against CD14 and CD80 and analyzed by flow cytometry.

Statistical analysis

The number of DCs (CD1a+ or S-100+) and macrophages (CD68+) infiltrating tumor-affected and non-affected lung in the patients was compared by paired two-tailed Student's *t* test. The expression of IL-4 and TNF-α in the tissues was assessed through χ^2 test for trends. Differences were considered significant when *P* was less than 0.05. All statistical analyses were performed using the Graphpad Software Prism 2.01 for Windows.

Results

Patients' characteristics

The patients' age, gender, smoking status, diagnosis, pathological tumor-node metastasis classification (pTNM) and clinical stage (CS) are listed in Table 1.

Distribution in situ of CD1a+ and S-100+ immature DCs and CD68+ macrophages

Patients' samples were analyzed as to the frequency of immature DCs (CD1a+ and S-100+) and macrophages (CD68+) in the primary tumor and in non-affected lung tissue by immunohistochemistry (Figs. 1, 2). Consistent and statistically significant differences were observed between these tissues, both in individual patients and as a group. The number of immature CD1a+ DCs per field (100×) within tumor ranged from 0 to 151.0 cells, with a mean, for the 15 patients, of 39.3, whereas in non-affected lung, the frequency of these cells ranged from 0 to 52.6 cells with a

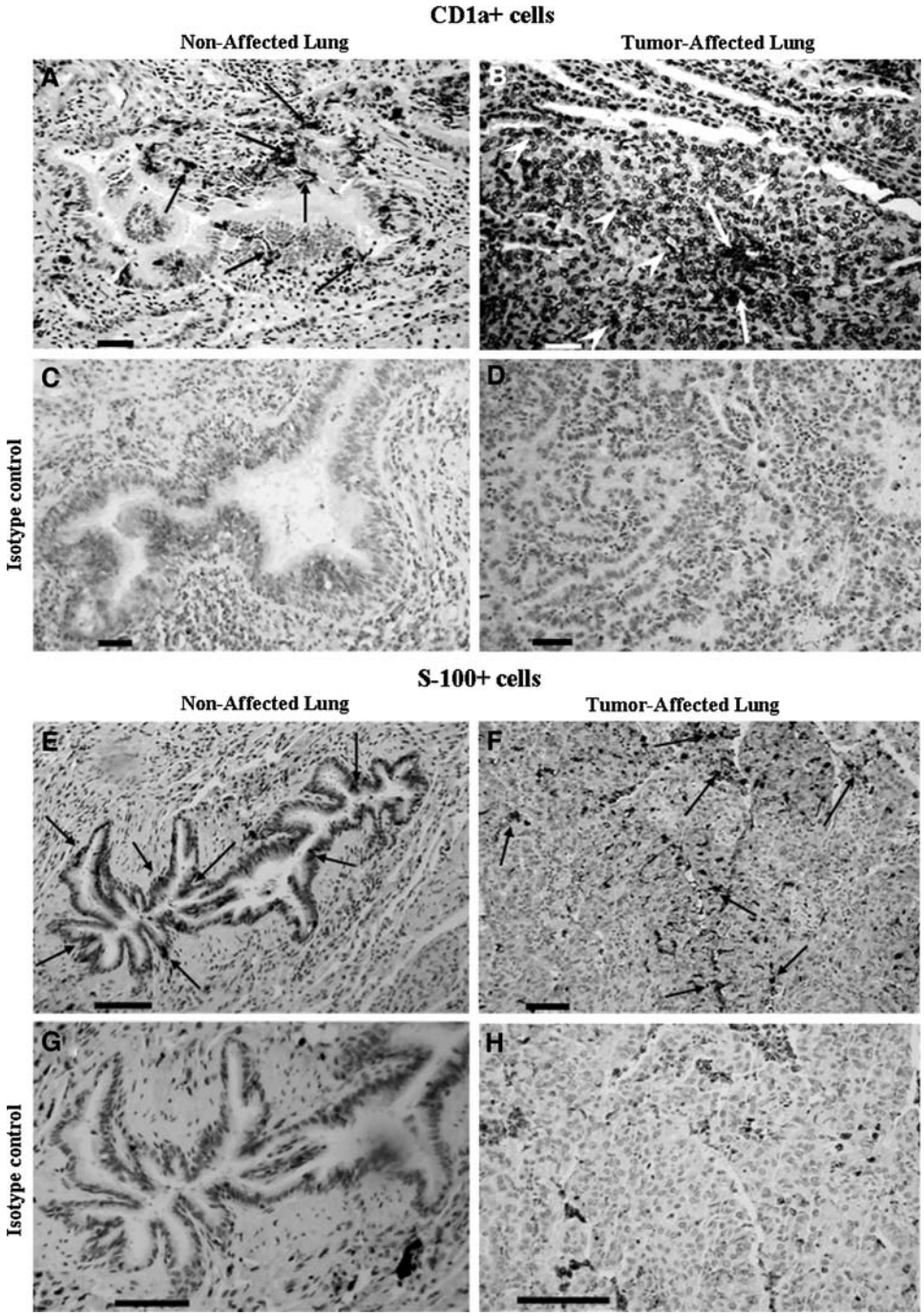

Fig. 1 Distribution and location of immature dendritic cells CD1a+ (**a** and **b**) and S-100+ (**e** and **f**) within non-affected lung tissue (**a** and **e**) and tumor-affected tissue (**b** and **f**). *Scale bar* = 100 μm. The distribution pattern of CD1a+ and S-100+ DCs in non-affected tissue was restricted to epithelia (*arrows* in **a** and **e**), whereas within tumor-affected tissue, these cells were either scattered (*white arrowheads* shown in **b** and *black arrows* in **f**) or aggregated (*white arrows* shown in **b**) around the neoplasia. In **c** and **d** are shown, respectively, isotype control of CD1a in non-affected and tumor-affected lung. In **g** and **h** are shown, respectively, isotype control of S-100 in non-affected and tumor-affected lung. *Scale bar* = 100 μm

mean of 6.2 ($P = 0.0371$). Likewise, immature S-100+ DCs in the tumor environment ranged from 5.6 to 152.0 cells/field with a mean of 59.3, whereas in non-affected lung their frequency ranged from 0 to 144.8 with a mean of 27.4 ($P = 0.0177$). No statistically significant correlation was found between the densities of immature DCs (both CD1a+ and S-100+) and the histological type of tumor, or with the tumor stage. Also macrophages were present with a higher frequency within the tumor (ranging from 53.4 to 538.6 cells, with a mean of 298.8 vs. a range of 50.0–380.0 with a mean of 172.4 in non-affected tissues, $P = 0.0108$). Interestingly, the frequency of CD68+ cells in adenocarcinomas was higher than their number in squamous-cell carcinomas ($P = 0.0048$) (Table 2).

Detection of IL-4 and TNF-α in situ

Cells of lung and tumor parenchyma were labeled similarly with the anti-IL-4 antibody, without any significant difference between these two tissues ($P = 0.5527$) (Fig. 3a, b).

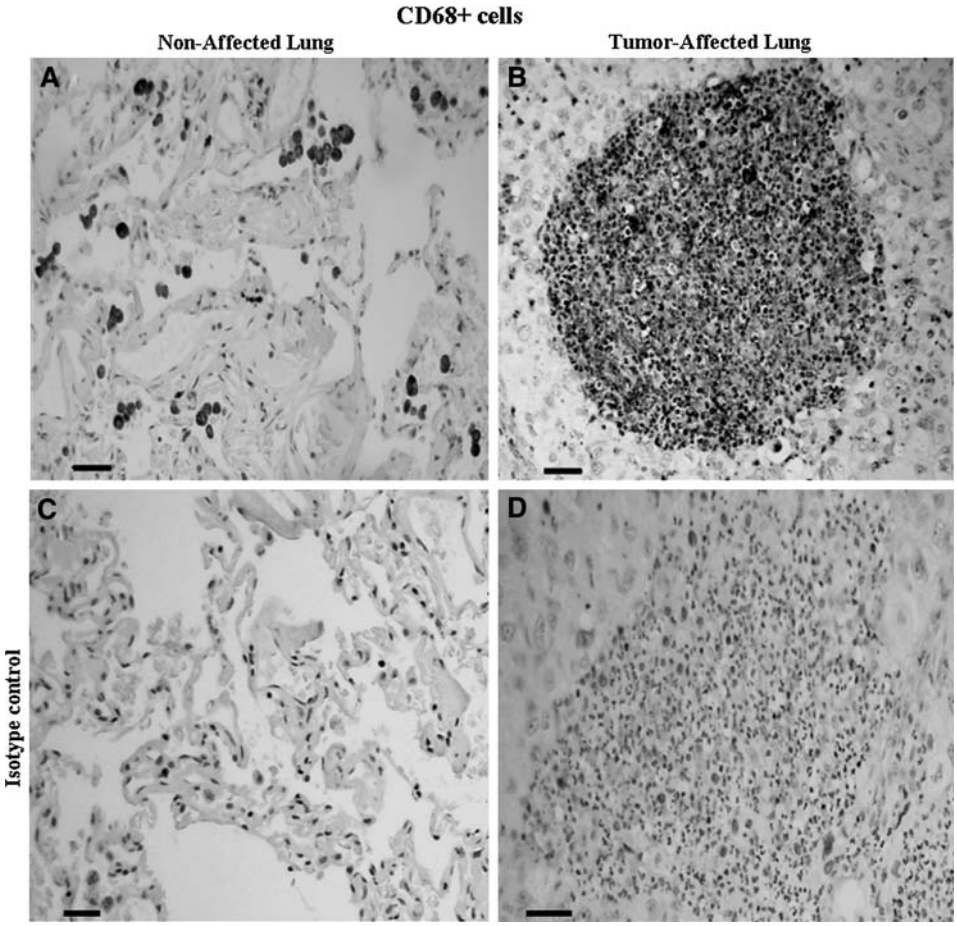

Fig. 2 Distribution and location of macrophages CD68+ within non-affected lung tissue (**a**) and tumor-affected tissue (**b**). In **c** and **d** are shown isotype controls of CD68 in non-affected and tumor-affected lung, respectively. *Scale bar* = 100 μm

Also macrophages in both the tissues were stained with the anti-IL-4 antibody (Fig. 3e), but, for these cells, the level of expression was significantly higher among non-affected lung samples ($P = 0.0097$). In non-affected lung, the expression of IL-4 could be detected in macrophages of all patients, one with low (1), three with moderate (2), and eight with high intensity (3) (Table 3). In contrast, in tumor-affected lung the expression of IL-4 could not be observed in two patients, in three patients its expression was low (1), in six, moderate (2) and in two, high (3) (Table 3).

Also TNF-α was detected in parenchymal cells from both the tissues (Fig. 4a, b), but contrasting with the IL-4 expression, labeling intensity for TNF-α was higher in tumor parenchymal cells than in non-affected lung parenchyma ($P = 0.0415$) (Table 3). While in non-affected lung, the expression of TNF-α could be detected in parenchymal cells in 9 of 12 patients analyzed [three with low (1); two with moderate (2); and four with high intensity (3)], in tumor-affected lung, the expression of TNF-α could be observed in all patients [(in two patients it was low (1), in three, moderate (2) and in eight, high (3)]. TNF-α was also detected inside macrophages, but for these cells, the high staining intensity was equal in both the tissues (Fig. 4e; Table 3).

As noted for the presence of DCs within the different histological types of tumor or the different tumor stages, no statistically significant correlation was found between the detection of IL-4 or TNF-α and the former parameters.

Immunophenotype of cells from pulmonary and neoplastic tissue

Patients were analyzed for the frequency of CD14−CD80+ cells in the primary tumor and in non-affected lung tissue by flow cytometry. Lung DCs were defined using the following criteria: at first, based on their side and forward scatter, lymphoid cells such as B and T were excluded from our analysis (Fig. 5). In the morphological analysis of the cells (forward and side scatter), no difference was detected between tumor-affected and non-affected tissues (data not shown). In the cell population that should include DCs, as judged by forward and side scatters (R2), the cells that were CD14− and expressed the co-stimulatory molecule, CD80 were considered as mature DCs. Lower frequency of phenotypicaly mature dendritic cells (CD14-CD80+) was found in tumor-affected lung when compared with non-affected lung ($P = 0.0235$) (Fig. 5). It is noteworthy that in this same population, the frequency of cells expressing the common myeloid DC marker, CD11c,

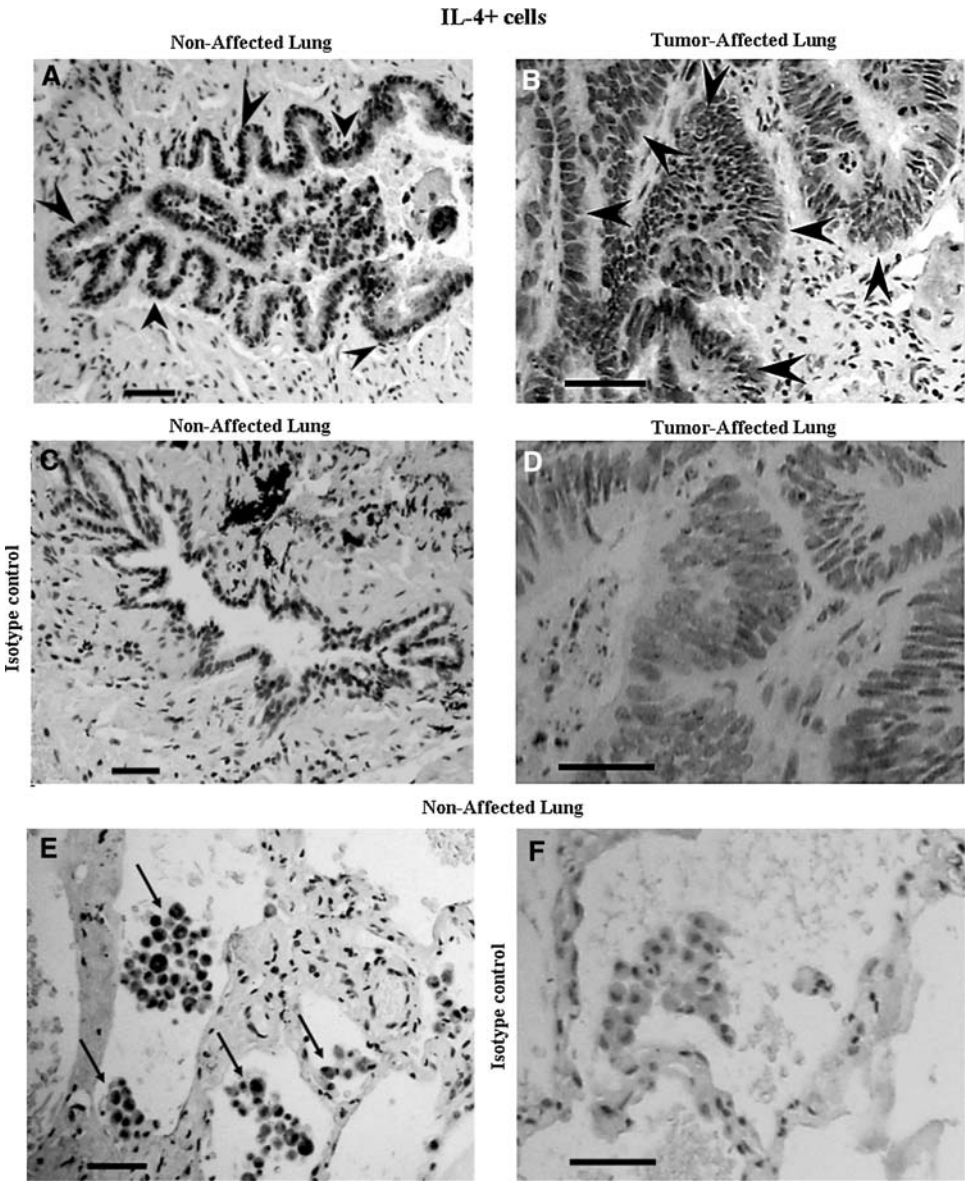

Fig. 3 Detection of IL-4 by immunohistochemistry in lung tissues. IL-4 production was observed in bronchiolar epithelium of non-affected tissues (*arrowheads* shown in **a**), tumor cells (*arrowheads* shown in **b**) and macrophages (*arrows* shown in **e**). In **c** and **d** isotype controls of IL-4 in non-affected and tumor-affected lung, respectively, are shown. In **f** the isotype control of IL-4 in non-affected lung (macrophages) is shown. *Scale bar* = 100 μm

was not different between tumor and non-affected lung (non-affected lung: 8.7% × tumor-affected lung: 10.2%; $P = 0.6884$). These comparisons of cell types' frequencies are not affected by the recovery of different cell numbers from each type of tissue, since absolute numbers of cells recovered from tumor samples were not significantly different from those recovered from non-tumor affected lung tissues in each patient. This is also reflected in the average cell recovery from each tissue type (tumor = $2.6 \times 10^7 \pm 2.2 \times 10^7$ cells/gram × non-tumor = $3.0 \times 10^7 \pm 2.4 \times 10^7$ cells/gram).

Discussion

The local microenvironment is probably a significant factor in the determination of the immune response pattern to tumors [17]. Among the various parameters that influence this phenomenon, the nature, frequency and activation state of antigen presenting cells should play a relevant role. In the present paper, we show that, indeed, there is a considerable alteration in the frequency and distribution of macrophages and dendritic cells within the tumor, when compared to the non-affected lung. Furthermore, we observed that the frequency of CD68+ macrophages was higher among adenocarcinomas than among squamous-cell carcinomas. This last phenomenon could be consequent to the usual location of both the tumor types, since adenocarcinomas tend to be more peripheral, whereas macrophages are physiologically more abundant [46]. For the other markers analyzed, the absence of correlation between their presence and tumor histological type and/or stage cannot be regarded as a definitive observation, since the sample size

Table 3 Staining intensity of sections subjected to immunohistochemistry for detection of IL-4 and TNF-α positive cells in lung tissues

Patient	IL-4				TNF-α			
	Non-affected lung		Tumor-affected lung		Non-affected lung		Tumor-affected lung	
	Parenchyma	Macrophages	Parenchyma	Macrophages	Parenchyma	Macrophages	Parenchyma	Macrophages
1	0	2	0	1	3	3	2	3
2	1	1	0	0	3	3	3	3
3	3	3	1	1	3	3	3	3
4	–	–	3	2	–	–	3	3
5	1	3	–	–	2	3	–	–
6	3	2	0	0	1	3	3	3
7	–	–	3	3	–	–	3	3
8	–	–	1	1	–	–	2	3
9	0	3	2	2	0	3	2	3
10	0	3	1	3	3	3	3	3
11	0	2	2	2	0	3	3	3
12	0	3	0	2	2	3	1	3
13	0	3	0	2	0	3	1	3
14	0	3	–	–	1	3	–	–
15	0	3	0	2	1	3	3	3
Mean	0.7	2.6#	1.0	1.5#	1.7*	3.0	2.5*	3.0

According to staining intensity, immunopositivity was scored from 0 to 3. Each marker was analyzed in 5 fields of one slide/patient

* $P = 0.0415$ in χ^2 test for trends, when non-affected versus tumor-affected lung macrophages were compared

$P = 0.0097$ in χ^2 test for trends, when non-affected versus tumor-affected lung macrophages were compared

– Not done

for each tumor type taken individually was not large enough to reach this conclusion.

Dendritic cells were identified in tissue sections by the expression of either S-100 or CD1a. The first marker, S-100, is a citosolic calcium-binding protein [23, 24] that is expressed in immature DCs [5,25] and is often used for detection of this type of cell in situ by immunohistochemistry [26–30] On the other hand, the CD1a molecule is the hallmark of a subset of DC, the Langerhans cells (LCs), that though initially described as a skin cell type [31, 32] is, today, also known to be present in the lung [21, 33]. It is noteworthy that the frequency of S-100+ cells was higher, in almost all samples analyzed, than that of CD1a+ cells, an observation that is compatible with the expression of this marker only by a subset of lung dendritic cells. Even higher was the frequency of macrophages in the tissues, again an expected observation [34]. Intriguing, however, was the observation that DCs, both CD1a+ and S-100+, were present with higher frequency within tumors. Furthermore, CD1a+ cells were those with the more pronounced increase in frequency. Considering that LCs are a subpopulation of DCs with specific functional characteristics [31], this preferential increase might affect the pattern of antigen presentation within the lungs.

Not only was the frequency of DCs affected in tumors, but also their distribution. While, DCs (both CD1a+ and S-100+) were predominantly placed in the bronchiolar epithelium in non-affected lung, that is in agreement with the literature [21, 33], in tumors, two distinct distribution patterns were observed: cells scattered throughout the tissue or cells aggregated in some areas. Such patterns of DCs distribution were present, frequently within the same patient and are similar to those described by Kurabayashi et al. [35]. Such patterns could be due to the architectural disorganization of tissues, caused simply by the tumor growth, but a "tumor-coordinated" distribution cannot be ruled out.

To explain the increased frequency of DCs within tumors, one could also argue that the local tumor growth, causing tissue damage, could lead to an enhanced recruitment of inflammatory cells to the tumor site. There, a microenvironment favorable to differentiation of precursors toward DCs would lead to the observed high frequency of these cells. This hypothesis is partially supported by the frequent detection of IL-4 within tumor samples, a cytokine with a clear role in the promotion of DC differentiation from monocytes [17, 19]. However, the intensity of IL-4 detection within tumors was not increased but decreased (mainly in the macrophage population), an observation that would not indicate such a preferential environment for DC differentiation in lung tumors.

Another possible explanation for this phenomenon would be the existence of a maturation deficit of tumor-infiltrating

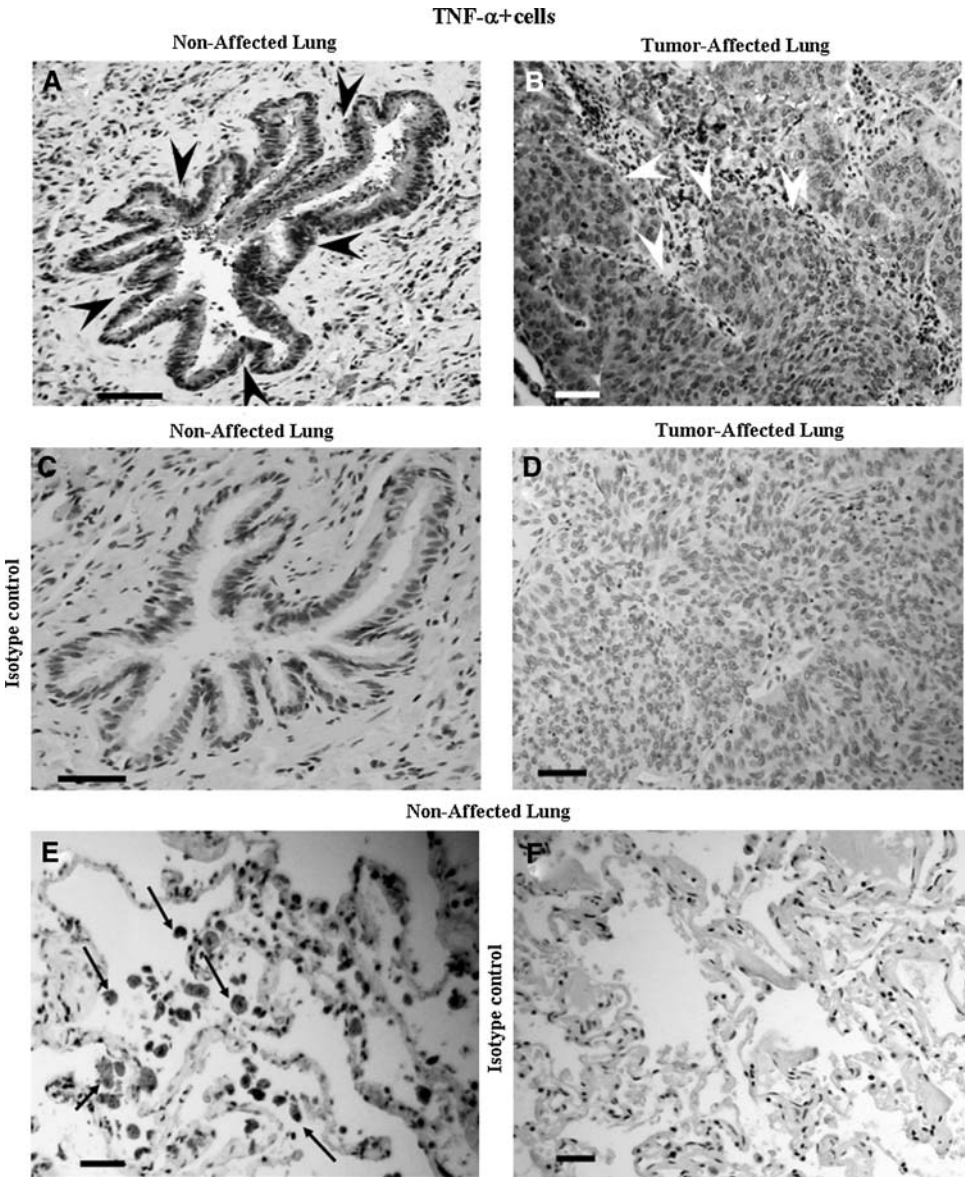

Fig. 4 Detection of TNF-α by immunohistochemistry in lung tissues. TNF-α production was observed in bronchiolar epithelium of non-affected tissues (*black arrowheads* shown in **a**), tumor cells (*white arrowheads* shown in **b**) and macrophages (*arrows* shown in **e**). In **c** and **d** isotype controls of TNF-α in non-affected and tumor-affected lung, respectively, are shown. In **f**, isotype control of TNF-α in non-affected lung (macrophages) is shown. *Scale bar* = 100 μm

DCs, a phenomenon that is frequently observed [36]. This maturation deficit could lead to an accumulation of immature cells within tissues, thus explaining our observations. Actually, in the flow cytometry study of cell suspensions obtained from tumor and from non-affected lung tissue samples, we noticed the presence of a higher frequency of CD14−CD80+ cells in non-affected lungs, an observation in agreement with this maturation deficit hypothesis. It is noteworthy that the usually used maturation marker, CD83, was not used for the determination of mature DCs because we have previously described that this marker is expressed, with high frequency, by non-hematopoietic cells within lung tissues, both tumor-affected and non-affected [47]. Furthermore, a possible enhanced maturation dependence of LCs, in order to leave peripheral tissues, could also explain their preferential increase within the tumor.

Paradoxically however, we detected high levels of TNF-α within tumors, mainly on parenchymal cells. Since this cytokine, in spite of being a growth factor for some tumors [37–39], is also a strong pro-inflammatory factor and important for DC maturation [19, 20], its increased levels in tumors would be contradictory with this hypothetical DC maturation deficit. Nevertheless, it is necessary to consider that, in the tumor microenvironment, there might be the simultaneous production of cytokines such as IL-10 and TGF-β that could block the maturation of local, immature DCs, as it has been shown in other tumors [40]. Furthermore, the detection of the cytokine does not indicate, necessarily, that it is exerting all its roles. The presence of tumor-infiltrating B lymphocytes (TIL-B), producing neutralizing antibodies to TNF [41], or the presence of soluble TNF receptors [42], could block some of this cytokine's biological activities in the tumor environment. Moreover, in spite

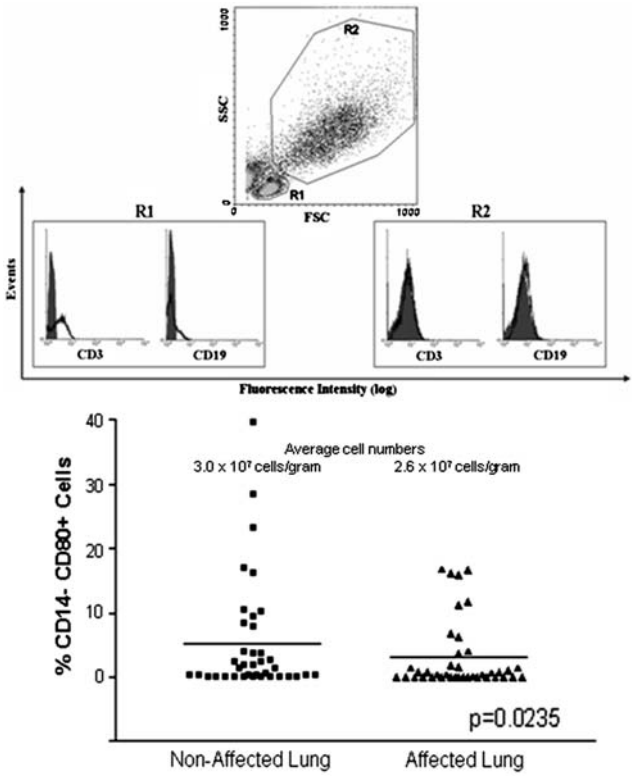

Fig. 5 Frequency of mature DCs within lung tissues. *Top* Dot-plot showing gate of cells analyzed, as defined by forward and side scatter patterns of cell suspensions obtained from lung tissues. *Middle* Histograms showing expression of CD3 (T cells) and CD19 (B cells) on cells gated in R1 and R2, in neoplastic tissue (filled histograms represent isotype controls). Note that the B (CD19+) and T (CD3+) lymphocytes are located in a region of cells that have a lower side scatter (reflecting lower granularity and complexity) as well as a lower forward scatter (reflecting cell size). Therefore, cells gated in R2 excluded both B and T lymphocytes from our analysis. *Bottom* graph The frequency of CD14−CD80+ cells gated in R2, compared by paired two-tailed Student's *t* test, showed a significant difference, with $P = 0.0235$. Cells numbers/tissue were not different (tumor = $2.6 \times 10^7 \pm 2.2 \times 10^7$ cells/gram × Non-tumor = $3.0 \times 10^7 \pm 2.4 \times 10^7$ cells/gram; $P = 0.464$)

of high immunopositivity for TNF in our samples, it is important to take into account that the antibody used in such analyzes was polyclonal, not being able to distinguish between free TNF and receptor-bound TNF [43]. Thus, we cannot exclude the possibility that the TNF detected in lung epithelial and tumor cells had been produced by tumor-infiltrating inflammatory cells in the tumor stroma [44, 45] and was bound to receptors present on the parenchymal cells. This would be in agreement with the high expression of receptors for TNF (TNF-R) in lung cancer cells [43], which could, actually, act as a "sink" for the cytokine, avoiding its action on immature DCs.

Altogether, the data presented here, showing an increase in immature dendritic cells and macrophage frequency within tumors, a decreased staining for IL-4 in tumor macrophages and an increase staining for TNF-α in tumor parenchimal cells, are compatible with a local microenvironment that does not favor the induction of effective immune responses against lung cancer. Although the presence of other cytokines within tumors could have significant impact upon the natural history of tumors, the pattern of expression of cytokines that have relevant roles in the differentiation and maturation of DCs could affect even more the evolution of tumors, since these cells are key players in the initiation and determination of immune response patterns [5]. The predominance of immature DCs within tumors could affect not only the induction of immune responses in draining lymph nodes but also the frequency and activation status of tumor-infiltrating lymphocytes, thus affecting significantly the interactions of tumor and immune cells. Anyhow, the data presented here warrant the further investigation of these parameters that were, however, not addressed in the present study.

In conclusion, the data presented here offer enough evidence to further support that the local microenvironment within tumors can modify the pattern of immune response to tumor antigens and should be considered in immunotherapeutic approaches to lung cancer.

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