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Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8

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In the course of a clinical trial consisting of intratumoral injections of dendritic cells (DCs) transfected to produce interleukin-12, the use of ^{111}In-labeled tracing doses of DCs showed that most DCs remained inside tumor tissue, instead of migrating out. In search for factors that could explain this retention, it was found that tumors from patients suffering hepatocellular carcinoma, colorectal or pancreatic cancer were producing IL-8 and that this chemokine attracted monocyte-derived dendritic cells that uniformly express both IL-8 receptors CXCR1 and CXCR2. Accordingly, neutralizing antihuman IL-8 monoclonal antibodies blocked the chemotactic attraction of DCs by recombinant IL-8, as well as by the serum of the patients or culture supernatants of human colorectal carcinomas. In addition, tissue culture supernatants of colon carcinoma cells inhibited DC migration induced by MIP-3β in an IL-8-dependent fashion. IL-8 production in malignant tissue and the responsiveness of DCs to IL-8 are a likely explanation of the clinical images, which suggest retention of DCs inside human malignant lesions. Impairment of DC migration toward lymphoid tissue could be involved in cancer immune evasion.

Key words: IL-8; dendritic cell; immunotherapy; chemotaxis; cancer

Malignant cells or tumor stroma produce cytokines that not only tamper with immunity, but also regulate other functions such as angiogenesis, invasiveness, metastasis and cell growth.[1] It seems that tumors kidnap normal systems of intercellular communication to support their own growth and spread. IL-8 is an ELR$^+$-CXC chemokine identified for its potent chemotactic activity on human granulocytes.[2] CXCR1 is quite specific for IL-8, while CXCR2 is a common receptor for many other ELR$^+$-CXC chemokines.[3] Mice do not express a homologous IL-8 molecule, thus preventing definitive experimentation in these animal models, but MIP-2 and KC chemokines bind to CXCR1 and mediate neutrophil chemotaxis in murine models.[4]

Human tumors from most tissue origins have been found to produce IL-8 in fairly large quantity,[5] probably reflecting the broad capability of normal cells to produce IL-8 under proinflammatory stress.[6] Mechanisms controlling mRNA transcription and DNA methylation are probably critically involved in derepressing IL-8 in tumors,[5,7] and it has been shown that IL-8 plays a significant role in tumor angiogenesis and metastasis.[3,5] The detailed molecular mechanisms underlying such activities are complex, but direct chemotactic effects on endothelial cells have been described.[8]

Artificial transfections support the relevance of IL-8 in these pathogenic mechanisms of cancer. However, most genetic experiments have been performed in immunodeficient mice able to graft human carcinomas.[5] Therefore, it is unknown whether tumor-derived IL-8 could interfere with antitumor immunity.

Vaccine formulations based on dendritic cells (DCs) loaded with different sources of tumor antigens are being tested in clinical trials.[9,10] Although increases in antitumor-specific immunity are frequently reported,[11] efficacy has not yet matched those seen in mouse models. When DCs are artificially released intratumorally, they can pick up antigens from the environment,[12] migrate to lymphoid tissues and present antigens to T cells.[12–15]

The antitumor activity of this strategy has been further enhanced by the transfection of IL-12 genes into the DCs.[12,13] A phase 1 clinical trial is being performed in our institution to assess safety and efficacy of intratumoral injection of IL-12-transfected DCs in advanced cancer patients. During the trial, we observed that IL-12-producing DCs injected into tumor nodules migrated very poorly out of the lesions. It was found that all patients included in the trial had high serum concentrations of IL-8 and that this chemokine attracts monocyte-derived DCs.

Material and methods

Patients

Fifteen patients with advanced metastatic digestive carcinomas originating from liver ($n = 7$), pancreas ($n = 3$), or colon ($n = 5$) were included in the study approved by the Spanish Medicine Agency (IL-12/00/002).

Autologous, immature and mature DCs were transfected with adenovirus expressing IL-12 genes (AFIL-12) and administered to patients by ultrasound-guided intratumoral injections. Three cohorts of 5 patients received 3 doses of 10^7, 2.5×10^7, or 5×10^7 transfected DCs at 21-day intervals. Detailed description of the clinical safety, efficacy and biologic effects will be reported on completion of this clinical study.[17]

Dendritic cell generation

Dendritic cells were generated from buffy coat-derived monocytes donated by healthy donors or from cancer patient leukapheresis products. Isolated mononuclear cells from these sources were subjected to positive selection using anti-CD14-conjugated paramagnetic beads and purified using the AutoMACSs and CliniMACs systems (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified monocytes were cultured for 7 days in AIM-V serum-free media (Gibco-BRL, Gaithersburg, MD) supplemented with GM-CSF (1000 U/ml; Novartis, Basel, Switzerland) and IL-4 (500 U/ml; R&D Systems, Minneapolis, MN). Fresh cytokines were added every two days to the differentiation cultures. DCs were

Abbreviations: DC, dendritic cell; DTH, delayed-type hypersensitivity; ELC, EBV-induced molecule 1 ligand chemokine; ELISA, enzyme-linked immunosorbent assay; MIP-3β, macrophage inflammatory protein 3β; MLR, mixed lymphocyte reaction; SLC, secondary lymphoid tissue chemokine.

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matured with clinical grade TNF-α (50 ng/ml; Boehringer Ingelheim, Ingelheim, Germany), IFN-α (1,000 IU/ml; Schering-Plough, Kenilworth, NJ) and PGE_2 (20 μg/ml; Pharmacia, Kalamozoo, MI) for 24 hr.

^{111}In labeling and imaging

Just before labeling, 1 mL of ^{111}In-oxinate (Mallinkrodt Medical, St. Louis, MO) was diluted with 0.4 mL of 0.1 M tris-HCl buffer (pH 7.2). AF-IL12-transfected DCs (5×10^6) were labeled with 100–400 μCi of ^{111}In-oxinate for 20 min at room temperature with occasional gentle shaking. After incubation, cells were washed 3 times and resuspended in saline. Cells were mixed with the nonlabeled DCs to complete the dose and were injected intratumorally. Scintigraphic images were acquired with a double-headed gamma camera (E CAM; Siemens, Erlangen, Germany).

Cell lines and IL-8 concentrations in sera and culture supernatants

HT29, Caco2, SW48, HepG2 and ASPC-1 cell lines were obtained from American Type Culture Collection (Rockville, MD). The concentration of IL-8 in supernatants from these cell lines, DCs and serum samples was determined by ELISA (BD Biosciences, San Diego, CA).

Immunofluorescence, FACS analysis and Western blot

Before immune staining, cells were incubated with PBS/human IgG (50 μg/ml; Beriglobina P; Aventis, King of Prussia, PA) for 20 min on ice to block Fc receptors. Subsequently, DCs were incubated with mAbs anti-CXCR1 and -CXCR2 (R&D Systems) or isotype-matched antibodies followed by an FITC-conjugated secondary antibody (Dako, Glostrup, Denmark). Samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences). For Western blot experiments,[16] mature and immature DCs as well as neutrophils from healthy donors were lysed in ice-cold Cellytic-M Mammalian Cell Lysis buffer (Sigma, St. Louis, MO), to which 5 μg/ml of leupeptin and aprotinin (Sigma) were added. Proteins (30 μg) were separated by 10% SDS-PAGE (Bio-Rad, Richmond, CA) and blotted onto nitrocellulose membranes. Blots were processed as previously described[16] with anti-CXCR1 and anti-CXCR2 antibodies (2 μg/ml; Serotec, Dusseldorf, Germany) and visualized by chemiluminiscence (Amersham Pharmacia, Uppsala, Sweden).

Chemotaxis assays in vitro

In vitro DCs migration was measured in Transwell Chambers (8 μm; Corning Costar, Corning, NY). Mature DCs (4×10^5) were cultured in the upper chambers and migration was assessed after 16 hr of culture at 37°C. The following migration stimuli were used in the lower chamber: MIP-3β (R&D Systems), human recombinant IL-8 (R&D Systems), different dilutions (1/2, 1/5 and 1/10) of HT29, Caco2 and SW48 supernatants collected from 80% confluent cultures and serum samples (5%, 10% and 50% v/v). When indicated, an antihuman IL-8 antibody (BD Pharmingen, San Jose, CA), which neutralizes IL-8 activity or control antibody, was added at 5 μg/ml to the lower chamber. Transmigrated cells in the lower chamber were quantified by counting them under microscopy using Neubauer chambers. Chemotactic index was calculated as number of migrated cells in the experimental conditions divided by number of migrated cells in the negative control (culture medium).

HT29 cell supernatant was also tested for its capacity to neutralize DC migration induced by MIP-3β (R&D Systems) in transwell chambers. In these experiments, DCs (4×10^5) were resuspended in HT29 supernatant with anti-IL8 mAb or control antibody (BD Biosciences) and added to the upper chambers while different concentrations of MIP-3β (0.1, 1, 10 and 100 ng/ml) were added to the lower chambers.

Results

DCs injected intratumorally fail to migrate out of human carcinomas

A clinical trial consisting of 3 intratumoral doses of monocyte-derived DCs adenovirally transfected to produce IL-12 is ongoing at our institution (IL-12/00/002). The clinical trial has included treatment of 17 patients suffering digestive cancers without toxicity. Evidence for biologic activity was detected in most patients with 2 cases of disease stabilization and 1 objective partial response.[17] In a series of 5 patients of this clinical trial, who have received 3 doses of 5×10^7 of such monocyte-derived DCs at 3 weekly intervals, 5×10^6 cells (10% of the total dose) were labeled with ^{111}In-oxinate. This technique is routinely used to trace adoptively transferred neutrophils and platelets for various clinical purposes. After ultrasound-guided administration of DCs, patients were scanned under a high-resolution gamma camera. This technique has been previously used by other authors to monitor migration of DCs after subcutaneous or intravenous injection during vaccination protocols for melanoma treatment.[18]

Patient 1 (Fig. 1) with metastatic colon cancer received IL-12-transduced immature DCs inside a liver metastasis. Patient 2, who suffered from metastatic cholangiocarcinoma, received a similar treatment to a bone metastasis, although in this case DCs were cultured during the last 24 hr before injection in the presence of clinical grade TNF-α, IFN-α and PGE_2. Maturation was monitored by assessing surface expression of CD80, CD83, CD86, HLA-DR and CCR7 (data not shown).

^{111}In biodistribution in these patients was observed in scintigram images taken 30 min, 24 hr and 48 hr after injection (Fig. 1). In patient 1, most of the dose remained intratumorally 15–30 min after injection, while a small fraction reached the lungs, presumably through vascular dissemination. Twenty-four and 48 hr later, most of the dose still remained intratumoral, while the intravascularly escaped DCs reached the spleen. Some other organs such as liver and bone marrow can be faintly visualized. This is consistent with a previous report showing that intravenously infused DCs were found first in lungs and later in the spleen and liver.[18]

Since immature DCs fail to express chemokine receptors guiding them to secondary lymphoid organs, we thought that providing a 24-hr maturation culture would help DCs to reach draining lymph nodes by inducing CCR7 expression.[19] However, as it can be seen in Figure 1, when mature DCs were used (patient 2), as with the immature DCs of patient 1, injected cells were retained inside the treated scapular metastasis. Again, part of the dose injected escaped to the lungs through the blood stream immediately after injection. Most of the remaining dose stayed inside the injected lesion for 24 and 48 hr (Fig. 1). Similar observations have been made in the other 3 patients with intrahepatic masses of pancreatic cancer, colon cancer and hepatocellular carcinoma (data not shown).

IL-8 is secreted by digestive carcinomas reaching high serum concentrations

It has been described in a number of reports that human malignancies, including colon adenocarcinomas, pancreatic cancer and primary liver cancer, produce and secrete IL-8.[5] Therefore, IL-8 could be a candidate molecule to mediate a chemotactic retention of DCs inside tumor tissue. To explore this possibility, IL-8 concentration was measured in serum samples from all patients included in the clinical trial. In these cases, IL-8 was detectable at concentrations between 19 and 601 pg/ml, whereas IL-8 was under the limit of detection (12 pg/ml) in all 15 control serum samples from healthy volunteers ($p < 0.001$; Fig. 2*a*). To confirm that IL-8 is secreted by human malignant cells, we examined IL-8 concentrations in tissue culture supernatants of 3 colon carcinoma cell lines (HT29, Caco2 and SW48), 1 hepatocellular carcinoma cell line (HepG2) and 1 pancreatic adenocarcinoma cell line (ASPC-1). IL-8 concentrations in tissue culture supernatants from HT29, Caco2, ASPC-1 and HepG2 cell lines were high

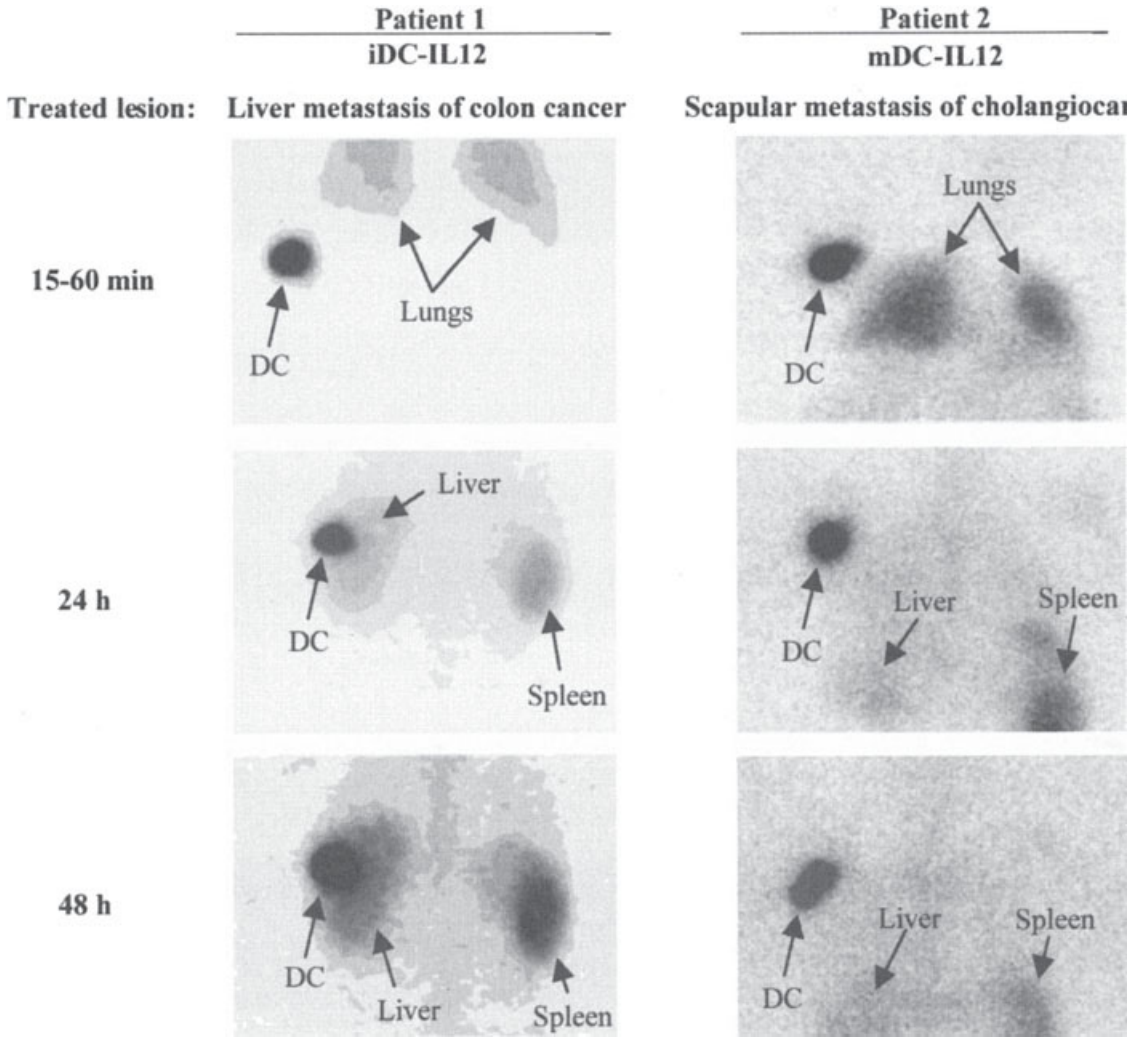


FIGURE 1 – Intratumorally injected DCs migrate poorly out of human malignant lesions regardless of their maturation status. Sequential gammagraphic follow-up of 2 patients after ultrasound-guided injection of 5×10^7 DCs transfected to produce IL-12 by means of recombinant adenovirus (AFIL-12). Ten percent of the dose (5×10^6 DCs) was labeled with ^{111}In-oxynate. Images were taken during the first hour and 24 hr and 48 hr after injection as indicated. Patient 1 received immature DCs, while patient 2 received mature DCs.

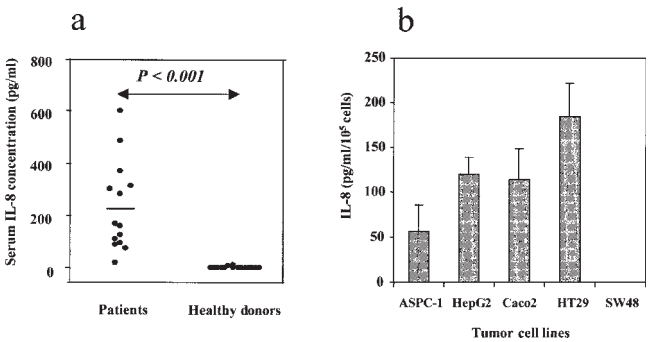


FIGURE 2 – IL-8 is secreted by digestive carcinomas. (*a*) IL-8 serum concentrations were measured by ELISA in 15 patients with digestive carcinomas and in 15 healthy controls. *p* was calculated with a Mann-Whitney U-test. (*b*) Production of IL-8 by human pancreatic (ASPC-1), hepatocellular (HepG2) and colon (CaCo2, HT29 and SW48) carcinoma cells. Culture supernatants were harvested from 10^5 cells after 24 hr of incubation and IL-8 content was measured by ELISA. Results representative of 3 independent experiments are shown as mean ± SD of triplicate cultures.

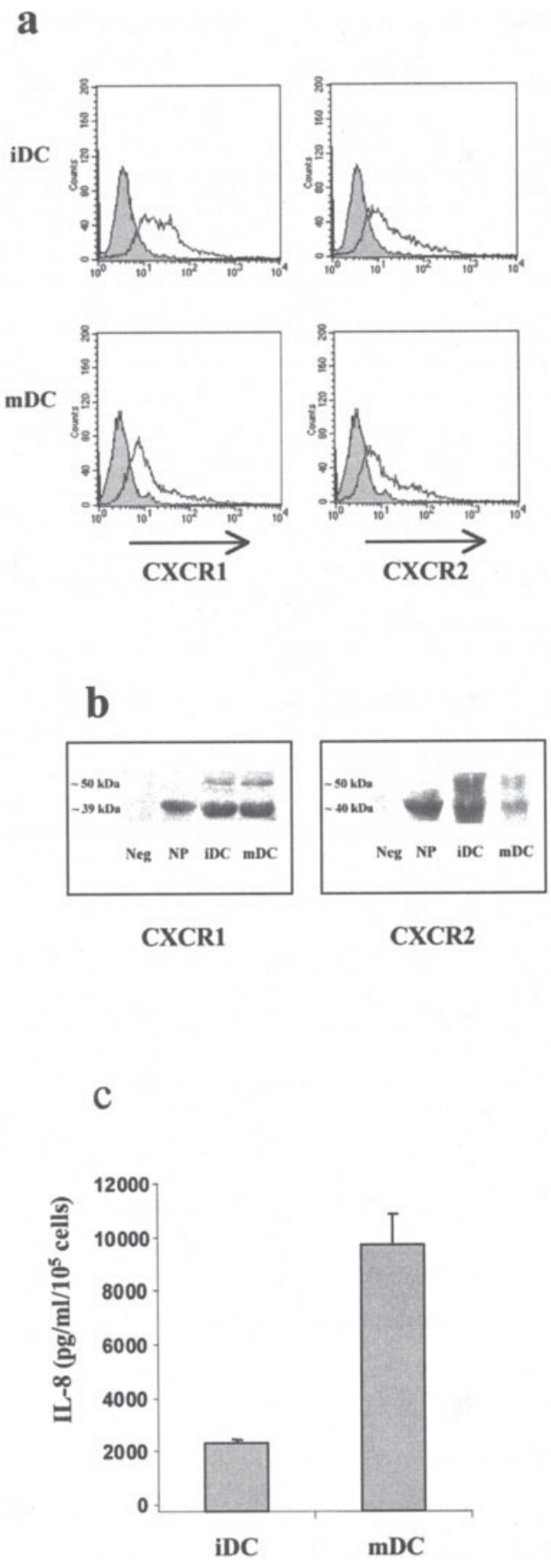
(50–200 pg/ml). However, SW48 colon carcinoma cell line did not produce detectable IL-8 (Fig. 2*b*), indicating that IL-8 production is a frequent but not constant feature of these types of cancer.

DCs are responsive to IL-8

If IL-8 is a factor directly involved in retaining DCs inside malignancies, DCs should express functional receptors for this chemokine on their plasma membrane. Previous studies had documented that CXCR1 is expressed by DCs as detected by Northern blot and flow cytometry.[19,20]

We were able to confirm that DCs expressed CXCR1 and in addition found coexpression of CXCR2 (Fig. 3*a*). Moreover, a decrease of CXCR1 expression was detected after maturation induced by TNF-α, IFN-α and PGE_2 (Fig. 3*a*), consistent with a previous report showing that CXCR1 expression is downregulated in the presence of LPS.[19]

Next, bands showing a molecular weight of 39 kDa were clearly detected by immunoblotting with antibodies to CXCR1 and CXCR2 in DCs and granulocyte lysates (Fig. 3*b*). In addition to the 39 kDa band described in neutrophils, we found a further, less intense band of approximately 50 kDa. The identity of this addi-



tional molecule reactive with the anti-CXCR1 and anti-CXCR2 mAbs is being currently explored, since it is undetectable in neutrophils and may be specific to DCs. Differentially regulated mRNA splicing is a possibility that has been recently reported for CXCR3.[21]

Both immature and mature DCs were found to produce IL-8 into the supernatant during tissue culture (Fig. 3*c*), suggesting that IL-8 could act in an autocrine or paracrine fashion. The role of IL-8 secreted by DCs at either maturation stage remains elusive, although increases during maturation (Fig. 3*c*) are probably involved in sustaining inflammation.[22] In the case of intratumorally injected DCs, production of IL-8 by the DCs themselves may contribute to their poor migration out of malignant tissue.

In our hands, expression of both CXCR1 and CXCR2 receptors correlated with the response of DCs to human recombinant IL-8 protein in a wide range of doses as detected in classic transwell migration assays (Fig. 4*a*). The ability of such recombinant protein to attract DCs was blocked with neutralizing mAb (Fig. 4*a*). Moreover, tissue culture supernatants of human colorectal carcinoma cell lines (HT29 and Caco2) attracted DCs in a fashion that was also inhibited by antihuman IL-8 mAb (Fig. 4*b*). Supernatants from SW48 cell line in which IL-8 had not been detectable failed to attract DCs (Figs. 2*b* and 4*b*). Most importantly, serum samples from the patients included in the clinical trial also attracted DCs in a mode dependent on IL-8 (Fig. 4*c*). A previous study has suggested that CXCR1 and CXCR2 were nonfunctional in DCs derived in culture with GM-CSF and IL-13 in spite of detectable expression of the receptors.[23] Reasons for the discrepancy between these data and our own might involve differentiation conditions of DCs in culture.

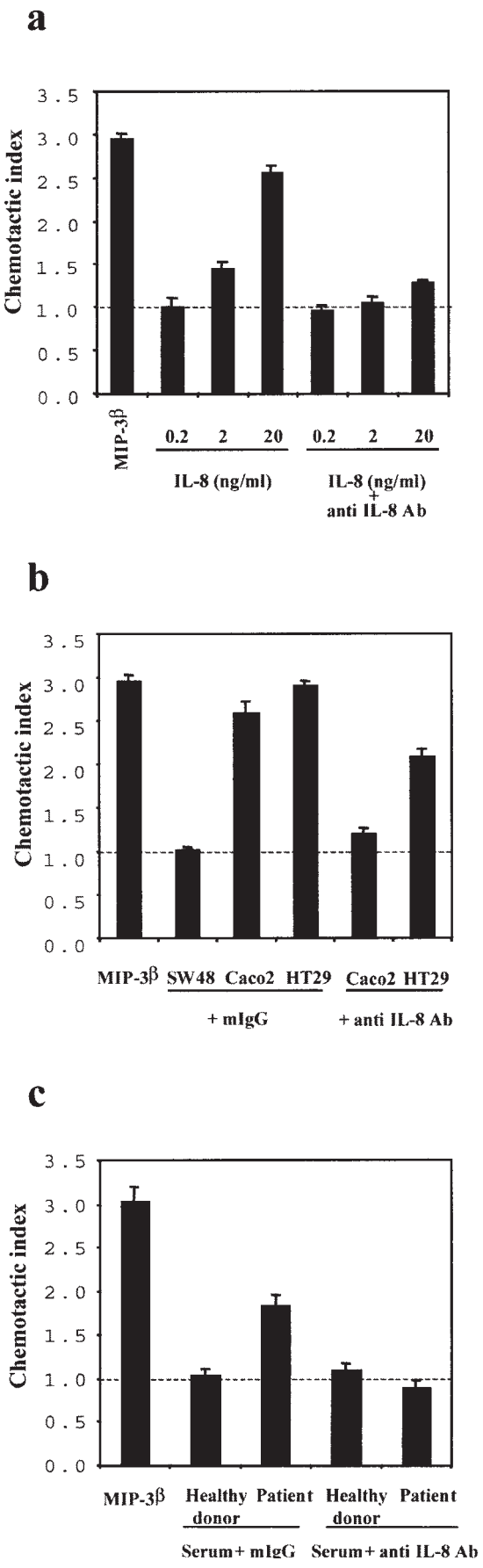
Tumor cells can prevent DCs migrating out of malignant lesions by producing IL-8

If IL-8 secretion by carcinoma cells is a factor in the poor migration of injected DCs out of tumors, IL-8 should oppose the attraction of DCs by other chemokines produced beyond the cancer. The main chemokines guiding DCs from peripheral, *i.e.*, tumor tissue, to secondary lymphoid organs are the CCR7 ligands, MIP-3β (ELC) and 6-C-kine (SLC).[24] We found that DCs can be retained by supernatants of colon cancer cells diminishing their response to MIP-3β in classical chemotaxis assays (Fig. 5). This effect was neutralized by anti-IL-8-specific mAb and could be overcome by higher concentrations of MIP-3β. Such *in vitro* experiments resemble the situation of tumors producing IL-8 that retain DCs, in such a way that DCs would not respond to chemokines guiding them to lymphoid organs.

Discussion

In the overall effort of clinical research to explore the potential of DCs to elicit antitumor immunity,[25] this study defines an unexpected limiting factor for the intratumoral route of DC administration consisting of a poor migration of DCs into lymphoid tissue. Intratumoral injection of DCs as a cancer treatment relies on several steps that take place once DCs are released inside the organ-

FIGURE 3 – Human dendritic cells express CXCR1 and CXCR2 and produce IL-8. (*a*) Surface expression of CXCR1 and CXCR2 on immature (iDCs) and mature (mDCs) dendritic cells were assessed by flow cytometry. Filled histograms represent cells stained with the isotype control antibody, whereas open histograms correspond to cells stained with anti-CXCR1 or -CXCR2 mAbs as indicated. (*b*) Western blot analysis of CXCR1 and CXCR2 expression in iDCs and mDCs. Blots were probed with mouse anti-CXCR1 and anti-CXCR2 mAbs. Cell lysates from human myoblasts (Neg) and neutrophils (NP) were respectively included as negative and positive controls. (*c*) IL-8 concentrations (mean ± SD) were measured by ELISA in 24-hr culture supernatants of immature and mature DCs (10^5 /ml).



ism: antigen capture from malignant cells,[26] migration into lymph nodes[13,27] and antigen presentation by activated DCs to T cells.[13,27,28] In mouse models, intratumoral injections of DCs can eradicate several transplanted tumors when DCs are pretransfected with genes encoding IL-12,[12,13] IL-7,[15] or CD40L.[14] In the case of IL-7, there is therapeutic evidence even in lung cancer arising in mice transgenic for an oncogene after being peritumorally injected with DCs engineered to produce IL-7.[29] In this regard, we have experimental evidence showing that intratumoral DCs transfected to produce IL-15 induce curative immunity in some mouse tumor models (data not shown). Even untransfected immature DCs when injected inside tumor tissue can induce systemic antitumor immunity that controls micrometastasis but not the primary tumor.[30] Thus, experimentation in rodents clearly suggests that the intratumoral route of administration had potential for translational research with DC-based immunotherapy. In these mouse models, DC migration from malignant to lymphoid tissue seems not to be a problem. DCs labeled with the GFP gene or fluorochromes reach the interfollicular areas of lymph nodes quite efficiently.[13,27,31]

Preclinical efficacy in rodents prompted clinical development of intratumoral injection of DCs that had been engineered to produce IL-12. In our studies in human patients harboring digestive carcinomas, deficient migration seems to be an important issue. Intratumoral retention was observed in scintigrams taken after intratumoral administration of tracing doses of ^{111}In-labeled DCs either with immature DCs or with DCs that had been matured with TNF-α, IFN-α and PGE_2. It is noteworthy that maturation under these stimuli promoted CCR7 expression and responsiveness to lymph node-homing chemokines (data not shown). Although more potent maturation-inducing agents such as Toll-like receptor agonists were considered, they were not included because clinical-grade products were not available. In this regard, adenovirus transfection of DCs is known to induce partial maturation of the cells[32,33] and IL-12 acting on a variant of the IL-12 receptor expressed by DCs enhances the maturation program.[34] As a result, DCs transfected with the IL-12-encoding adenovirus should experience an additional set of activation/maturation signals dependent on the virus and on the autocrine effects of IL-12.

The sequential scintigrams performed to the patients cannot completely disregard that a minority of DCs could have reached draining lymph nodes. Moreover, part of the DCs reaching the spleen could have stayed long enough inside the tumor mass as to be loaded with tumor antigens. Nonetheless, with these clinical images in mind, we found that the serum of all patients in the clinical study contained significant quantities of the IL-8 chemokine. Several reports had demonstrated that IL-8 was secreted by malignant cells in human digestive carcinomas,[5,35–37] while genetic evidence in mice indicated a role for IL-8 in angiogenesis and metastasis. We thought that the chemotactic activity of IL-8, previously characterized on polymorphonuclear leukocytes (PMNs),[6] monocytes[23] and endothelial cells,[8] could be involved in intratumoral retention of DCs, provided that DCs would respond to IL-8.

The IL-8 receptor CXCR-1 had been found on DCs in previous studies,[19,20,23] but somehow DCs differentiated from monocytes with GM-CSF and IL-13 were not chemoattracted by IL-8 in spite of receptor expression.[23] In our hands, both IL-8 receptors CXCR-1 and CXCR-2 are expressed on DCs differentiated with GM-CSF and IL-4 from $CD14^+$ immunomagnetically purified monocytes in

FIGURE 4 – Human DCs are chemoattracted by IL-8 secreted by tumor cells. Migration DCs in transwell migration assays. In these experiments, DCs (4×10^5) were set across 8 μm pore transwells exposed to (*a*) different concentrations of recombinant human IL-8, (*b*) HT29, Caco2 and SW48 culture cell supernatants (10% v/v) and (*c*) serum from a patient (5% v/v) containing 30 pg/ml of IL-8. When indicated, a neutralizing antihuman IL-8 antibody or control mouse IgG mAb were added. Migration is expressed as chemotactic index (mean ± SD).

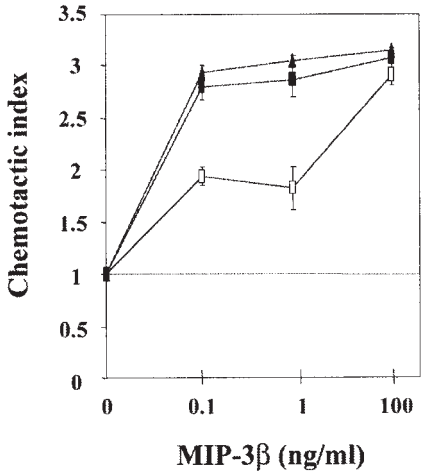


FIGURE 5 – Tumor-derived IL-8 inhibits DC migration induced by MIP-3β. Chemotaxis assay in which mature DCs (4×10^5) were set in transwell experiments as those in Figure 4 to respond to increasing concentrations of MIP-3β (0.1, 1 and 100 ng/ml) in the lower chamber. In this case, DCs were cultured in the upper chamber with control medium (filled triangles) or with HT29 cell supernatant, either with control mouse IgG (open squares) or with antihuman IL-8 mAb (filled squares). Results are presented as chemotactic index (mean ± SD).

serum-free media. Importantly, these DCs are attracted by IL-8. This apparent discrepancy can probably be reconciled if the differentiation conditions are taken into account and it raises interesting points on the existence of nonfunctional CXCR-1 receptors under certain conditions. Indeed, all these studies have been performed on *ex vivo* differentiated DCs and it would be interesting to know what is the case with DCs isolated from human tissues that are the relevant ones in the physiology of the immune system.

The finding that IL-8 can attract DCs might be relevant for DCs recruitment into inflamed tissue or for DC-endothelium crosstalk.[38] During inflammation, IL-8 can be involved in colocalizing PMN leukocytes and professional antigen presenting cells at the microbial entry site. There are lines of research that indicate an important function for this rendezvous of leukocytes at the initiation of adaptive immunity in inflammatory foci.[10] It seems wise for DCs to reach tissues containing a likely source of dangerous antigens, requiring eventual presentation to the immune system.

It is noteworthy that, in spite of the production of IL-8 in tumor tissue, there is not a massive infiltrate of neutrophils inside most malignancies. This probably reflects the fact that leukocyte extravasation needs adherent proinflammatory vascular endothelium in addition to the IL-8 chemotactic signal.[39]

Apart from migration, we have not found effects of IL-8 on other functions of DCs. Maturation is not modified in our hands by IL-8, at least up to 100 ng/ml. Exposure to IL-8 neither changes the basal level of surface expression of CD86, MHC-II and CD83 on immature DCs, nor the level of maturation induced with TNF-α, IFN-α and PGE_2 (data not shown). In addition, similar concentrations of IL-8 do not change the MLR-stimulating activity of these DCs (data not shown). We have also found that IL-8 at 4 ng/ml did not decrease expression levels of CXCR1 and CXCR2 (data not shown), thus indicating that IL-8 does not desensitize DC to itself. However, it has been shown that IL-8 activates neutrophil functions other than migration, such as the respiratory burst,[40] thus suggesting that IL-8 could modulate other maturation-unrelated functions of DCs.

This study provides strong *in vitro* evidence that IL-8 can chemotactically retain DCs and therefore this molecule is a good candidate to mediate sequestration of DCs inside human tumors, at least in the patients included in this trial.

Chemokine retention of DCs inside the malignancies could be a major factor for the weakness of the immune responses elicited in the trial. Other factors potentially downsizing the efficacy of the intratumoral route, at least in digestive carcinomas, are the local production of TGF-β and VEGF that can downregulate DC functions by means of preventing maturation/activation of these cells.[41,42] It is important to mention that IL-8 might not be the only factor involved in DC retention inside the tumor. Other possibilities include the poor lymphatic vessel drainage of malignant tissue, the impairment of the natural pathways of DC circulation and the possible interplay of other factors with chemotactic activity on DCs. Tumors seem to have learned how to exploit the attraction/retention of leukocytes actively, as recently shown for regulatory T cells[43] and for DCs.[44] Interestingly, at least in certain models, local VEGF at the tumor mass seems to subvert the differentiation of the attracted/retained DCs toward endothelial lineage, while turning them into tolerogenic antigen presenting cells.[44]

It would be interesting to study whether IL-8 secretion by malignant cells could tamper with endogenous DCs and therefore provide tumors with immune escape mechanisms. It is noteworthy that IL-8 tumor transfectants have been studied in animals devoid of adaptative immune systems and therefore immune-interfering properties of IL-8 could have been overlooked. The lack of IL-8 in the mouse genome has been an important limiting factor in this regard.[45]

As a whole, our findings have direct implications for explaining the active retention of DCs injected intratumorally with an immunotherapeutic aim. It is possible that IL-8 might also be involved in retaining endogenous DCs inside malignancies. In either case, deficient migration into lymphoid tissue could be preventing efficacious cross-priming of tumor antigens.[26,46,47]

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