Dendritic Cells Infiltrating Human Non-Small Cell Lung Cancer Are Blocked at Immature Stage[1]

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The efficacy of immune response to control human cancer remains controversial. It is particularly debated whether and to what extent the capacity of tumor-infiltrating dendritic cells (DC) to drive immunization can be turned off by transformed cells, leading to tumor-specific tolerance rather than immunization. To address this issue, we have characterized the DC isolated from human non-small cell lung cancer (NSCLC). These biopsy specimens contained CD11c^{high} myeloid DC (mDC), but also CD11c^{-} plasmacytoid DC (pDC) and a third DC subset expressing intermediate level of CD11c. Compared with peripheral blood, CD11c^{high} tumor-infiltrating DC (TIDC) displayed a "semi-mature" phenotype, and TLR4 or TLR8 stimulation drove them to mature partially and to secrete limited amounts of cytokines. In contrast, most tumor-infiltrating pDC were immature but underwent partial maturation after TLR7 activation, whereas TLR9 ligation triggered low secretion of IFN-α. CD11c^{int} mDC represented ~25% of total DC in tumoral and peritumoral tissues and expressed low levels of costimulatory molecules contrasting with high levels of the immunoinhibitory molecule B7-H1. Finally, the poor APC function of total TIDC even after TLR stimulation and the migratory response of both tumor-infiltrating mDC and pDC toward CCL21 and SDF-1 in vitro suggested their ability to compromise the tumor-specific immune response in draining lymph nodes in vivo. Further studies will be required to establish the specific role of the three TIDC subsets in tumor immunity and to draw conclusions for the design of therapeutic strategies. ***The Journal of Immunology,*** **2007, 178: 2763–2769.**

Most human tumors are infiltrated by immune cells, and features of the local immune response have often been found to correlate with clinical evolution of the disease (1, 2). Dendritic cells (DC),[3] after recognition of their unique role in the initiation of tumor-specific T cell response (3), have become the focus of particular interest. Early publications reported that the high density of CD1a^{+} immature DC infiltrating various types of cancers, including lung tumors, was associated with a better prognosis (4–7). However, the role of tumor-infiltrating DC (TIDC) turned out to be more complex with the discovery of different DC populations (8), the realization of the functional diversity and extensive plasticity of DC (9), and the understanding that tumors develop various strategies to escape immune surveillance, including the subversion of DC toward tolerance rather than immunization (10). When new markers became available, it was observed that both myeloid DC (mDC) and plasmacytoid DC (pDC) were present in some cancers, but that most TIDC were immature. It was also shown that several tumor-derived factors concur to inhibit DC maturation, including IL-10, IL-6, VEGF, TGF-β, lactic acid, and PGE_2 (reviewed in Ref. 10). Triggering the maturation of TIDC was therefore proposed as a therapeutic objective to counter tumor-driven tolerance and to boost immunization. However, abnormal differentiation of TIDC appears to be an additional factor responsible for tumor immune escape and it remains controversial whether these cells might be activated to ever become efficient APC. In mice, TIDC appear to be largely refractory to maturation signals, even after isolation from the tumor microenvironment (11, 12). To further characterize the functional capacities of human TIDC, we have now isolated such cells from human non-small cell lung cancer (NSCLC) and studied their phenotype and behavior before and after activation by TLR ligands in vitro.

Materials and Methods

TLR ligands and blocking Abs

A total of 25 ng/ml LPS from *Escherichia coli* (Sigma-Aldrich) was used to stimulate mDC via TLR4, 10 μg/ml CpG 2216, or CpG 2006 (MWG Biotec) to stimulate pDC via TLR9 and 10 μM R848 (Schering-Plough) to stimulate mDC via TLR8 and pDC via TLR7. A total of 10 μg/ml mouse anti-human IL-10R (clone 3C5.2b; Schering-Plough) was used to block this cytokine.

Preparation of human lung single-cell suspension

Human primary NSCLC surgical samples were obtained from the Centre Hospitalier Lyon-Sud (Pierre-Bénite, France) in agreement with the Hospital bioethical protocols and after receiving written informed consent from all patients. No tumor treatment had been administered before surgery. Peritumoral tissues corresponded to noninfiltrated lung situated at >10 cm from the tumor. Tissues were mechanically dilacerated and single-cell suspensions were obtained after enzymatic digestion (1 mg/ml collagenase A; Roche Diagnostics) and 100 IU/ml type I DNase (Sigma-Aldrich)) for 1 h at 37°C and 5% CO_2 under magnetic agitation in serum-free RPMI 1640 medium. Single-cell suspension was filtered through a 0.7-μm cell strainer (BD Biosciences) and cells were washed twice in 5% FCS/PBS medium.

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Received for publication July 25, 2006. Accepted for publication November 9, 2006.

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[1] This work was supported by a grant from the Institut National du Cancer-Canceropole 2004–05.

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[3] Abbreviations used in this paper: DC, dendritic cell; mDC, myeloid DC; pDC, plasmacytoid DC; TIDC, tumor-infiltrating DC; NSCLC, non-small cell lung cancer; PMC, pulmonary mononuclear cell; BDCA, blood DC Ag; SDF, stromal cell-derived factor; int, intermediate.

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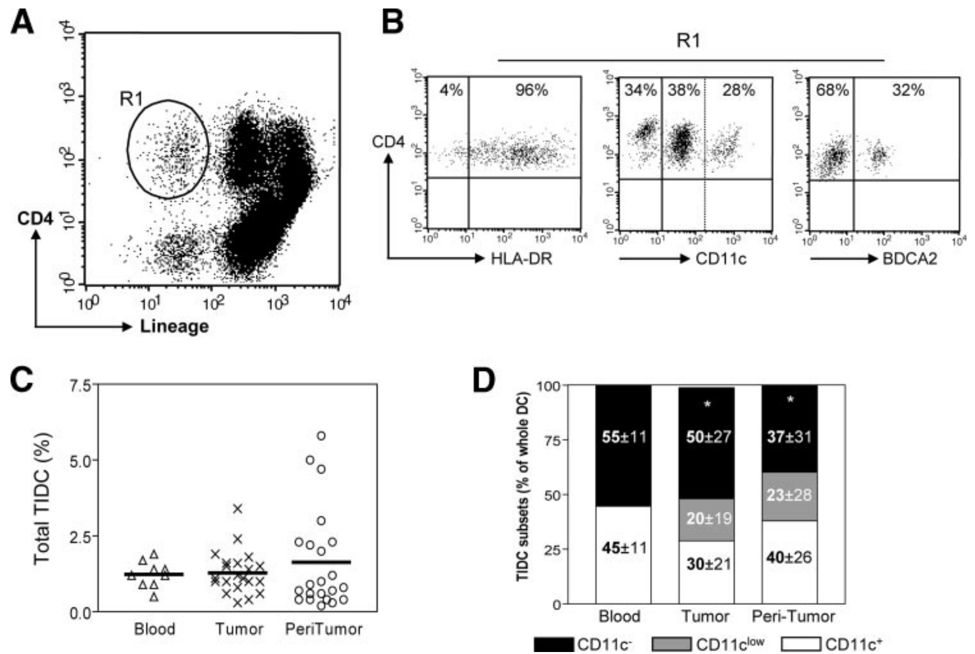

FIGURE 1. Three subsets of DC ($CD11c^+$, $CD11c^-$, and $CD11c^{int}$) are detected in NSCLC and peritumoral lung tissues using flow cytometry. *A*, Tumoral and nontumoral lung tissues were analyzed for the presence of $CD4^+$/$lineage^-$ (CD3, CD14, CD19, CD35, CD56 and HEA mAbs) cells. *B*, The different subsets of DC were characterized for their expression of HLA-DR, CD11c, and BDCA-2. *C*, Total $CD4^+$/$lineage^-$ TIDC were enumerated in 22 samples of NSCLC and peritumoral lung tissue and compared with normal peripheral blood. *D*, The proportion of the three subsets of DC was analyzed in the same samples.

RBC and dead cells were discarded by centrifugation on a Ficoll gradient to obtain pulmonary mononuclear cells (PMC).

Analysis of TIDC phenotype

TIDC were phenotypically characterized on a FACSCalibur (BD Biosciences) equipped with a doublet-discrimination module and using CellQuest Pro software (BD Biosciences). Briefly, TIDC were gated according to their negativity for a FITC-coupled mix of Abs including CD3, CD14, CD16, CD19, CD35, CD56, and HEA. ($lineage^-$) and their positivity for Cy5-coupled CD4 as previously described (13). Then, PE-coupled mAbs were used to further characterize the gated TIDC populations: CD11c and blood DC Ag (BDCA-2) Abs were used to distinguish the tumor-infiltrating mDC and pDC subsets HLA-DR, CD80, CD86, and CD83, B7-H1 and CCR7 were used to analyze their maturation stage, and finally, CCR2, CCR5, CCR6, CXCR3, and CXCR4 were used to study their chemokine receptor profile. Positivity threshold was systematically defined with isotype-matched control Abs. All Abs were purchased from BD Biosciences except CD3 (DakoCytomation), CD4 (Beckman Coulter-Immunotech), BDCA-2 (Miltenyi Biotec), B7-H1 (clone MIH1; eBioscience), and CCR7 (R&D Systems). Human PBMC from healthy donors were used as control normal cells.

Cytokine quantification

Supernatants of 1×10^6 cells PMC cultured for 24 h in flat-bottom 24-well plates (BD Labware) either alone or with TLR ligands with or without anti-IL-10R mAb were harvested and IL-12p40, TNF-α, and IL-10 were quantified using the OptEIA kits (BD Pharmingen), whereas IFN-α was quantified by the Cell Com IFN-α2 kit (Beckman Coulter).

Chemotaxis assay

TIDC migratory capacity was evaluated using 5-μm pore size Transwell plates (Costar). Briefly, human recombinant chemokines at 1 μg/ml (MCP-4, MIP-1α, MIP-3α, MIP-3α, IP-10, or MIG) or 100 ng/ml (stromal cell-derived factor (SDF)-1) concentration in RPMI 1640 medium supplemented with 2% FCS were added to 24-well plates under 500 μl. A total of 5×10^5 PMC were added to the Transwell inserts in a volume of 100 μl. Plates were incubated at 37°C for 2 h and migrated cells were harvested and stained for FACS analysis to distinguish mDC ($lineage^-$/$CD4^+$/$CD11c^+$) and pDC ($lineage^-$/$CD4^+$/$CD11c^-$). Results are expressed as migration index corresponding to the ratio of the number of cells migrating in response to chemokines to the number of cells migrating in response to culture medium alone.

Purification of DC

For T cell activation experiments, TIDC from PMC suspension were purified using a FACSVantage cell sorter (BD Biosciences). Briefly, TIDC were first enriched by immunomagnetic depletion (Dynabeads; Dynal Biotech) using anti-CD3 (OKT3), anti-CD14 (MOP9.25), anti-CD19 (4G7) ascites, and anti-CD16 (ION16), anti-CD35 (CR1), anti-CD56 (NKH1) (all from Beckman Coulter) and, for tumor cell removal, anti-human epithelial Ag (Miltenyi Biotec) purified mAbs. The purity of TIDC was ~80%. TIDC were then stained and gated based on their negativity for a FITC-coupled Ab mix containing anti-CD3, anti-CD14, anti-CD16, anti-CD19, anti-CD56, and anti–human epithelial Ag and on their positivity for Cy5-coupled anti-CD4 and PE-coupled anti-CD11c and anti-BDCA-4 to stain mDC and pDC, respectively. Control blood DC were enriched from human PBMC from healthy volunteers and mDC were distinguished from pDC following the protocols described. The purity of blood DC was ~95%.

MLR assay

Sorted TIDC cultured alone or with TLR ligands for 24 h were harvested and numbered. Various numbers of these TIDC were cocultured with 2×10^4 allogenic naive $CD4^+$ T cells ($CD4^+CD45RA^+$) for 4 days in round-bottom 96-well plates (BD Labware). Cell cultures were pulsed for 24 additional hours with 1 μCi/well of [H^3]thymidine (Amersham Biosciences), and radioactivity incorporation was measured with a TopCount apparatus (PerkinElmer).

Statistical analyses

Data are presented as mean ± SD. Statistical analyses were performed using GraphPad software. Student's *t* test was used to compare tumoral, peritumoral, and blood samples.

Results

CD11c expression allows detection of three subsets of DC in NSCLC and peritumoral lung tissues

When total single-cell suspensions recovered from 32 NSCLC biopsy specimens were analyzed for the presence of $CD4^+$/$lineage^-$ cells, around 1% (1.248 ± 0.124%) of TIDC were observed. (Fig. 1, *A* and *C*). Among total TIDC, 96% of which express high levels of HLA-DR (Fig. 1*B*, *left panel*), BDCA-2 staining demonstrated the presence of both BDCA-2^- mDC and BDCA-2^+ pDC (Fig. 1*B*, *right panel*). Moreover, according to the intensity of CD11c expression, three discrete populations of TIDC were clearly detected (Fig. 1*B*, *middle panel*). Compared with corresponding nontumoral lung tissues, NSCLC biopsy samples contained a slightly lower proportion of total DC (1.248 ± 0.124 vs 1.632 ± 0.351; $p = 0.146$) (Fig. 1*C*). Interestingly, when CD11c expression by $CD4^+lineage^-$ DC was analyzed, only $CD11^-$ and $CD11^{high}$ DC

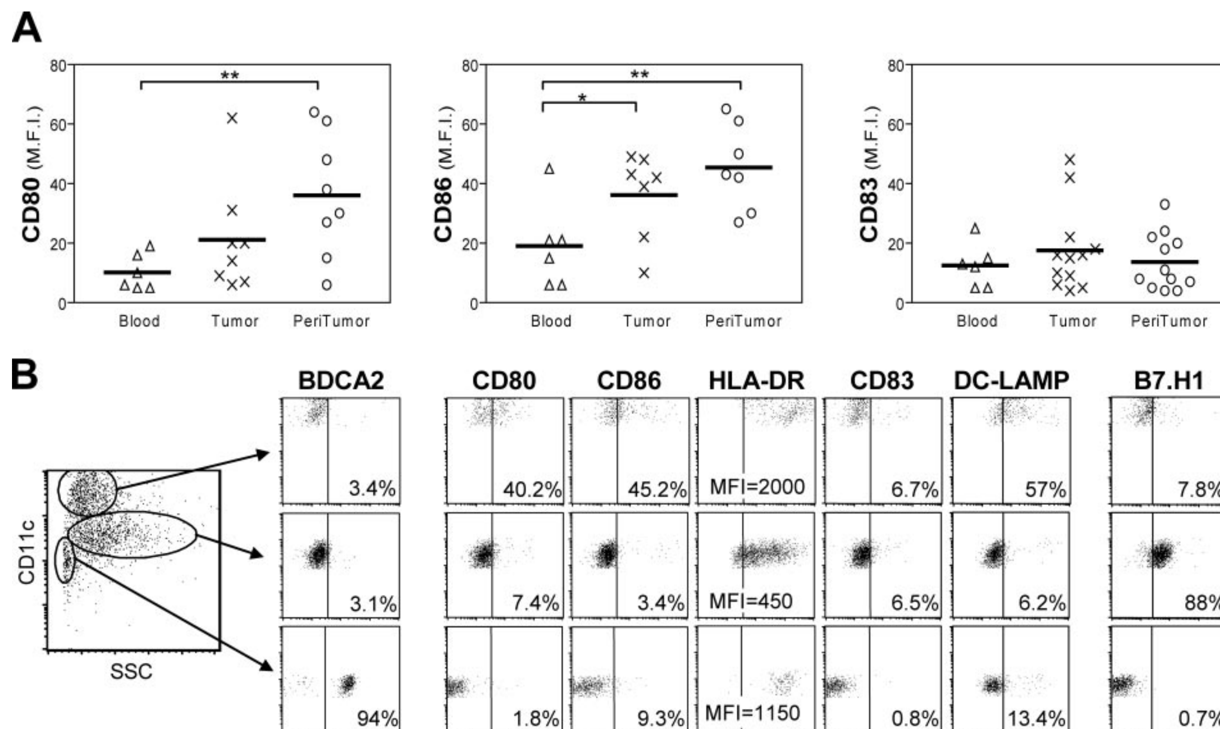

FIGURE 2. DC isolated from NSCLC and peritumoral lung tissues have a relatively immature phenotype. *A*, DC isolated from tumoral and nontumoral lung tissues express low although significantly higher levels of B7-1 and B7-2 molecules compared with blood DC. The expression of CD83 is similarly low in TIDC, in peritumoral DC, and in blood DC. *B*, CD11c^{int} TIDC correspond to particularly immature DC that express high levels of the immunomodulatory molecule B7-H1. *, $p < 0.05$, significantly different.

were found in peripheral blood of normal donor, representing 55% of pDC and 45% of mDC, respectively. In tumor fragments, 50% of TIDC were CD11c$^-$ pDC, whereas the remaining 50% corresponded to mDC being either CD11^{int} (20%) or CD11^{high} (30%) (Fig. 1*D*). In peritumoral lung fragments, proportions were 37% for CD11c$^-$ pDC, 23% for CD11^{int} mDC, and 40% for CD11^{high} mDC. These data establish that according to the expression of CD11c, TIDC and peritumoral DC contain in addition to classical pDC and mDC about one-third of CD11c^{int} mDC that are not found in normal blood.

TIDC freshly isolated from NSCLC are relatively immature

To characterize the stage of maturation of TIDC, we analyzed the expression levels of the costimulatory molecules CD80/B7-1 and CD86/B7-2 and of the DC activation marker CD83 on total DC isolated from blood from NSCLC biopsies and from peritumoral lung tissues. As shown in Fig. 2*A*, when compared with blood DC, TIDC and peritumoral DC expressed modestly but significantly more CD80 ($p = 0.096$ and $p = 0.006$, respectively) and CD86 ($p = 0.028$ and $p = 0.0035$, respectively), whereas the difference was not significant for CD83. Focusing on TIDC, we next compared the expression levels of these markers as well as of HLA-DR and CD208/DC-LAMP in the three DC subsets identified according to CD11c staining (Fig. 2*B*). The CD11c^{high} mDC subset turned out to express the higher although limited level of all five markers and therefore to display an incompletely mature phenotype. CD11c^{int} cells did not express significant amounts of BDCA-2, corresponding indeed to mDC. Although being clearly HLA-DR$^+$, most CD11c^{int} mDC were immature as they expressed none of the activation markers CD80, CD86, CD83, or CD208/DC-LAMP. The same observation was made for CD11$^-$ pDC. Interestingly, only CD11c^{int} TIDC were found to express the coinhibitory molecule B7-H1 (Fig. 2*B*). Altogether, most TIDC appeared to be immature, and this was particularly striking for the CD11c^{int}/B7-H1$^+$ mDC subset.

TIDC subsets reach different levels of maturation in response to activation by TLR ligands

Considering the apparent immaturity of most TIDC, we wondered whether these cells would be driven to mature after TLR stimulation. Therefore, the level of expression of maturation markers was followed in the three subsets after culture of tumor single-cell suspensions with TLR4 (LPS), TLR7/8 (R848), or TLR9 (CpG ODN) ligands (Fig. 3*A*). Before culture, according to the mean fluorescence intensity of CD80, CD83, and CD86 and in agreement with our reported observations, pDC appeared to be the most immature subset, followed by CD11c^{int} mDC and then by CD11c^{high} mDC. CD11c$^-$ pDC that express TLR7 and TLR9 responded only to R848, showing a modest up-regulation of CD86. mDC are known to express TLR4 and TLR8, but not TLR9. Accordingly, LPS and R848 up-regulated the expression of CD86 and CD83 on CD11c^{high} mDC, but R848 only increased the levels of CD80. CD11c^{int} mDC appeared to be particularly refractory to maturation, as expression of CD80 could only be weakly increased by R848. A switch of chemokine receptor expression from CCR6 to CCR7 is another change that accompanies DC maturation. R848 increased the expression of CCR7 by the three TIDC subsets, whereas LPS was active on the CD11c^{high} DC only (Fig. 3*A*). In conclusion, the three TIDC subsets underwent only partial phenotypic maturation after TLR activation.

TIDC produce limited amounts of cytokines in response to TLR ligands

To extend our study of the functional maturation of TIDC, we next measured the cytokines released by PMC either spontaneously or after stimulation by TLR ligands. Recognizing that several types of

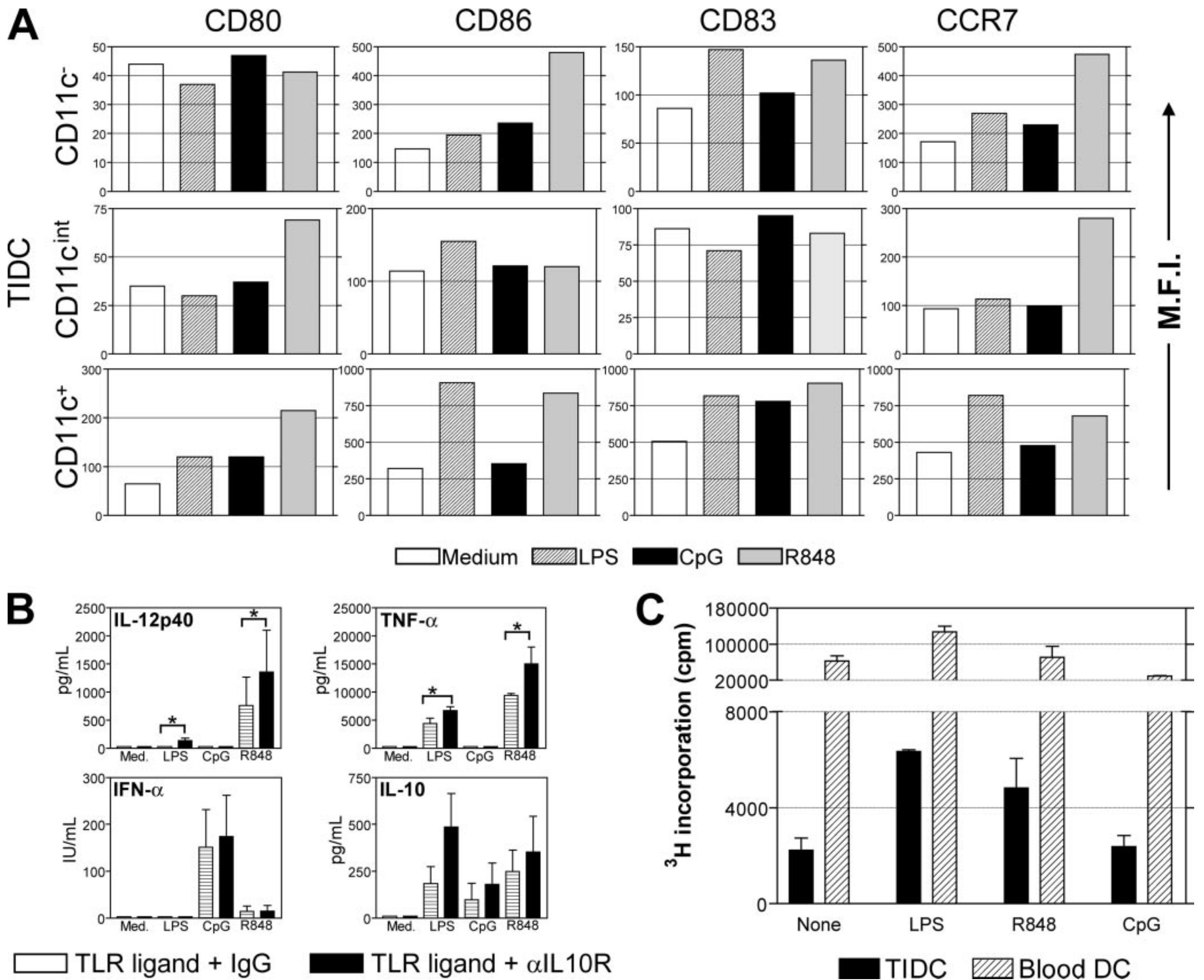

FIGURE 3. TIDC and peritumoral DC undergo partial phenotypic maturation in response to TLR ligands and have a weak capacity to present Ag to naive T cells. *A*, PMCs depleted of tumoral cells were activated with LPS, CpG, or R848. Activation of $CD11c^-$, $CD11c^{int}$, and $CD11c^+$ DC was monitored separately by analyzing the expression of B7-1, B7-2, CD83, and CCR7 by FACS. Data are representative of four independent experiments. *B*, Supernatants of PMC, either nonactivated or activated with TLR ligands alone or in the presence of blocking IL-10R Ab were harvested, and production of IL-12p40, IFN-α, TNF-α, and IL-10 was quantified by ELISA. $*$, $p < 0.05$, significant difference. Data are representative of four independent experiments. *C*, Sorted NSCLC TIDC, either nonactivated or activated for 24 hours with LPS, CpG, and R848 were cocultured with naive T cells (DC to T cell ratio = 1:5) for 4 days. Cultures were pulsed for 24 additional hours with $[H^3]$thymidine and radioactivity incorporation was measured. Data are representative of two independent experiments.

cells were present in these cultures, we measured IL-12p40 that is mainly produced by myeloid cells, including mDC and macrophages, in response to TLR4 and TLR8 stimulation, and type I IFN, the largest amount of which is secreted by pDC in response to TLR9 triggering. We also monitored the secretion of TNF-α and IL-10, a proinflammatory and anti-inflammatory cytokine, respectively. Moreover, given the reported role of IL-10 in inhibiting mouse TIDC maturation in mouse tumor models (11), we analyzed whether a blocking anti-IL-10R mAb would increase the secretion of cytokines in response to TLR activation. Fig. 3*B* shows that before activation and in either the absence or the presence of anti-IL-10R mAb, none of these cytokines was detected in culture supernatants. Without blocking IL-10R Ab, only R848 could induce the secretion of detectable amounts of IL-12p40. In the presence of blocking mAb, LPS stimulated very weak secretion of IL-12p40, whereas the secretion induced by R848 was slightly but significantly increased. Of note, whichever the condition of activation, IL-12p70 was never detected in the supernatant of TIDC or peritumoral DC cultures (data not shown). As anticipated, CpG ODN did not trigger the production of IL-12p40. Similar results were obtained for TNF-α except that even alone, LPS stimulated low but detectable production of this cytokine. Significant amounts of type I IFN were secreted after TLR9 activation only, and this response was not amplified by blocking IL-10R. Lastly, IL-10 itself was easily detected following activation with each TLR ligand, but production was not significantly increased by IL-10R blocking Ab. Overall, tumor-infiltrating mDC and pDC appeared to respond partially to TLR activation by the production of IL-12p40 and IFN-α, respectively. Moreover, different types of cells present in tumor biopsy samples, including DC, secreted TNF-α and IL-10 in response to activation by various TLR ligands. Finally, IL-10 appeared to be responsible in part for the limitation of IL-12p40 and TNF-α production by TIDC.

TIDC are poor APCs, even after TLR stimulation

Considering the low level of costimulatory molecules expression and proinflammatory cytokines secretion by TIDC even after TLR

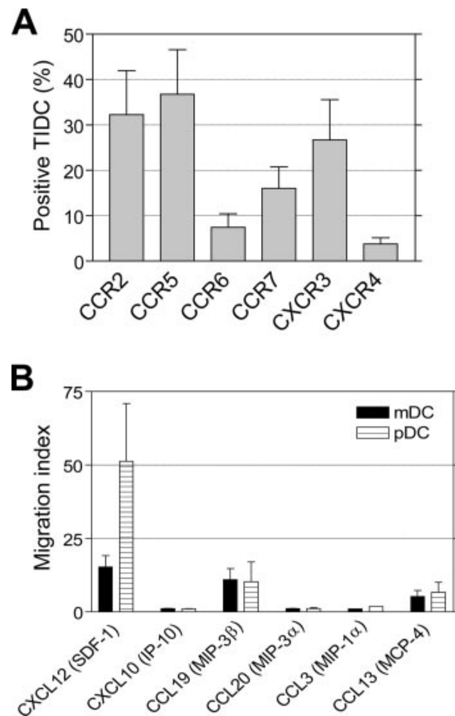

FIGURE 4. DC isolated from NSCLC and peritumoral lung tissues migrate in response to chemokines. *A*, FACS analysis detects significant amounts of CCR-2, CCR-5, CCR-7, and CXCR-3 at the surface of total TIDC. *B*, When TIDC were incubated in Transwell inserts for 2 h at 37°C, both CD11c$^+$ tumor-infiltrating mDC and CD11c$^-$ tumor-infiltrating pDC migrated vigorously toward CCL19 and CXCL12. Migration index was determined by the ratio of specific migration to spontaneous migration in medium alone. Data represent three independent experiments.

stimulation, we next analyzed to what extent TIDC would activate naive T cells in MLR. Fig. 3*C* shows that naive T cells proliferate only weakly in the presence of freshly isolated TIDC when compared with peripheral blood DC. Preactivation by LPS or R848 increased only modestly the APC capacity of TIDC, whereas CpG ODN had no effect on subsequent T cell proliferation. Kinetic experiments confirmed that the low thymidine uptake measured in presence of TIDC was not due to delayed proliferation of T cells (data not shown).

TIDC migrate in response to chemokines

The relative immaturity of TIDC and their accumulation at the tumor site have raised questions regarding their capacity to migrate in response to chemokines to reach the tumor-draining lymph nodes. We therefore analyzed the profile of chemokine receptors expressed by the TIDC and correlated these results with their capacity to migrate in response to the corresponding ligands. By FACS analysis, between 25 and 35% of freshly isolated CD4$^+$lineage$^-$ TIDC stained positive for CCR2, CCR5, CXCR3, around 15% for CCR7, and $<$10% for CXCR4 and CCR6 (Fig. 4*A*). Next, tumor PMC migration was analyzed in vitro. Both tumor-infiltrating mDC and pDC were found to migrate in response to MCP-4, to MPI-3β and to SDF-1, the latter being particularly efficient for pDC. In contrast, there was no detectable migration in response to MIP-1α, MIP-3α, and IP-10 (Fig. 4*B*). Those experiments established that freshly isolated TIDC are motile and respond to chemokines, in particular to those that should drive them into draining lymph nodes in vivo.

Discussion

We (N. Freymond, submitted for publication) and others (14–18) have analyzed the immunohistochemical features of the immune response that takes place at the site of human lung cancer. To better understand the role of NSCLC TIDC, we now provide new phenotypic and functional data about those cells.

First, we observed that three subsets of DC that can be distinguished according to the levels of CD11c they express. We found that around 1% of cells isolated either from tumor or from peritumoral lung tissues displayed a lineage$^-$/CD4$^+$/HLA-DR$^+$ phenotype corresponding to DC. Using different criteria to identify the DC, Demedts et al. (19) concluded that ~3% of PMCs extracted from nontumoral fragments of lungs resected for various reasons (mostly lung cancer) were DC. Their work identified three lung DC subsets as CD3$^-$/CD19$^-$/low autofluorescence cells expressing BDCA-1 (mDC1), BDCA-2 (pDC), or BDCA-3 (mDC2). In this study, by focusing on lineage$^-$/CD4$^+$ total DC, we could clearly distinguish three subsets of TIDC: CD11c^{high} and CD11c^{int} mDC accounted for about half of total DC, whereas CD11c$^-$ pDC represented the second half. Several publications have established the infiltration of human lung cancer by mDC, but this is the first report showing the presence of pDC in this type of tumor and in peritumoral lung tissue as well. The presence of pDC in NSCLC was not necessarily unexpected, as the presence of BDCA-2$^+$/CD123$^+$ pDC in single-cell suspension obtained from human nontumoral lung tissue was recently demonstrated (19), and because both mDC and pDC have been found in bronchoalveolar lavage from noncancer patients (20). Infiltration of pDC has been reported in several types of cancers, including cervical carcinoma (21), head and neck cancer (22), melanoma (23), ovarian cancer (24, 25), and breast cancer (26), where tolerizing or proangiogenic rather than immunizing functions have been ascribed to them (24–26). Thus, the role and signification of pDC infiltration in lung cancer raise the same issues as for other cancers. Although BDCA-2 expression allowed us to recognize pDC with both protocols, the relationship between mDC1 and mDC2 on one hand, and CD11c^{high} and CD11c^{int} DC in contrast deserves some comment. The CD11c^{high} DC described likely include all mDC1 and ~70% of mDC2 that express high levels of CD11c (27), and their counterpart was easily observed in normal peripheral blood. In contrast, CD11c^{int} mDC were present in tumoral and peritumoral lung tissue, but not in normal blood. The low level of CD11c expression suggests these DC may correspond to the minority of BDCA-3$^+$/CD11c^{dim} mDC2 isolated from nontumoral lung (27). Noticeably, CD11c^{int} mDC expressed the coinhibitory molecule B7-H1. B7-H1 (PD-L1) together with B7-DC (PD-L2) represent the two identified ligands for the programmed cell death 1 receptor (PD-1) which plays a negative costimulatory function on activated T cells (28, 29). B7-H1 is expressed by T cells, B cells, monocytes, and by peripheral tissues, including some cancer cells (reviewed in Ref. 30) but also by DC that are poorly immunogenic (31) or even suppress T cell activation (32). It was recently demonstrated that DC infiltrating ovarian tumors express B7-H1 and that blockade of B7-H1 molecules by specific Abs enhances the efficiency of DC-based antitumor immunotherapy (33). Therefore, whether CD11c^{int}/B7-H1$^+$ DC represent a population of TIDC involved in the suppression of tumor-specific immune response through the PD-1-PD-L1 signaling pathway represents an intriguing possibility. However, the actual functions of this TIDC subset as well as the potential relationship between CD11c^{int}/B7-H1$^+$ TIDC and TGF-β^+ immature TIDC that support the local proliferation of T regulatory cells (34) remain to be investigated.

Our second observation was that most NSCLC TIDC are immature and poorly sensitive to TLR stimulation. Both the proportion and mean fluorescence intensity of CD80, CD83, CD86, and CD208/DC-LAMP staining indicated that TIDC were globally immature. When analyzed separately, $CD11C^{high}$ mDC and pDC represented the most and the less mature subset, respectively, whereas $CD11c^{int}$ expressed intermediate level of those maturation markers. Remarkably, the coinhibitory molecule B7-H1 was detected on the majority of $CD11c^{int}$ TIDC, whereas being completely absent from either $CD11C^{high}$ mDC or $CD11C^{-}$ pDC. The absence of cytokines reflecting secretion by either mDC (IL-12p40, TNF-α) or pDC (IFN-α) in the supernatant of PMC cultured without stimulation further confirmed the nonactivated status of freshly purified TIDC. Our data are in agreement with immunohistochemical studies showing that the large majority of TIDC have an immature phenotype in different types of cancers, (26, 35–37), with the possible exception of colon carcinomas (38, 39). Although most NSCLC-TIDC appeared immature in situ, it remains questionable whether TIDC can be induced to mature. Based on the up-regulation of CD80, CD83, and CD86 expression after stimulation with TLR ligands, $CD11c^{high}$ mDC were found to represent the most responsive subset, whereas $CD11c^{int}$ mDC and $CD11c^{-}$ pDC were poorly sensitive to maturation. CCR7 was weakly expressed on the three subsets but clearly up-regulated by TLR ligands. When production of cytokines by total PMC exposed to TLR ligands was analyzed, there was indirect evidence that mDC could secrete modest amounts of IL-12p40 and TNF-α in the presence of TLR4 or TLR8 ligands. Both mDC and monocytes/macrophages, which express TLR8, likely participated to the production of these cytokines, but their relative contribution could not be evaluated in our experimental setting. In the presence of CpG ODN, the limited amounts of IFN-α was most likely secreted by pDC, as they represent the only cells among PMC known for producing type I IFN upon TLR9 stimulation. Lastly, the anti-inflammatory cytokine IL-10 was secreted in response to all three TLR ligands tested, and experiments with blocking anti-IL-10R mAb demonstrated that IL-10 was in part responsible for the limitation of proinflammatory cytokines secretion by TIDC. Although NSCLC do not appear to secrete IL-10 by themselves, they have been shown to produce soluble factors that increase the secretion of this cytokine by mDC, monocytes/macrophages, and epithelial cells and that reduce the Ag presentation capacity of human DC generated from monocytes in vitro (40).

The immaturity of freshly isolated TIDC and their limited capability to express costimulatory molecules and to secrete proinflammatory cytokines suggested that they would be poor APCs. This suggestion was directly confirmed in MLR, as even after TLR stimulation, TIDC turned out to induce very weak T cell proliferation.

Third, we have shown that NSCLC TIDC are able to migrate in response to chemokines. Key aspects of DC functions depend on the maturation-dependent switch of capacity to migrate to and from peripheral tissues (41). However, the profile of chemokine receptors expressed by DC has often been difficult to interpret because it does not always correlate with the functional migratory response. In this regard, the absence of migration to the inflammatory chemokine CCL20, but also to CCL3 and CXCL10 despite detection of CCR5 and CXCR3, respectively, might be indicative of their capacity to leave inflammatory tumor sites (42). More significantly, both tumor-infiltrating mDC and pDC migrated in vitro in response to CCL19 and CCL21, the two chemokines known to drive DC toward secondary lymphoid organs. The importance of functional CCR7 expression might be inferred from published data obtained both in vitro and in vivo. First, this receptor was also found at the surface of DC matured in the presence of mycophenolic acid that display an immature phenotype and an altered capacity to activate T cells very similar to what we observed with TIDC (43). Second, the expression of CCR7 and its ligands appears to be required for Ag transport by DCs to draining lymph nodes in vivo to establish peripheral tolerance in skin noninflammatory conditions (44) or to induce oral tolerance (45). Thus our data suggest that, notwithstanding their immaturity, tumor-infiltrating mDC and pDC have the intrinsic ability to emigrate from the tumor to the draining lymph node in vivo.

Altogether, the limited Ag-presentation capacity of most TIDC in NSCLC, even after TLR stimulation, and their theoretical capability to migrate to draining lymph nodes suggest that they may be suboptimal APC for initiating and supporting tumor immune rejection at the primary site as well as in draining lymph nodes.

Acknowledgment

We thank S. Saeland for discussion of the data and critical review of the manuscript. Canceropole 2004–05.

Disclosures

The authors have no financial conflict of interest.

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