CHARACTERIZATION OF DENDRITIC CELL SUBSETS IN LUNG CANCERS

MICROENVIRONMENT

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ABSTRACT

The aim of this study was to seek evidence for a correlation between mediators present in lung cancers microenvironment and subsets of dendritic cells (DCs) infiltrating these tumors. We used immunohistochemistry and recently available antibodies to define the phenotype of DCs present in surgical biopsies from 12 patients with lung carcinomas and evaluated both at mRNA and protein levels the local expression of chemokines potentially involved in the recruitment of these cells. We also used real time PCR to analyze the expression of mRNA coding for cytokines known to influence DCs maturation in vitro.

Different subsets of myeloid DCs were present in lung cancers, but no plasmocytoid DCs were identified. Both Langerhans cells and CD1a+/Langerin− cells were interspersed among tumor cells, in numbers that were correlated to the amounts of CCL20 produced in these tumors. In most specimens, DC-SIGN+ DCs were also present at the periphery of the tumor beds. No DC-LAMP+ DCs were identified and CD83+ DCs were rarely present in the tumor stroma. All tumors expressed IL-10, TGF-β and VEGF, whereas IL-12 was virtually absent. Thus, various types of DCs infiltrate lung carcinomas and display an immature phenotype, presumably because of the inhibitory cytokine microenvironment.

Key words: chemokines; cytokines; immunohistochemistry; Langerhans cells; PCR
INTRODUCTION

Promising results have been previously reported using DC based vaccination against particularly immunogenic malignant tumors such as metastatic melanomas and renal carcinomas [1, 2]. Since, numerous clinical trials have evaluated various strategies for using DCs in immunotherapy for patients with advanced cancers, including lung cancer [3-5]. Although, a T cell mediated immune response has been repeatedly shown in these patients, the clinical effectiveness of these therapeutic approaches was, however, generally limited [3]. Thus, questions have been raised concerning the type and activation state of human DCs that should be used to induce an effective anti-tumoral immunity.

Indeed, DCs are highly heterogeneous cell populations, and numerous subsets of DCs with different functional capacities have been identified [3, 6]. Thus, myeloid DCs can be derived in vitro from CD34+ precursors or from blood monocytes, and these DCs can differentiate into Langerhans cells (LCs), particularly in the presence of TGF-β [3, 6]. Plasmacytoid DCs are derived from lymphoid precursors, express the IL-3R (CD123) at their surface and are dependent for their growth on the presence of IL-3 [3, 6]. DCs are also functionally heterogeneous according to their state of maturation [3, 6]. Thus immature DCs (such as myeloid DCs normally present in peripheral tissues and LCs in the epidermis or the respiratory tract mucosa) capture antigens but weakly stimulate T lymphocytes. In the presence of particular signals, such as LPS or various cytokines, these cells mature into potent lymphostimulatory cells, a process that is associated with up-regulation of costimulatory molecules (CD80, CD86, CD40, DC-LAMP, CD83), and the switch of chemokine receptors expressed at their surface. Immature DCs, particularly LCs are CCR6+ and respond to CCL20 (MIP-3α) where as mature DCs are attracted by CCL19 (MIP3-β) or CCL21 (SLC) chemokines following de novo expression of CCR7 [7]. It should be stressed, however, that even these phenotypically mature DCs can elicit a tolerogenic immune response, by
preferentially activating regulatory T-cells [8]. Indeed, a critical characteristic of fully mature DCs is the production of pro-inflammatory cytokines, particularly IL-12, which plays a critical role in the induction of an efficient TH1 immune response, including anti-tumor cytotoxic immunity [3, 9].

The microenvironment in which DCs are present has important bearing on the maturation process, and therefore affects the final outcome of the immune response [8]. Thus, IL-10, TGF-β, VEGF or prostanoids inhibit the maturation of DCs at different levels, whereas other factors such as LPS, IFN-γ or early interactions with CD40 ligand expressing T-cells stimulate their functional capacities [3, 6, 8].

It is known that tumors escape surveillance by the immune system through various mechanisms, including the inhibition of the recruitment and/or function of host’s DCs, and the local production of immunosuppressive factors [10, 11]. Thus, the precise knowledge of the tumor microenvironment, which vary in different tumor types, is important to design optimal DC based therapeutic strategies against cancer.

We have previously reported that various numbers of DCs infiltrate lung carcinomas and suggested that these cells were functionally immature [12]. More recently novel markers have emerged that allow the identification in tissue sections of a broader spectrum of DC subpopulations as well as better assessment of their functional status. In this study, we have used these new tools to further characterize DC populations present in non small cell lung carcinomas, and evaluate the local expression of chemokines that may play a role in the recruitment of these cells. We also analyzed by real time PCR the production in the tumor microenvironment of factors that are known to have a critical influence on DC function.
METHODS

Tissue specimens

Biopsies of primary lung carcinomas were obtained from 12 patients (9 men; 3 women; mean age 64 ± 8 yr, all smokers) at the time of thoracic surgery. The histologic types of cancer were: squamous cell carcinoma (n=5), adenocarcinoma (n=5); undifferentiated large cell carcinoma (n=2). No patient had received chemotherapy or radiotherapy at the time of evaluation. The study was approved by our institution review board.

Processing of tissues and morphologic assessment

Lung tumor biopsies were immediately snap frozen and stored in liquid N₂ until use for isolation of RNA or immunohistochemical evaluation. The histopathologic features of the frozen specimens were evaluated using cryostat sections stained with hematoxylin and eosin. The entire tumoral surface of each section was quantified using an image analysis system (Microvision, Histolab, Evry, France). In all cases, a second tissue fragment was fixed in Bouin-Hollande solution, processed by routine techniques and used for diagnostic purposes. Two paraffin-embedded specimens were also used for immunostaining with anti-CCR6 antibody.

Isolation of RNA and cDNA synthesis

Total RNA from each sample was isolated using Rneasy Mini kit according to the manufacturer’s instructions (Qiagen, GmbH, Hilden, Germany) and quantified by measurement of absorbance at 260 nm. cDNA was synthesized using an Omniscript reverse transcriptase kit according to the manufacturer’s instructions (Qiagen).

Quantification of cDNAs by real-time PCR

Ten µl of each cDNA sample was amplified in the presence of 25 µl Taqman universal PCR master mix (Perkin-Elmer, Foster City, CA), 2.5 µl assays-on-demand containing specific primers and probe for each gene target evaluated (CCL20, CCL19, GM-CSF, IL-12,
IFN-γ, IL-10, TGF-β, IL-4, VEGF) obtained from Perkin-Elmer and 22.5 µl of water (50 µl, final volume). For amplification of the internal positive control (GAPDH), reaction mixture contained 25 µl Taqman universal PCR master mix (Perkin-Elmer), 10 µl cDNA, 200nM each specific forward (5’-ACC CCT GGC CAA GGT CAT C) and reverse (5’-AGG GGC CAT CCA CAG TCT TC) primers, 200nM TaqMan probe [5’-(6-FAM)- AGG ACT CAT GAC CAC AGT CCA TGC CAT (MGB)] and water (50 µl, final volume). In all cases, cycling parameters were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Amplifications of the specific cDNA and the internal positive control were always performed at the same time and in parallel for all samples. Gene specific PCR products were measured by means of an ABI PRISM 7000 Sequence Detection System (Perkin-Elmer). The number of cycles required to reach threshold fluorescence (Ct) was determined, and the quantity of sequences initially present was calculated by extrapolation onto the standard curve. All reactions were performed in duplicate, and the mean of the 2 values was used for calculations. Target gene expression was normalized between different samples based on the values of the expression of the internal standard.

**Immunohistochemical techniques**

Monoclonal antibodies (mAbs) used in this study were: anti-CD11c (BU15, Immunotech, Marseille, France); anti-CD1a (BL6, Immunotech); anti-Langerin (DCGM4, Immunotech); anti-CCR6 (53103.111, R&D Systems; Abingdon, UK); anti-DC-LAMP (104.G4, Immunotech); anti-CD83 (HB15a, Immunotech); anti-IL-3R (S-12, Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-CD3 (UCHT1, Immunotech); anti-CD19 (J4.119, Immunotech). Immunoglobulin IgG1 (679.1Mc7, Immunotech) and IgG2b (MOPC-195, Immunotech) control antibodies were used to assess non-specific binding. Purified goat polyclonal antibodies used were: anti-MIP-3α (CCL20); anti-MIP-3β (CCL19); anti-DC-SIGN and anti-CCR7 (all from Santa Cruz).
Immunohistochemical staining was performed as previously described [12], using Vectastain ABC-alkaline phosphatase system (Vector, Burlingame, CA, USA) and the fast red substrate. Immunostaining for CCR6 on 2 paraffin embedded specimen was performed as previously described [13]. The intensity of immunostaining was graded from – (absent) to +++ (strongly positive). Complete agreement in scoring was obtained between 2 independent observers. To determine the numbers of CD1a+ and Langerin+ cells within a given biopsy, positive cells were counted using a light microscope at a magnification of 400 (40X objective and 10X eyepiece), and the entire surface of serial sections stained with the 2 antibodies was evaluated. Results are expressed as cells/mm² of the tissue section. Interobserver variation was <10%.

Statistical analysis

Results are presented as mean ± SD, unless otherwise stated. Comparisons were made using the Mann-Whitney U-test, except for histologic type and lymph node staging which were made by Fisher exact test. Correlation analyses between the number of CD1a+ cells infiltrating tumor specimens and the chemokines and cytokines studied was performed using the Spearman correlation coefficient. A p value of < 0.05 was considered significant.
RESULTS

Surface phenotype of dendritic cells infiltrating non small cell lung carcinomas

Various numbers of CD11c+ DCs (i.e. myeloid DCs) were present in all non small cell carcinoma specimens. In contrast, no CD123+ (IL3-R) DCs (i.e. plasmacytoid DCs) were observed in the tumor tissues evaluated in this study, although some endothelial cells were CD123+, as confirmed by anti-CD31 immunostaining on serial sections (not shown).

The lung tumor specimens were variably infiltrated by CD1a+ DCs. The distribution of these CD1a+ cells was not uniform within a given tumor and positive cells were essentially localized within tumor nodules interspersed among tumor cells (Figure 1A). Interestingly, the 12 samples evaluated could be separated into 2 groups according to the numbers of CD1a+ cells infiltrating the tissue sections: 6 samples were heavily infiltrated by CD1a+ DCs, whereas few CD1a+ cells were identified in the remaining 6 specimens (Table 1, 18.9 ± 8.4 cells/mm² and 2.3 ± 1.2 cells/mm² respectively, p<0.01 comparing the 2 groups). The numbers of CD1a+ cells present within a tissue specimen were not correlated with the histologic type or the lymph node staging of the tumor obtained after surgery (Table 1, p>0.2). Langerin+ cells were unequivocally observed in several tumor tissue specimens and had the same distribution as that of CD1a+ cells (Figure 1B). Strikingly, however, on serial tissue sections, the numbers of Langerin+ cells were always lower than that of CD1a+ cells (Table 1). Furthermore, few or no Langerin+ cells were noted in 5 specimens, including 2 lung tumors heavily infiltrated by CD1a+ cells (Table 1).

Interestingly, DC-SIGN+ cells were also observed in 7 lung tumor biopsies (Table 1). On serial tissue sections, these cells were shown to be CD1a negative and were localized in areas immediately surrounding the tumor infiltrates (Figure 1C). Furthermore, these DC-SIGN+ cells were, without exception, CD68 negative (Figure 1D). Conversely, no DC-LAMP+ DC was observed in any of the 12 lung tumors evaluated, although in 5 tumors, type II
pneumocytes adjacent to the tumor nodules strongly reacted with the anti-DC-LAMP antibody (Figure 1E). Scant CD83⁺ DC were observed in 10/12 tumors, essentially in the connective tissue stroma surrounding the tumor nodules (Figure 1F). These cells had a characteristic dendritic shape and were CD3⁻ and CD19⁻ (not shown).

Expression of chemokines and chemokine receptors in lung carcinomas

mRNA coding for CCL20 was detected in all tissue specimens, although in variable amounts. Strikingly, the amounts of CCL20 mRNA were quantitatively higher in the 6 specimens heavily infiltrated by CD1a⁺ cells compared to that measured in the remaining 6 samples containing few CD1a⁺ cells (14.1 ± 13.3 and 2.4 ± 1.6 respectively, p<0.05, Table 2 and Figure 2). Immunostaining with anti-MIP-3α antibody demonstrated that tumor cells were the main cell type that expressed CCL20, although the intensity of this expression was heterogeneous from one tumor specimen to another. Tumors that were heavily infiltrated by CD1a⁺ DCs were strongly positive for CCL20 and were graded from ++ (moderately positive) to +++ (strongly positive) by 2 independent observers (Figure 3A), whereas 5 of 6 of the biopsies containing few CD1a⁺ cells were negative (-) for this chemokine and the remaining specimen reacted weakly (+) with anti-CCL20 antibody (Figure 3C and D). Furthermore, the number of CD1a⁺ cells present in tumor specimens and the amounts of mRNA coding for CCL20 were strongly correlated (r=0.95, p<0.001). Surprisingly, however, CD1a⁺ DCs present in the tumor specimens were always CCR6 negative, although CCR6⁺ lymphocytes were clearly present in the same samples (Figure 3A inset). No CCR6⁺ DCs were observed in 2 paraffin embedded tumor specimens expressing high amounts of CCL20 mRNA (not shown).

mRNA coding for CCL19 was also detected in all specimens studied here. No difference in the level of expression of CCL19 mRNA was observed, however, comparing tumors heavily infiltrated by CD1a⁺ cells and the specimens containing few CD1a⁺ cells (8.6 ± 10.6
and 5.2 ± 4.7 respectively, p>0.2, Table 2). Immunostaining with anti-CCL19 antibody was performed in 11 lung tumor biopsies and was positive in 5 specimens (Figure 4A). A part from tumor cells, bronchiolar epithelial cells, endothelial cells and some lymphocytes were also positive for this chemokine. No correlation was observed between the expression of CCL19 mRNA or CCL19 immunostaining and the presence of CD83+ DCs or DC-SIGN+ cells. Finally, the only CCR7+ cells present in 4 tumor specimens were endothelial cells as confirmed by morphological analysis and CD31 immunostaining (Figure 4B).

Cytokine pattern expressed in the tumoral microenvironment

To evaluate the potential effects of tumor microenvironment on DCs present in lung carcinomas, we measured by real time PCR the expression of mRNAs coding for GM-CSF, IFN-γ, IL-12, IL-4, IL-10, TGF-β and VEGF in the 12 lung tumor biopsies. As we previously reported, GM-CSF mRNA was detected in all samples, but in greater amounts in the tumor biopsies heavily infiltrated by CD1a+ cells (18.2 ± 16.6 and 1.5 ± 1.6 respectively, p<0.05, Table 2, Figure 2). The number of CD1a+ cells was strongly correlated with the amounts of mRNA coding for GM-CSF expressed in all 12 samples (r=0.94, p<0.001). mRNAs coding for VEGF, TGF-β, IL-10 and IFN-γ were expressed in all specimens, regardless of the number and phenotype of DCs present in the tumors (Table 2) and no correlation was observed with the number of CD1a+ cells present in tumor specimens (r <0.3 and p >0.3 for all cytokines). IL-4 and IL-12 mRNAs were detected in only 2 of 12 specimens for each cytokine (Table 2). Notably, the expression of IL-4 mRNA was not associated with the presence of DC-SIGN+ DCs in the same tumor biopsies.
DISCUSSION

In this study we have shown that: 1) different subsets of immature myeloid DCs, but not plasmacytoid DCs, infiltrate non small cell carcinomas; 2) MIP-3α (CCL20) produced locally may play a role in the recruitment of CD1a⁺ cells in these tumors; 3) lung carcinomas produce several factors that are known to inhibit DCs function.

**Phenotype of DCs infiltrating lung carcinomas**

All tumor specimens evaluated here contained CD1a⁺ DCs, although in variable numbers from one tumor to another. As we previously reported [14], these cells were essentially localized within tumor nodules and interspersed among tumor cells. Here, we have shown that this population of DCs can be further divided into CD1a⁺ Langerin⁺ cells (i.e. LCs) and CD1a⁺ Langerin⁻ cells. Similar CD1a⁺ subsets have been also identified in breast carcinomas [15, 16]. The CD1a⁺ Langerin⁻ cells could be precursors of LCs, or the intratumoral counterpart of the inflammatory dendritic epidermal cells described in some skin disorders [6, 17].

We also identified another population of DCs that was CD1a⁻ and expressed DC-SIGN, a C-type lectin involved in capture of antigen and interactions with T-lymphocytes [18]. These cells were present in 7/12 lung carcinomas and were always localized at the periphery of the tumor beds. Beside their dendritic morphology, DC-SIGN⁺ cells were always CD68 negative, as assessed using serial tissue sections, further confirming that they were not macrophages. DC-SIGN⁺ DCs were very recently identified in thyroid carcinomas and melanomas, and these cells were also localized at the periphery of the tumors [19, 20]. Finally, although it was reported that IL-4 was an important factor for the expression of DC-SIGN [21], mRNA coding for this cytokine was not detected by real time PCR in the 7 specimens that were infiltrated by this DCs subset, suggesting that other factors are involved in DC-SIGN
expression at the surface of DCs in vivo. The significance of the presence of these DC-SIGN+ DCs in human tumors remains to be studied.

Very few CD83+ DCs were observed in the connective stroma of lung carcinomas, indicating that most of the DCs subsets infiltrating these tumors were immature DCs. These cells had a characteristic dendritic shape and were not stained with anti-CD3 or anti-CD19 antibodies, further confirming that they were not activated T or B lymphocytes. Consistent with this idea, no tumor infiltrating DC expressed DC-LAMP, another marker of DC maturation. These results are in agreement with our previous observations that CD1a+ cells in lung cancers were CD40, CD80 and CD86 negative [12]. Strikingly, however, epithelial cells in the peritumoral tissue, particularly type II pneumocytes, intensely expressed DC-LAMP. The expression of DC-LAMP by type II pneumocytes in normal human lung has been very recently reported [22]. Thus, DC-LAMP should no more be regarded as specific for DCs, as it was initially thought.

Plasmacytoid DCs infiltrating other types of carcinomas were recently identified, and it has been suggested that these cells may be involved in the impaired immune response against tumors and adverse outcome [20, 23]. In contrast, plasmacytoid DCs were never identified in the lung carcinomas studied here. This did not reflect technical problems, since CD123 positive endothelial cells (as confirmed by the staining of these cells for the CD31 marker) were shown to be present in the same tissue sections. Interestingly, Conejo-garcia et al. have recently described, in an animal model, a population of DCs that migrate to endothelium and express markers of both dendritic and endothelial lineage [24]. Thus, although, CD123+ cells identified in our study had a characteristic endothelial shape, we cannot exclude that these cells represent the counterpart of endothelial DCs.

Chemokine production by lung carcinomas
In the current study, all tumor specimens expressed mRNA coding for CCL20, but the expression was significantly correlated with the number of CD1a+ cells infiltrating lung carcinomas. Evaluation of the expression of this chemokine at the protein level, using immunohistochemistry, showed that tumor cells were the main source of this chemokine, and further confirmed the correlation between the intensity of this expression and the burden of tumor CD1a+ infiltrating DCs. Expression of CCL20 has also been reported in other tumors, although a correlation with the presence of a particular subset of DCs was not established [16, 19]. Interestingly, in some experimental models, the expression of CCL20 in tumors was associated with the local recruitment of DCs, although the final outcome on the anti-tumor response was controversial [25-28].

Strikingly, we failed to identify CCR6+ DCs in any tumor specimen, despite repeated efforts, whereas CCR6+ lymphocytes were observed in the same tissue sections, suggesting that the expression of CCR6 at the surface of DCs was weak and not detectable by the antibodies used here. In this regard, although a correlation between the expression of CCL20 and the infiltration of breast carcinomas by CD1a+ DCs was reported, the expression of CCR6 receptor by these cells was not evaluated [16]. Similarly, although the in situ expression of CCR6 receptor has been assessed by immunohistochemistry in some pathological tissues, the specimens were paraffin embedded and antigen retrieval procedures were performed previously to immunostaining [19, 29]. Indeed, little data is currently available concerning the detection of CCR6 at the surface of DCs in snap frozen tissues. To further address this point, 2 paraffin embedded tumor specimens expressing high amounts of CCL20 mRNA were stained with anti-CCR6 antibody and were shown to contain no positive DCs. Thus, the expression of CCR6 antigen at the surface of DCs was probably below the threshold of immunohistochemical techniques used.
CCL19 chemokine mRNA was expressed in all lung carcinomas evaluated. In contrast to CCL20 expression, several cell types, apart from tumor cells, were stained with the anti-CCL19 antibody. This production of CCL19 is somewhat surprising, since few mature (CD83+)
DCs were observed in lung carcinomas, and no correlation could be established between CCL19 and the presence of any subset of DCs present in tissues evaluated, including the number of CD1a+ cells. CCL19 expression may play a role in the recruitment of naïve T-cells or NK cells, a hypothesis that was not evaluated here [7, 30]. It should be noted, however, that the only CCR7+ cell type identified were endothelial cells. Thus, additional studies are needed to define the role of this chemokine in lung cancer, and to determine whether CCL19 expression is beneficial or detrimental for anti-tumoral immunity.

**Cytokine microenvironment in lung carcinomas**

We confirmed our previous finding that GM-CSF was expressed in non small lung carcinomas, and that the levels of expression correlated with the numbers of CD1a+ infiltrating cells [14]. These findings suggest that CCL20 is not the only mediator involved in the recruitment of DCs in lung cancers. Consistent with the immature phenotype of DCs infiltrating lung carcinomas, the balance of the cytokine pattern in the tumor microenvironment was, however, clearly shifted toward an inhibitory milieu for DCs maturation. Thus, in all specimens, mRNAs coding for IL-10, TGF-β and VEGF were detected, regardless the subsets of DCs present in the tumor, and no correlation was observed with the number of CD1a+ cells. These 3 factors are potent inhibitory mediators of DCs maturation and function, and have been associated to various extents with a defective anti-tumor immune response [10, 11, 31, 32]. In contrast, although IFN-γ mRNA was present in all tumors, only 2 samples contained mRNA coding for IL-12, a critical cytokine for mounting efficient cytotoxic anti-tumor immune responses both in animal models and in human trials [3, 9]. Taken together, the virtual absence of IL-12 and the predominance of inhibitory factors
of DC maturation suggest that the tumor microenvironment is unlikely to be propitious for T-cell activation.

This study is limited, however, by the small number of specimens evaluated and results obtained here, although clear cut, should be further confirmed. Nevertheless, these results shade new light on DC biology in lung cancers, and show that tumor microenvironment is essentially inhibitory of DC maturation. These results should be considered when elaborating strategies using DC based immunotherapy for patients with lung cancers.
ACKNOWLEDGMENTS

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REFERENCES


Table 1. Phenotype of DCs infiltrating lung carcinomas*

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*: ADC: adenocarcinoma; SCC: squamous cell carcinoma; LCC: large cell carcinoma. Numbers for CD1a and Langerin are presented as cells/mm². Data for DC-SIGN are scored as – (absent) to ++ (numerous) cells. § p>0.2 comparing histologic type and lymph node staging obtained after surgery (pN) between the specimens respectively containing numerous and few CD1a⁺ cells. All patients were M0.
Table 2. Cytokine mRNAs expressed in tumor microenvironment*

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<td>1</td>
<td>3.4</td>
<td>5</td>
<td>0.8*</td>
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<td>0.4</td>
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*Data obtained by real time PCR are expressed as number of copies. § p<0.05 comparing the specimens respectively containing numerous (1-6) and few (7-12) CD1a⁺ cells.
FIGURE LEGENDS

Figure 1. Phenotype of DCs infiltrating lung carcinomas. (A) Adenocarcinoma tumor nodule containing numerous CD1a⁺ cells. (B) Another section of the same tumor showing infiltration by Langerin⁺ cells (x 250). (C) A squamous cell carcinoma showing the presence of DC-SIGN⁺ cells surrounding the tumor nodules. (D) A serial section from the specimen shown in panel C is negative with an anti-CD68 antibody (x 125). (E) DC-LAMP immunostaining. No positive DCs were observed. Note that the epithelial cells adjacent to the tumor nodules are strongly positive (x 125). (F) Scant CD83⁺ cells are present in this adenocarcinoma (x 300).
Figure 2. The expression of CCL20 (A) and GM-CSF (B) mRNAs in tumors that were heavily infiltrated by CD1a⁺ cells (■) and tumors containing few CD1a⁺ cells (□). Results of real time PCR are expressed as number of copies. P<0.05, comparing the 2 groups of tumors.
Figure 3. Comparison between the production of CCL20 by lung cancers and the presence of CD1a+ cells. (A) A squamous cell carcinoma showing strong positive reaction of tumor cells with anti-CCL20 antibody. Although no CCR6+ DCs were identified some lymphocytes were CCR6 positive in the same sample (Inset). (B) Serial section of the same tissue specimen containing numerous CD1a+ cells among tumor cells. (C) Adenocarcinoma weakly positive for CCL20 immunodetection. (D) Few CD1a+ cells were present in serial tissue section from the tumor shown in panel C. (x 250).
Figure 4. (A) Immunostaining for CCL19 in an adenocarcinoma showing positive tumor nodules. (B) A squamous cell carcinoma stained with anti-CCR7 antibody. Note that only endothelial cells are positive (x 250).
Abbreviations used in this study are: