Featured Article

Dendritic Cell Infiltration and Prognosis of Early Stage Breast Cancer

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ABSTRACT

Purpose: Although dendritic cells (DC) and T cells can infiltrate primary breast carcinoma, it remains unclear whether the immune response influences the clinical outcome.

Experimental Design: T lymphocytes and DC infiltration within primary tumors was investigated in 152 patients with invasive nonmetastatic breast cancer. CD1a, CD3, CD68, CD123, CD207/Langerin, and CD208/DC-LAMP expression was assessed with semiquantitative immunohistochemical analysis. Expression of chemokines involved in DC migration (MIP-3a/CCL20, MIP-3b/CCL19, and 6Ckine/CCL21) was also examined. The correlation between these markers and the characteristics of the tumors, as well as relapse-free and overall survival was analyzed. Significant prognostic parameters were then tested in a validation series.

Results: Infiltration by immature CD207/Langerin$^+$ DC was found in a third of the cancers and did not correlate with clinicopathological data. Presence of mature CD208/DC-LAMP$^+$ DC (56%) and CD3$^+$ T cells (82%) strongly correlated with lymph node involvement and tumor grade. Among the chemokines analyzed, only the presence of MIP-3b/CCL19 in 57% of the tumors correlated with prolonged overall survival. CD123$^+$ plasmacytoid DC (pDC) infiltrated 13% of the primary tumors. Their presence was strongly associated with shorter overall survival (93% *versus* 58% at 60 months) and relapse-free survival (90% *versus* 37% at 60 months) and was found to be an independent prognostic factor for overall survival and relapse-free survival and confirmed in an independent validation series of 103 patients.

Conclusions: Infiltration by pDC of primary localized breast tumor correlates with an adverse outcome, suggesting their contribution in the progression of breast cancer.

INTRODUCTION

Breast cancer is the most frequent malignant tumor of women in Western countries. The prognosis of early breast carcinoma is influenced by several clinical and biological variables. Among these, signs of early dissemination such as the presence of tumor cells in regional lymph nodes and, possibly, in bone marrow are well-established, adverse prognostic factors (1, 2). In addition, the phenotypic and molecular characteristics of the tumor, especially the histologic grade, the hormone receptor expression, and HER-2/neu amplification, are prognostic factors for relapse and death and predictive factors for resistance to hormone and cytotoxic therapy (3). Recently, level of cyclin E (4) and *in situ* mRNA expression profile have also been linked to the survival of breast cancer patients (5).

Several lines of evidence suggest that the immune response may also influence the progression of tumors. The concept of tumor immunosurveillance, which was proposed more than 40 years ago (6), has been supported in humans by epidemiologic studies revealing a correlation between clinical immunosuppression and cancer development (7). Tumor immunosurveillance was recently shown through the use of tumor-prone and immunodeficient mice (8, 9). The capacity of both innate resistance and adaptative immunity to affect the progression of tumors has been shown in several mouse models (10, 11) and, more recently, in patients receiving tumor-specific vaccine (12). However, the role of the T-cell–mediated immune response in controlling cancer progression remains poorly documented.

In primary breast cancer, an efficient antitumor immune response has not been shown, although dendritic cells (DC) infiltrate the tumors (13) and antibodies directed against p53 (14) or HER-2/neu (15) have been detected in the sera of patients. Indeed, unlike other tumor types, the incidence of breast cancer is not altered in immunocompromised patients (16), and some nonspecific immunostimulating therapies have been suggested to worsen the prognosis (17). Immature DC have been recently shown to infiltrate primary breast carcinoma, whereas mature DC were only found at the periphery of the

tumor (13, 18). However, the clinical relevance of this observation remains unclear because immature DC infiltration in primary breast carcinoma does not correlate with improved survival (19, 20) in contrast to other tumor types (21–23).

The present study confirms that the frequent infiltration of primary breast carcinomas by myeloid DC does not correlate with prognosis. However, a strong correlation was observed between plasmacytoid DC (pDC) infiltration and poor survival, suggesting a contribution of these immune cells in tumor progression.

PATIENTS AND METHODS

Patient Selection

The 1996 Series.   All clinical and biological data on early breast cancer were collected prospectively and included in a regularly updated computer database at Centre Léon Bérard since 1996. The first 152 patients with invasive nonmetastatic breast cancer treated in the Centre Léon Bérard since 09/01/1996 were analyzed. Patient characteristics are presented in Table 1. The median follow-up of the series is 60 months (range 2 to 72).

The 1997 Validation Series.   The first 103 patients with invasive nonmetastatic breast cancer treated at the Centre Léon Bérard since 06/01/1997 were analyzed. Patient characteristics are presented in Table 1 and show that, although similar, the tumor size was on average smaller with a lower frequency of lymph node invasion than in the 1996 series. The median follow-up of the series is 58 months (range 6 to 68 months).

Table 1   Patient characteristics

	n (%)	
	The 1996 series	Validation series
Age	56 (30–89)	54 (31–83)
Tumor size		
0	17 (11)	9 (8)
1	44 (29)	59 (57)
2	45 (30)	31 (30)
3	15 (10)	2 (3)
4	31 (20)	3 (3)
Number of involved lymph nodes		
0	61 (40)	61 (59)
1–3	52 (35)	29 (28)
4–8	18 (12)	8 (8)
>8	21 (13)	6 (6)
SBR		
1	40 (26)	21 (20)
2	67 (44)	53 (51)
3	45 (30)	30 (29)
ER		
0	37 (24)	21 (20)
+	115 (76)	78 (75)
PgR		
0	43 (28)	28 (27)
+	109 (72)	62 (69)
ER & PgR		
0	23 (15)	11 (11)
HER2 *		
0–2[+]	131 (87)	92 (92)
3[+]	21 (13)	8 (8)

Immune markers

	Proportion of positive cells									
	0	+	++	+++	Total[+]	0	+	++	+++	Total[+]
Cells in the stroma										
CD3	27 (17)	63 (41)	38 (25)	24 (16)	125 (82)	24 (23)	33 (32)	32 (31)	13 (13)	78 (77) ‡
CD68	30 (20)	91 (60)	25 (17)	5 (3)	121 (70) †			ND		
DC Lamp	67 (44)	55 (36)	26 (17)	3 (2)	84 (56) †	35 (35)	35 (35)	23 (23)	8 (8)	66 (66) ‡
Langerin	106 (70)	33 (22)	9 (6)	4 (3)	46 (30)			ND		
CD1a	111 (73)	23 (15)	13 (9)	5 (3)	41 (27)			ND		
CD123	132 (87)	18 (12)	2 (1)	0	20 (13)	93 (90)	11 (11)	0	0	11 (11)
On tumor cells and cells in the stroma										
MIP3β	66 (43)	32 (21)	42 (28)	11 (7)	85 (57) †	55 (54)	10 (10)	31 (31)	5 (5)	56 (56)‡
6CK	141 (93)	8 (5)	3 (2)	0	11 (7)			ND		

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; ND, not determined.

* Not available in 4 patients.

† CD68, DC Lamp, and MIP3β expression was not interpretable in one sample.

‡ CD3 expression was not interpretable in 2 samples. DC Lamp and MIP3β in 3 samples.

Treatment. All of the patients were treated according to the following procedures: radical mastectomy for central tumors or tumors larger than 3 cm, conservative surgery followed by radiotherapy for the remaining patients. Adjuvant chemotherapy with anthracyclins was given to node-positive patients and to node-negative patients with two or more of the following criteria: tumor larger than 3 cm, Scarff-Bloom-Richardson grading index (SBR; a pathological index of tumor aggressiveness) of grade 2 to 3, and negative estrogen receptor, and progesterone receptor expression. Adjuvant chemotherapy with anthracyclins was given to T4d tumors. Tamoxifen 20 mg/day was given for 5 years in patients with estrogen receptor or progesterone receptor expressing tumors.

Immunohistochemical Staining

Paraffin-embedded breast tumors tissues were used for analysis. Slides were reviewed, and the blocks containing invasive carcinoma were serially sectioned at a thickness of 4 μm. After deparaffinization and rehydratation, incubating the slides in 5% hydrogen peroxide in sterile water blocked endogenous peroxidases. For heat-induced antigen retrieval, tissue sections were boiled in 10 mmol/L citrate buffer pH6 with either a microwave oven for 15 minutes [anti-CD3 rabbit polyclonal (Dako, Trappes, France); anti-CD1a mouse clone 010 (Beckman-Coulter/Immunotech, Marseille, France); anti-CD208/DC-LAMP rat clone 1010E1 and anti-CD207/Langerin mouse clone 310F7 (Schering-Plough, Dardilly, France); and anti-hCCL19 goat polyclonal (R&D Systems, Minneapolis, MN)] or a water bath at 97°C for 40 minutes [anti-hCCL21 polyclonal (R&D Systems)]. No antigen retrieval was done for the following antibodies: anti-CD123 mouse clone 107D2 (Schering-Plough), anti-BDCA2 mouse clone 104C12 (Schering-Plough), and anti-CD68 mouse clone PGM1 (Beckman-Coulter/Immunotech). Nonspecific binding was blocked with the Ultratech Kit blocking solution (Beckman-Coulter/Immunotech) for 5 minutes except for anti-CD123 (10 minutes), anti-CD1a, and anti-hCCL19 antibodies (15 minutes). The slides were then incubated at room temperature for one hour with the primary antibodies from the list above. These antibodies were used directly (anti-CD1a and anti-CD68-PGM1) or were diluted with an antibody diluent solution (Chem Mate, Dako) at 1 of 25 (anti-hCCL19), 1 of 50 (anti-hCCL21), 50 μg/mL (anti-CD207/Langerin), 1 of 200 (anti-CD3), 0.5 μg/mL (anti-CD208/DC-LAMP), 5 μg/mL (anti-CD123), and 1 of 150 (anti-BDCA2). For the negative control slides, the primary antibody was replaced by isotype-matched control monoclonal antibody or nonimmune serum. After rinsing in PBS, the slides were incubated with a biotinylated secondary antibody bound to a streptavidin peroxidase conjugate (Ultratech HRP DAB kit, Beckman-Coulter/Immunotech or LSAB[+] kit, Dako) for anti-hCCL19 and anti-hCCL21. Bound antibody was revealed by adding the substrate 3,3′-diamino benzidine. Sections were counterstained with hematoxylin and analyzed independently by two investigators blinded for the clinical outcome of the patients.

Semiquantitative Evaluation of the Stainings

On the observation of the first 30 cases, an arbitrary grading system was defined for each antibody in which the density of positive cells within the tumor was assessed semiquantitatively on the whole tissue section. This classification allowed the stratification of the tumors as positive or negative for CD123 or in three groups for CD68, CD3, CD1a, CD207/Langerin, and CD208/DC-LAMP. For the three-groups scale, 20 tumors were first classified according to the number of positive cells in at least 5 low-power fields (×100) within the high staining spots as negative, low ([+]), intermediate ([++]), or high ([+++]) positive. Slide representative for each group was used as reference during the analysis of the subsequent cases. For antibodies against hCCL19 and hCCL21, both the percentage of positive cells (either tumoral or infiltrating cells) and the intensity of the staining (three grades) on the whole tissue section were assessed semiquantitatively. Two pathologists blind for clinical data scored independently all of the slides according to the established criteria. The two investigators reviewed the few cases of discordance (<5% for each marker) to reach a consensus.

Statistical Analysis

The correlation between the clinicobiological data and the phenotype of both tumor and stromal cells within the tissue was done with the χ^2 test or Fisher exact test in Table 2. The correlation between the different phenotypic markers of immune cells was also tested with the Pearson test. Survival curves were plotted with the Kaplan Meier method, and we used the log-rank test to compare survival. Multivariate analysis of prognostic factors for overall survival and relapse-free survival was done with the Cox model. All statistical analysis was done with the procedures of the SPSS 10.02 package.

RESULTS

Immune Cell Infiltration, Chemokine, and Chemokine Receptor Expression in Breast Tumors. Table 1 describes the presence and phenotype of immune cells, as well as the chemokine and chemokine receptor expression pattern in the 1996 series of 152 patients with primary invasive nonmetastatic breast tumors. Examples of immunohistochemical stainings obtained for markers with clinical relevance are presented in Fig. 1. One hundred and twelve tumors from this series were infiltrated by DC. Present in almost one-third of the tumors, CD207/Langerin^{+} and CD1a^{+} DC displayed tight contacts with cancer cells (Fig. 1A; data not shown). CD207/Langerin and CD1a expressions were highly correlated ($r = 0.71$, $P < 0.0001$). Fifty-six percent of the tumors contained CD208/DC-LAMP^{+} mature DC (Fig. 1B), which were consistently located within CD3^{+} T-cell infiltrates. Indeed, a strong correlation was observed between CD208/DC-LAMP expression and CD3^{+} T-lymphocyte infiltrates (Fig. 1A; $r = 0.73$, $P < 0.0001$). Thirteen percent of the tumors were infiltrated by CD123^{+} small racquet-shaped cells with an eccentric nucleus that correspond to the typical morphology of pDC (Fig. 1C; ref. 24). Using BDCA2 staining on paraffin sections, these CD123^{+} cells were also found to express BDCA2, a marker specific for pDC (ref. 25; Fig. 1D). Identification of pDC was additionally confirmed by double CD123/BDCA2 staining of frozen sections originating from the same tumors (data not shown). Of note, CD123^{+} pDC were never found within CD3^{+} T-cell infiltrates, but sometimes occurred in the vicinity of tumor cells (Fig. 1, C and D).

Table 2 Correlations between tumor characteristics and T-cell or DC infiltration

		CD3 (%)					DC Lamp (%)				CD123 (%)		
	n (%)	0	+	++	+++	*P*	0	+	++/+++	*P*	0	+/++	*P*
Age													
<35	5 (3)	0	0	60	40		60	40	0		100	0	
35–50	41 (27)	22	34	22	22		75	12	12		85	15	
>50	106 (70)	17	45	24	13	0.33	68	24	8	0.55	87	13	0.03
T													
0	17 (11)	35	41	6	18		82	18	0		94	6	
1	44 (29)	18	45	18	18		52	30	18		91	9	
2	45 (30)	11	42	24	22		73	18	9		87	13	
3	15 (10)	26	33	40	0		93	0	7		93	7	
4	31 (20)	13	39	39	10	0.14	71	29	0	0.06	74	26	0.25
n													
0	61 (40)	21	54	16	8		59	31	10		89	11	
>0	91 (60)	15	33	31	21	0.004	34	40	26	0.005	86	14	0.61
SBR													
1	40 (26)	30	42	20	7		59	26	15		97	3	
2	67 (44)	15	51	24	10		48	37	15		82	18	
3	45 (30)	11	27	31	31	0.004	27	44	29	0.03	84	16	0.06
HER2													
0–2	131 (87)	22	40	26	12		48	37	15		89	11	
3	21 (13)	3	24	34	38	0.004	23	33	43	0.009	79	21	0.12
Hormone receptors													
0	23 (15)	9	17	26	48		22	26	52		83	17	
+	129 (85)	19	46	25	10	0.0001	48	38	13	0.0001	88	12	0.51

NOTE. *P* of a χ^2 test or Fisher's exact test analysis of the correlation between clinical and biological parameters and CD3, DC Lamp, and CD123 expression.

Abbreviation: T, tumor size.

Interestingly, an anti-MxA monoclonal antibody did not stain pDC in any of the 20 tumors of the 1996 series infiltrated with pDC, suggesting that those cells were not secreting type I IFN (data not shown; ref. 26). CCL20 was not detectable in this series (data not shown). CCL19 and CCL21 expression was observed both in tumor cells and DC (Table 1; data not shown). Of note, CCL21 expression was also observed occasionally in lymphatic endothelial cells (data not shown).
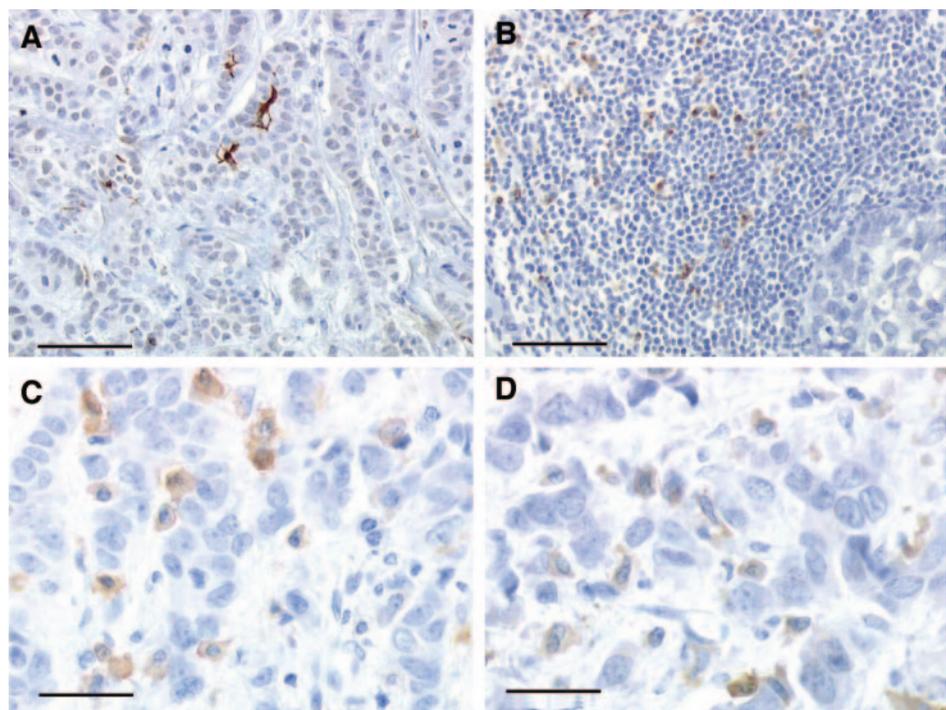

Fig. 1 Immunohistochemical staining with various antibodies of paraffin-embedded invasive breast cancer tissue sections. *A*, CD207/ Langerin: Langerhans-type CD207/ Langerin$^+$ DC establish intimate contact with tumor cells. *B*, CD208/ DC-LAMP: mature CD208/DC-LAMP$^+$ DC lying within a cluster of lymphocytes at the margin of the tumor. CD123 (*C*) and BDCA2 (*D*): CD123$^+$ cells are small racquet-shaped cells with an eccentric nucleus that are spread among tumor cells. Cells located in the same areas with a similar shape also express BDCA2. *Bars* correspond to 100 μm (*A* and *B*) or 50 μm (*C* and *D*), respectively.

Infiltration with Immune Cells and Clinicobiological Presentation of the Tumors. CD1a, CD68, CD207/Langerin, CCL21, and CCL19 expression did not significantly correlate with the clinical and/or histologic variables of the primary tumors ($P > 0.01$). The presence of CD3$^+$ infiltrating T cells and CD208/DC-LAMP$^+$ cells significantly correlated with axillary lymph node involvement, a high SBR histologic grade, HER2/neu^{+++} expression in tumor cells, and lack of hormone receptor expression. Conversely, the presence of pDC did not correlate with tumor size, nodal stage, SBR grade, or hormone receptor status (Table 2).

Survival and Immune Cell Infiltration. As expected, both overall survival and relapse-free survival were significantly reduced in patients with large tumors, nodal involvement, and high SBR grade (Table 3). In addition, in univariate analysis, the presence of CD123$^+$ cells was identified as an adverse prognostic factor for both overall survival and relapse-free survival, whereas the presence of CCL19 was significantly associated with an improved overall survival but not relapse-free survival (Fig. 2; Table 3). In contrast, no significant correlation was found between expression of CD1a, CD207/Langerin, CD3, CD208/DC-LAMP, hCCL21, or CD68 and either overall survival or relapse-free survival (data not shown).

Tumor size, nodal status, SBR, hormone receptor expression, HER2/neu^{+++}, CD123, and MIP-3b/CCL19 expression were tested in multivariate analysis. Lymph node involvement and CD123$^+$ pDC infiltration were independent, adverse prognosis factors for relapse-free and overall survival (Table 4). The presence of CCL19 was an independent, positive prognostic factor for overall survival only (Fig. 2; Table 4). Survival curves illustrate the striking differences of both relapse-free and overall survival between patients with or without pDC infiltration of their tumors (Fig. 2).

To confirm the prognostic value of the presence of CD123$^+$ pDC in primary breast carcinoma, we tested this variable in a validation series of the 103 first patients included in the prospective database of 1997. Eleven (11%) of the tumors contained pDC, as compared with 13% in the test series described above. Overall survival at 58 months was 93% in the pDC-negative subgroup *versus* 73% in the pDC$^+$ subgroup ($P = 0.05$). Relapse-free survival at 58 months was 89% in the pDC-negative subgroup *versus* 37% in the pDC$^+$ subgroup ($P = 0.03$; Fig. 3).

DISCUSSION

The results presented in this study show that primary breast carcinomas are frequently infiltrated by immature and/or mature myeloid DC but only rarely by pDC. The presence of pDC in the primary tumor was found to be a major, yet unreported prognostic factor for clinical outcome in this series of early breast cancer.

DC are professional antigen-presenting cells that play a sentinel role in both peripheral organs and in peripheral blood. On triggering by microbial products or by tissue damage, they migrate to secondary lymphoid organs where they present antigen to T cells (27). Antigen presentation leads to T-cell proliferation, which results in either immunity or tolerance depending on the stage of maturation of the presenting DC (28). The nature of the T-cell response is also dependent on the subpopulation of DC involved. Indeed, two major human DC populations have been recognized, the myeloid DC and the pDC. They differ in many aspects, including their origin, their migratory ability and tissue localization, their capacity to discriminate and to respond to different pathogens, and the profile of cytokines they secrete (29). In response to various stimuli, myeloid DC and pDC can polarize the type of T-cell response toward a Th1 or a Th2 response, depending primarily on interleukin-12 secretion (30). The two DC subtypes also contribute in different ways to link acquired and innate immune responses, with myeloid DC-activating B cells (31) and NK cells (32), and pDC producing large amounts of natural IFNs in response to viruses (33).

Given the functional differences defined by both the activation status and the subpopulations under consideration, we have revisited the role of DC in the biology of early breast cancer. For this purpose, tumors were retrieved from files of patients who had their primary breast cancer resected at the
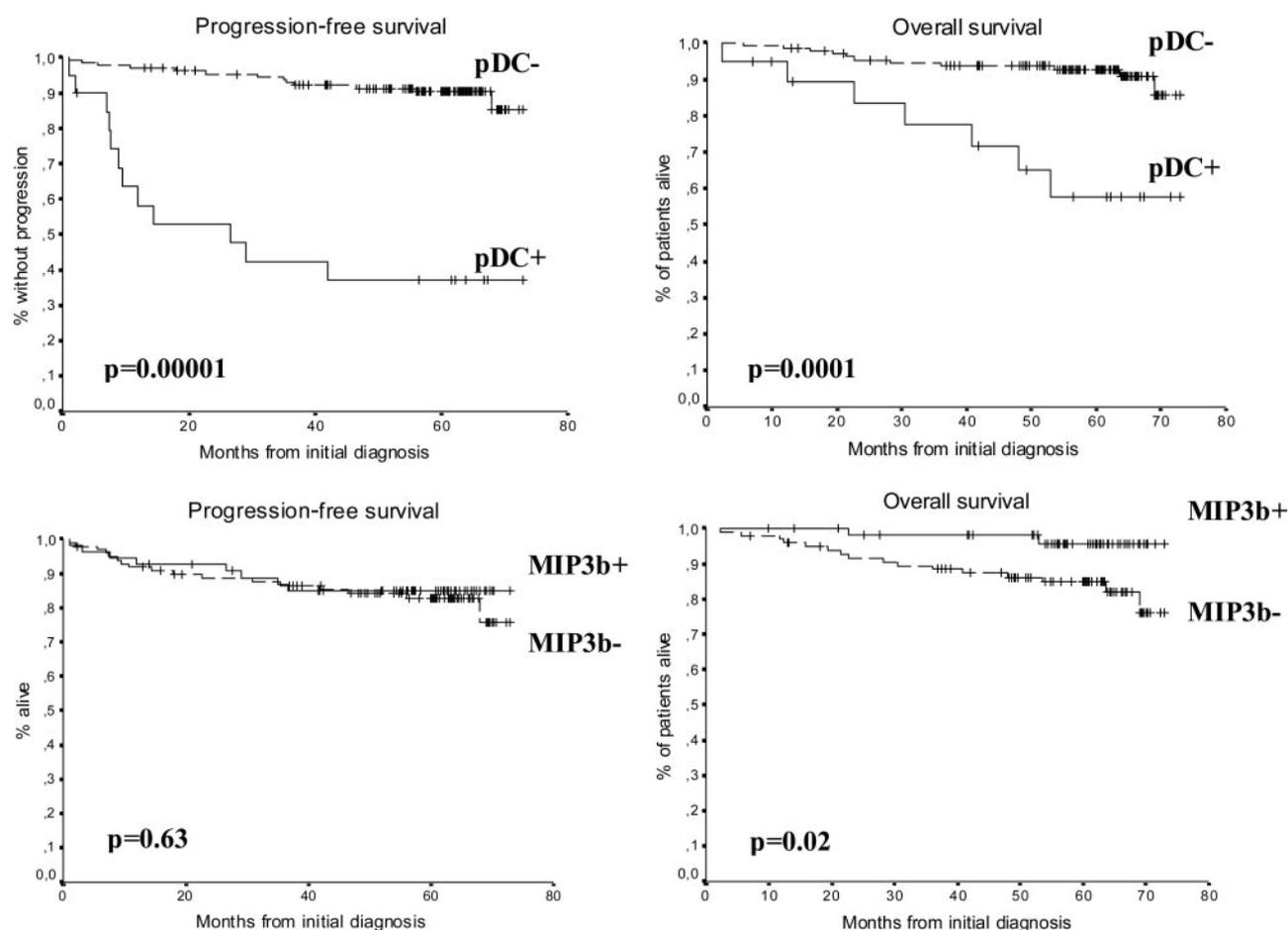
Table 3 Prognostic parameters for survival in univariate analysis

		Relapse-free survival		Overall survival	
	n (%)	5-year survival (%)	log-rank	5-year survival (%)	log-rank
Age					
<35	5 (3)	80		100	
35–50	41 (27)	80		90	
>50	106 (70)	90	0.42	87	0.56
T					
0	17 (11)	94		100	
1	44 (29)	95		92	
2	45 (30)	88		88	
3	15 (10)	70		90	
4	31 (20)	65	0.01	73	0.04
Ax. node involved					
0	61 (40)	92		93	
1–8	70 (47)	87		89	
>8	21 (13)	54	0.0001	74	0.007
SBR					
1	40 (26)	100		100	
2	67 (44)	78		85	
3	45 (30)	86	0.01	81	0.02
HR					
0	23 (15)	85		80	
+	129 (85)	86	0.83	90	0.07
HER2					
0, + or ++	131 (87)	86	0.02	90	0.08
+++	21 (13)	67		80	
DC Lamp*					
0/+	122 (80)	86		90	
++/+++	29 (19)	93	0.43	86	0.54
CD123					
0	132 (87)	90		93	
+/++	20 (13)	37	0.00001	58	0.0001
CCL19/MIP3β					
0/+	98 (64)	83		85	
++/+++	54 (36)	85	0.68	95	0.02

* In one tumor, DC Lamp expression was not interpretable.
Abbreviations: T, tumor size; Ax, axillary; HR, hormone receptor.

Fig. 2 Overall survival and relapse-free survival of patients from the test series according to the presence of CD123^{+} pDC and MIP-3b/CCL19^{+} cells in primary breast carcinoma.

regional Cancer Center (Centre Léon Bérard). This prospective and exhaustive series included the first 152 patients with primary invasive nonmetastatic breast carcinomas that were diagnosed in 1996. The tumor characteristics were comparable with those from other series of patients in large comprehensive cancer centers in terms of tumor size, nodal involvement, HER2/neu[+++] overexpression, and SBR grading. HER2/neu[+++] was also found to correlate with high SBR grading and low hormone receptor expression (data not shown), as well as with a poor relapse-free survival. Several parameters were selected for investigation: CD1a and CD207/Langerin, two markers of Langerhans-type immature DC; CD123, a marker of pDC; and CD208/DC-LAMP, a molecule expressed specifically by mature DC (34). Immunostaining was also done to evaluate the expression of hCCL20, hCCL19, and hCCL21, which are known to drive immature and mature DC migration, respectively (35). In addition, CD3^{+} lymphocyte and CD68^{+} macrophage infiltrates were studied.

Langerhans-type DC were detected in about one-third of primary breast tumors, in contrast with our previous report, where all of the frozen tissue sections were infiltrated by CD1a^{+} and/or CD207/Langerin^{+} DC (13). This discrepancy

might be because of differences in patient populations or may reflect a lower sensitivity of immunostaining on paraffin-embedded tissue sections. It has been reported by several groups that the density of CD1a^{+} DC in various types of cancer is associated with an improved prognosis (21–23, 36, 37). However, in agreement with previous reports in primary breast adenocarcinomas (19, 20), no correlation with overall survival or relapse-free survival was observed in the present series.

Table 4 Multivariate analysis of prognostic factors for survival

	β	SE	P	Relative risk
Relapse-free survival				
Presence of CD123^{+} cells	2.53	0.44	0.0001	12.6
Number of involved nodes	0.12	0.02	0.0001	1.13
SBR	0.59	0.32	0.06	1.73
Overall survival (with MIP3b)				
Presence of CD123^{+} cells	2.66	0.58	0.0001	14.3
Number of involved nodes	0.119	0.03	0.0001	1.13
Presence of MIP3β^{+} cells	−2.14	0.78	0.006	0.12

Progression-free survival
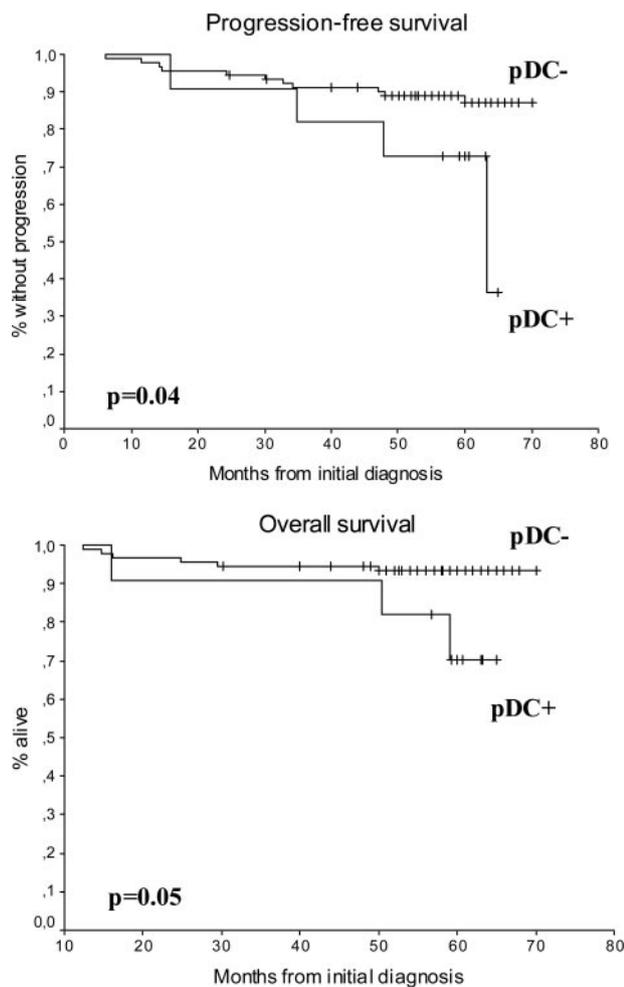

Overall survival

Fig. 3 Overall survival and relapse-free survival of patients from the validation series according to the presence of CD123$^+$ pDC in primary breast carcinoma.

Over half of primary breast tumors analyzed were infiltrated by mature (CD208/DC-LAMP$^+$) DC. The striking compartmentalization of immature tumor-infiltrating DC within tumor bed and mature tumor-infiltrating DC within peritumoral clusters of T cells was confirmed in this study (13, 18). A strong association between the presence of CD208/DC-LAMP$^+$ and CD3$^+$ cells was observed, but the density of both mature DC and T-cell infiltrates did not correlate with the prognosis. Whether the CD208/DC-LAMP$^+$ tumor-infiltrating DC are of myeloid or plasmacytoid origin remains to be formally determined, but the nonoverlapping localization of CD208/DC-LAMP$^+$ and CD123$^+$/BDCA-2$^+$ cells on serial tissue sections (data not shown) suggests they are of myeloid origin. The direct correlation with axillary lymph node metastasis, SBR, HER2/neu, and inverse correlation with hormone receptor status suggest the association of CD3 and CD208/DC-LAMP infiltrate with more aggressive tumors. In particular, immune infiltrate could either reflect HER2/neu immunogenicity or indicate a pathogenic role of immune cells that may contrib-

ute to HER2/neu$^+$ tumor progression. However, neither CD3 nor CD208/DC-LAMP was significantly associated with overall survival or relapse-free survival in univariate analysis in the whole series.

CCL21 and CCL19, two ligands for CCR7, were expressed (by tumors cells, stromal cells, or both) in 7% and 57% of the samples, respectively. Although the expression of these two chemokines did not correlate with tumor size, nodal status, SBR grade, or hormone receptor status, CCL19 expression was associated in both univariate and multivariate analyses with a favorable overall survival (98% rate at 5 years) but not with relapse-free survival. The mechanism underlying this observation is unclear: CCL19 may attract mature DC and T lymphocytes that could contribute to control tumor progression (38–41). However, no correlation was observed between tumor-infiltrating DC or CD3$^+$ T-cell infiltrates and either CCL19 expression or prognosis (data not shown).

The main observation of this study is the adverse prognostic value of CD123$^+$ pDC infiltration in the tumor for overall survival and relapse-free survival. The presence of pDC in breast metastatic lymph node (42), in malignant ascites (43), and in primary melanoma (44) has previously been reported, but no attempt has been made thus far to correlate the pDC infiltration with clinical data. Patients with pDC infiltrates in the primary tumor had a 58% and 73% overall survival at 5 years in the test and the validation series, respectively, and only a 37% relapse-free survival in both series. In marked contrast, patients without pDC infiltrate had a favorable evolution: subgroups of patients with T$_{1-2}$, T$_{3-4}$, and N$^+$ tumors all had an overall survival >90% at 5 years in this series with a long follow-up.

Different nonexclusive mechanisms may account for the association between tumor pDC infiltration and poor prognosis. Infiltration by pDC may merely mark a subset of aggressive primary breast cancers with an intrinsic propensity to metastasize. Alternatively, tumor-infiltrating pDC may enhance tumor growth by the production of angiogenic factors (17, 45), proteases and/or motility factors that can modify the microenvironment of breast cancer (46). Finally, tumor-infiltrating pDC may facilitate tumor progression by interfering with the immune response as follows: (*a*) tumor-infiltrating pDC may induce T-cell tolerance either directly (47) or through the generation of tumor-specific, interleukin-10–secreting CD4$^+$ (47) and/or CD8$^+$ T-suppressor cells (48); (*b*) tumor-infiltrating pDC may deviate the tumor-specific T-cell response toward a Th2 phenotype (30), which seems less effective against tumors; and (*c*) through natural IFN secretion, tumor-infiltrating pDC may alter the presentation of tumor-associated antigens to T cells by myeloid DC. Indeed, pDC may inhibit the differentiation of monocytes into DC, reduce the antigen-presentation capacity of myeloid DC (49), or even convert myeloid DC into mediators of immune tolerance. This last hypothesis is unlikely, however, given the absence of MxA in tumor-infiltrating pDC, which suggests that they are not actively secreting type I IFN *in situ* (26). Nevertheless, the understanding of the mechanisms by which pDC contribute to tumor progression could reveal a new therapeutic target. The key role of CXCL12 in recruiting

pDC that express CXCR4 in malignant ovarian ascites was established recently (43). However, such a function for CXCR4 ligand seems to be less evident in breast tumors, as no correlation could be found between the presence of this chemokine and the infiltration of pDC (data not shown). Whether blood vessels of some breast tumors overexpress adhesion molecules such as PNAd that may help pDC precursors to extravasate (50) remains to be determined.

In conclusion, this first description of a strong correlation between pDC infiltration in breast tumor and poor prognosis may provide a novel prognostic marker for primary breast cancer that could assist in deciding how to optimize the use of current therapies. A larger multicentric study is being set up to confirm these data. Furthermore, by suggesting a role for the immune system in the control of primary breast adenocarcinoma, these findings open new perspectives for the treatment of cancer patients.

REFERENCES

1. Solomayer EF, Diel IJ, Salanti G, et al. Time independence of the prognostic impact of tumor cell detection in the bone marrow of primary breast cancer patients. Clin Cancer Res 2001;7:4102–8.

2. Schnitt SJ. Traditional and newer pathologic factors. J Natl Cancer Inst Monogr 2001;(30):22–6.

3. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000;124:966–78.

4. Keyomarsi K, Tucker SL, Buchholz TA, et al. Cyclin E and survival in patients with breast cancer. N Engl J Med 2002;347:1566–75.

5. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.

6. Burnet FM. Immunological aspects of malignant disease. Lancet 1967;1:1171–4.

7. Keast D. Immunosurveillance and cancer. Lancet 1970;2:710–2.

8. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol 2001;2:293–9.

9. Shankaran V, Ikeda H, Bruce AT, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature (Lond) 2001;410:1107–11.

10. Pardoll DM. Immunology. Stress, NK receptors, and immune surveillance. Science (Wash DC) 2001;294:534–6.

11. Lanier LL. A renaissance for the tumor immunosurveillance hypothesis. Nat Med 2001;7:1178–80.

12. Bancereau J, Schuler-Thurner B, Palucka AK, Schuler G. Dendritic cells as vectors for therapy. Cell 2001;106:271–4.

13. Bell D, Chomarat P, Broyles D, et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. J Exp Med 1999;190:1417–26.

14. Lenner P, Wiklund F, Emdin SO, et al. Serum antibodies against p53 in relation to cancer risk and prognosis in breast cancer: a population-based epidemiological study. Br J Cancer 1999;79:927–32.

15. Disis ML, Cheever MA. HER-2/neu protein: a target for antigen-specific immunotherapy of human cancer. Adv Cancer Res 1997;71:343–71.

16. Stewart T, Tsai SC, Grayson H, Henderson R, Opelz G. Incidence of de-novo breast cancer in women chronically immunosuppressed after organ transplantation. Lancet 1995;346:796–8.

17. Stewart TH, Tsai SC. The possible role of stromal cell stimulation in worsening the prognosis of a subset of patients with breast cancer. Clin Exp Metastasis 1993;11:295–305.

18. Suzuki A, Masuda A, Nagata H, et al. Mature dendritic cells make clusters with T cells in the invasive margin of colorectal carcinoma. J Pathol 2002;196:37–43.

19. Lewko B, Zoltowska A, Stepinski J, Roszkiewicz A, Moszkowska G. Dendritic and cancer cells in the breast tumors– an immunohistochemical study: short communication. Med Sci Monit 2000;6:892–5.

20. Lespagnard L, Gancberg D, Rouas G, et al. Tumor-infiltrating dendritic cells in adenocarcinomas of the breast: a study of 143 neoplasms with a correlation to usual prognostic factors and to clinical outcome. Int J Cancer 1999;84:309–14.

21. Furukawa T, Watanabe S, Kodama T, et al. T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. Cancer (Phila) 1985;56:2651–6.

22. Ambe K, Mori M, Enjoji M. S-100 protein-positive dendritic cells in colorectal adenocarcinomas. Distribution and relation to the clinical prognosis. Cancer (Phila) 1989;63:496–503.

23. Goldman SA, Baker E, Weyant RJ, et al. Peritumoral CD1a-positive dendritic cells are associated with improved survival in patients with tongue carcinoma. Arch Otolaryngol Head Neck Surg 1998;124:641–6.

24. Grouard G, Rissoan MC, Filgueira L, et al. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J Exp Med 1997;185:1101–11.

25. Dzionek A, Sohma Y, Nagafune J, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. J Exp Med 2001;194:1823–34.

26. Haller O, Kochs G. Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. Traffic 2002;3:710–7.

27. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature (Lond) 1998;392:245–52.

28. Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000;191:411–6.

29. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002;2:151–61.

30. Rissoan MC, Soumelis V, Kadowaki N, et al. Reciprocal control of T helper cell and dendritic cell differentiation. Science (Wash DC) 1999;283:1183–6.

31. Dubois B, Bridon JM, Fayette J, et al. Dendritic cells directly modulate B cell growth and differentiation. J Leukoc Biol 1999;66:224–30.

32. Zitvogel L. Dendritic and natural killer cells cooperate in the control/switch of innate immunity. J Exp Med 2002;195:F9–14.

33. Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell 2001;106:259–62.

34. de Saint-Vis B, Vincent J, Vandenabeele S, et al. A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced on DC maturation, is transiently expressed in MHC class II compartment. Immunity 1998;9:325–36.

35. Dieu MC, Vanbervliet B, Vicari A, et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J Exp Med 1998;188:373–86.

36. Tsujitani S, Furukawa T, Tamada R, et al. Langerhans cells and prognosis in patients with gastric carcinoma. Cancer (Phila) 1987;59:501–5.

37. Giannini A, Bianchi S, Messerini L, et al. Prognostic significance of accessory cells and lymphocytes in nasopharyngeal carcinoma. Pathol Res Pract 1991;187:496–502.

38. Vicari AP, Ait-Yahia S, Chemin K, et al. Antitumor effects of the mouse chemokine 6Ckine/SLC through angiostatic and immunological mechanisms. J Immunol 2000;165:1992–2000.

39. Sharma S, Stolina M, Luo J, et al. Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. J Immunol 2000;164:4558–63.

40. Sharma S, Stolina M, Zhu L, et al. Secondary lymphoid organ chemokine reduces pulmonary tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 2001;61:6406–12.

41. Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, et al. T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine: augmentation of dendritic cell-based immunotherapy. Cancer Res 2001;61:2062–70.

42. Horny HP, Feller AC, Horst HA, Lennert K. Immunocytology of plasmacytoid T cells: marker analysis indicates a unique phenotype of this enigmatic cell. Hum Pathol 1987;18:28–32.

43. Zou W, Machelon V, Coulomb-L'Hermin A, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med 2001;7:1339–46.

44. Vermi W, Bonecchi R, Facchetti F, et al. Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. J Pathol 2003;200:255–68.

45. Leek RD, Lewis CE, Whitehouse R, et al. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 1996;56:4625–9.

46. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161–74.

47. Kuwana M, Kaburaki J, Wright TM, Kawakami Y, Ikeda Y. Induction of antigen-specific human CD4(+) T cell anergy by peripheral blood DC2 precursors. Eur J Immunol 2001;31:2547–57.

48. Gilliet M, Liu YJ. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. J Exp Med 2002; 195:695–704.

49. Zou W, Borvak J, Wei S, et al. Reciprocal regulation of plasmacytoid dendritic cells and monocytes during viral infection. Eur J Immunol 2001;31:3833–9.

50. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001;159:237–43.