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Tumor-Expressed B7-H1 and B7-DC in Relation to PD-1+ T-Cell Infiltration and Survival of Patients with Cervical Carcinoma

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Abstract

Purpose: The interaction between programmed cell death 1 (PD-1), expressed by activated effector or regulatory T cells, and B7-H1 (PD-L1) and B7-DC (PD-L2) results in the inhibition of T-cell function. The aim of this study was to determine B7-H1, B7-DC, and PD-1 expression in cervical carcinoma.

Experimental Design: A tissue microarray of a well-defined group of 115 patients was stained with antibodies against B7-H1 and B7-DC. Three-color fluorescent immunohistochemistry was used to study the number and phenotype of tumor-infiltrating T cells expressing PD-1. Additional analyses consisted of *in vitro* T-cell suppression assays.

Results: B7-H1 was expressed in 19%, and B7-DC was expressed by 29% of the 115 tumors. PD-1 was expressed by more than half of both the infiltrating CD8+ T cells and CD4+Foxp3+ T cells, irrespective of B7-H1 or B7-DC expression by tumors. The expression of B7-H1 did not show a direct impact on patient survival. However, subgroup analysis revealed that patients with a relative excess of infiltrating regulatory T cells displayed a better survival when the tumor was B7-H1 positive ($P = 0.033$). Additional studies showed that the presence of B7-H1 during the activation of CD4+Foxp3+ regulatory T cells impaired their suppressive function in a functional *in vitro* assay.

Conclusions: B7-H1 is expressed on only a minority of cervical cancers and does not influence the survival of patients with cervical cancer. PD-1 is expressed by a vast number of infiltrating CD8 T cells, suggesting that blocking of PD-1 could have therapeutic potential in cervical cancer patients. (Clin Cancer Res 2009;15(20):6341–7)

Cervical cancer is the second most common cancer in women worldwide (1). It develops as a result of an uncontrolled persistent infection with a high-risk type of human papilloma virus (HPV), in particular, types HPV16 and HPV18 (2). The occurrence of HPV-induced cancer is strongly associated with failure to mount a strong HPV-specific type 1 T-helper and cytotoxic T-lymphocyte response (3–5), the lack of CD8+ T cells migrating into the tumor cell nests, the induction of HPV16-specific regulatory T cells, and the influx of regulatory T cells into the tumor (6, 7). Moreover, the ratio between the tumor-infiltrating CD8+ T cells and coinfiltrating CD4+Foxp3+ regulatory T cells is an independent prognostic factor for overall survival (8), indicating the key role of these different types of T cells in cervical cancer.

Activated T cells can express the programmed cell death 1 (PD-1) receptor, which can bind B7-H1 (PD-L1) and B7-DC (PD-L2). B7-H1 could be induced to express by a wide variety of immune cells and nonhematopoetic cell types, whereas B7-DC is expressed mainly on activated macrophages and dendritic cells (9). Upon simultaneous engagement of both, the T-cell receptor and PD-1–negative immunoregulatory signals are transferred to the T cells, resulting in a decreased effector response and T-cell tolerance (10). PD-1/B7-H1 interactions have been shown to inhibit a wide range of immune responses against pathogen, tumor, and self-antigens (11, 12).

More recently, it has been reported that B7-H1 and B7-DC are exploited by tumors to evade immune responses. B7-H1 is found to express on cell surface in most human cancers, and this expression was correlated with poor clinical prognosis in renal, gastric, ovarian, breast, and esophageal carcinomas (13–17). The role of B7-DC in the suppression of immune responses remains controversial (18). Because of the strong association between tumor-infiltrating lymphocytes (TIL) and the prognosis of cervical cancer (6, 8) and the fact that PD-1 has been reported to be expressed by tumor-infiltrating CD8+

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Translational Relevance

The extent of tumor infiltration by T cells and the ratio between the several different subtypes is an independent prognostic factor with respect to the survival of patients with cervical cancer. We have studied B7-H1 and programmed cell death 1 (PD-1) in cervical cancer because the B7-H1–PD-1 axis has been implicated in tumor escape. Our data show that more than half of the tumor-infiltrating CD8+ T cells are positive for PD-1, indicating that these T cells may have become exhausted and die in the event they interact with B7-H1 expressed on tumor cells or antigen-presenting dendritic cells. Given the interest to target PD-1 or B7-H1 for the immunotherapy of cancer our observation bears direct impact on the immunotherapeutic treatment of patients with cervical cancer.

T cells, CD4+ T cells, and regulatory T cells (10), we studied the expression and function of B7-H1, B7-DC, and PD-1 in cervical cancer. Here, we show that PD-1 is expressed on a substantial number of tumor-infiltrating CD8+, CD4+, and regulatory T cells. B7-H1, however, is expressed in only a small group of cervical cancer patients and does not confer a survival disadvantage. Interestingly, when the tumors of this group of patients are infiltrated with a high number of tumor-infiltrating CD4+Foxp3+ regulatory T cells, the expression of B7-H1 may bestow a survival benefit.

Materials and Methods

B7-H1 and B7-DC staining of tissue array. A previously described tissue array containing 115 cervical cancer samples of patients, all of whom underwent a radical hysterectomy, was used for the B7-H1 and B7-DC staining (8). Standard immunohistochemical staining was done using antibodies against human B7-H1 (clone 5H1) and B7-DC (R&D Systems). The tissue array sections were deparaffinized, and antigen retrieval was done using EDTA. To reduce nonspecific binding, sections were incubated overnight at 4°C with 10% rabbit serum. The B7-H1 and B7-DC antibodies were used in 1:200 and 1:800 dilutions, respectively. The Powervision detection system was applied (DAKO). The tissue array was evaluated and scored by two experienced researchers (R. Karim; E.S. Jordanova) independently. Expression groups were defined based on the presence or absence of membranous staining.

Three-color immunostaining for CD8, Foxp3, and PD-1. Eight cases with B7-H1–positive and eight cases with B7-H1–negative cervical cancer specimens were selected from the tissue array based on a comparable amount of tumor-infiltrating T cells to avoid a potential bias between the two groups. The simultaneous immunohistochemical staining of three different epitopes applied to 4-μm, formalin-fixed, paraffin-embedded tissue sections has been reported by us before (6, 8). Briefly, the sections were incubated overnight with a mix of anti-CD8 (4B11; mouse IgG2b; Novo Castra), anti-Foxp3 (clone 236A/E7; Abcam), and anti–PD-1 (R&D Systems) after antigen retrieval with EDTA. Slides were washed and incubated with a combination of the fluorescent antibody conjugates goat anti-mouse IgG2b–Alexa-546, goat anti-rabbit IgG1–Alexa-488, and donkey anti-goat–Alexa-647. Alexa Fluor conjugates were obtained from Molecular Probes. The images were captured with a confocal Laser Scanning Microscope (Zeiss LSM510, Zeiss). Ten images were scanned per slide. For each case, one successive negative control slide was included. The intraepithelial TIL count was presented as the number of cells per square millimeter.

In vitro ***analysis of the effect of B7-H1 on PD-1–positive regulatory T cells.*** A previously isolated HPV16-specific CD4+FoxP3+ regulatory T-cell clone (7) C148.31 and an influenza-specific CD4+Foxp3- T-helper clone (B1.50) were stained with goat anti–PD-1 (R&D Systems), followed by anti-goat biotin (Dako) and streptavidin-allophycocyanin (APC) (eBioscience). PD-1 expression was analyzed by flow cytometry. The effect of B7-H1 on the proliferative response of these two clones was assessed by stimulating 25,000 T cells with 1 μg/mL plate-bound anti-CD3 (OKT-3; Ortho Biotech) and 1 μg/mL plate-bound anti-CD28 (clone L293; BD Biosciences) in the presence or absence of 5 μg/mL plate-bound recombinant human B7-H1/Fc chimera (R&D Systems). The effect of B7-H1 on the regulatory capacity of the CD4+Foxp3+ T-cell clone was tested in a classic suppression assay (7) in which the clone C148.31 was stimulated with 1 μg/mL plate-bound anti-CD3 in the presence or absence of 5 μg/mL plate-bound recombinant human

Table 1. Patient characteristics and relations to B7-H1 and B7-DC expression

Characteristic	Category	*n* (%)	B7-H1, *n* (%)			B7-DC, *n* (%)		
			Negative	Positive	*P*	Negative	Positive	*P*
FIGO stage	Ib1	56 (49)	43 (77)	13 (23)	0.345	45 (82)	10 (18)	0.013
	Ib2/II	59 (51)	50 (85)	9 (15)		35 (59)	24 (41)	
Histopathology	SCC	88 (77)	68 (77)	20 (23)	0.099	67 (76)	21 (24)	0.012
	ADC/ADSC	26 (23)	24 (92)	2 (8)		12 (48)	13 (52)	
Lymph nodes	Negative	84 (74)	65 (77)	19 (23)	0.183	60 (72)	23 (28)	0.488
	Positive	29 (26)	26 (90)	3 (10)		19 (66)	10 (34)	
Tumor size (mm)	<40	66 (57)	55 (83)	11 (17)	0.614	49 (75)	16 (25)	0.276
	≥40	42 (37)	33 (79)	9 (21)		27 (64)	15 (36)	
Vasoinvasion	Negative	69 (64)	54 (78)	15 (22)	0.462	45 (66)	23 (34)	0.279
	Positive	39 (36)	33 (85)	6 (15)		30 (77)	9 (23)	
Infiltration depth (mm)	<15	65 (57)	50 (77)	15 (23)	0.338	45 (70)	19 (30)	1.0
	≥15	49 (43)	42 (86)	7 (14)		35 (71)	14 (29)	
HPV type	HPV16	58 (58)	50 (86)	8 (14)	0.122	42 (74)	15 (26)	0.524
	HPV18	24 (24)	16 (67)	8 (33)		15 (63)	9 (38)	
	Other	17 (17)	14 (82)	3 (18)		13 (76)	4 (24)	

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma; ADC, adenocarcinoma; ADSC, adenosquamous carcinoma.

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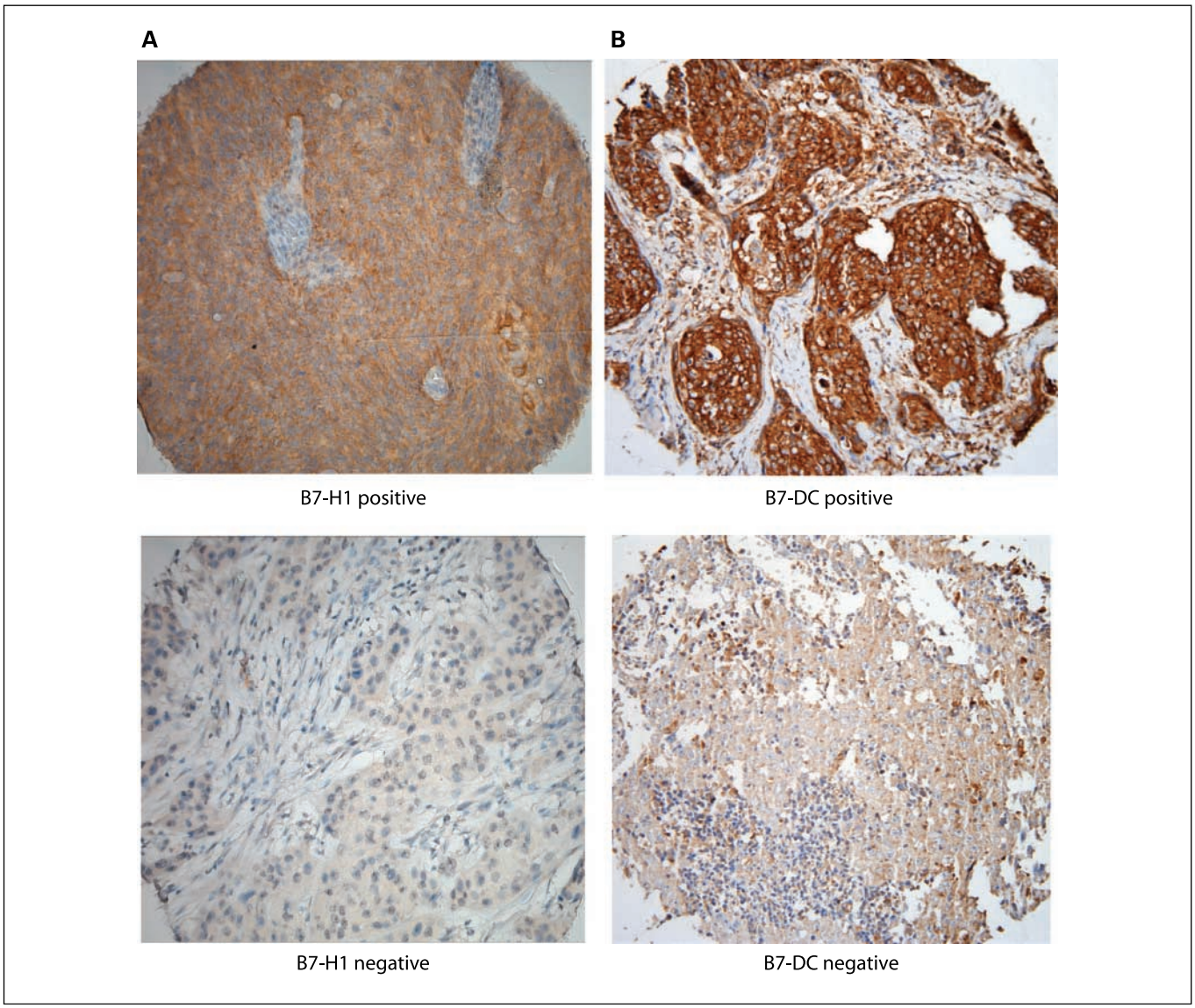

Fig. 1. Immunohistochemical staining of human cervical cancer tissues using B7-H1 and B7-DC antibodies. Expression was defined based on the presence or absence of membranous staining. Specimens with tumor cell surface B7-H1 expression (*A, top*) and tumor with no B7-H1 staining (*A, bottom*), and tumor cell surface B7-DC expression (*B, top*) and tumor with no membranous B7-DC staining (*B, bottom*).

B7-H1/Fc chimera for 24 h. Subsequently, the stimulated C148.31 regulatory T cells were washed to prevent spillover of B7-H1 and put into a coculture with CD4+CD25- responder cells in the presence of 1 μg/mL soluble anti-CD3 and APC (7). After 48 h, the supernatants of triplicate wells were harvested and pooled for the analysis of IFN-γ production by the activated responder cells using enzyme-linked immunosorbent assay (ELISA).

Statistical analyses. Correlations between B7-H1 or B7-DC expression with clinicopathologic parameters or the (high or low) number of tumor-infiltrating cells was done by the χ^2 or, where appropriate, the Fisher's exact test. Patient groups were based on the median (50th percentile) of the numbers of infiltrating immune cells per square millimeter because none of the data for the TIL subtypes followed a normal distribution pattern. Analyses of differences in the numbers of subpopulations of PD-1+ TIL in B7-H1–positive or–negative tumors were done by the nonparametric Mann-Whitney test. All reported *P*s are two sided. A $P < 0.05$ was considered significant. Cumulative 5-y survival rate was calculated by the Kaplan-Meier method and analyzed by the log-rank test. Statistical analyses were done with the SPSS software package 16.

Results

Expression of B7-H1 and B7-DC by cervical cancer cells. To assess the expression and impact of B7-H1 and B7-DC in cervical cancer, we studied a group of 115 well-characterized patients whose clinicopathologic characteristics are shown in Table 1. The mean age of the patients was 48.5 years, with a range between 24 and 87 years at the time of surgery. Fifty-one patients received postoperative radiotherapy because of either tumor-positive lymph nodes or a combination of two of the following parameters: depth of infiltration ≥ 15 mm, tumor size ≥ 40 mm, and presence of vasoinvasion. At the end of the 5-year follow-up period, 23 patients had died of disease, 85 were alive, 5 patients

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Table 2. Correlations of B7-H1 and B7-DC with tumor-infiltrating epithelial T cells

Intraepithelial infiltration	Category*	*n* (%)	B7-H1, *n* (%)			B7-DC, *n* (%)		
			Negative	Positive	*P*	Negative	Positive	*P*
CD8+	Low	34 (34)	31 (91)	3 (9)	0.162	23 (68)	11 (32)	0.824
	High	66 (66)	52 (79)	14 (21)		46 (70)	20 (30)	
CD4+	Low	46 (46)	42 (91)	4 (9)	0.060	31 (75)	15 (25)	0.829
	High	54 (54)	41 (76)	13 (24)		38 (70)	16 (30)	
Foxp3+	Low	38 (41)	36 (95)	2 (5)	0.022	27 (71)	11 (29)	1.000
	High	55 (59)	42 (76)	13 (24)		38 (69)	17 (31)	
CD8+/Treg ratio	Low	49 (54)	40 (82)	9 (18)	0.778	33 (67)	16 (33)	0.820
	High	42 (46)	36 (86)	6 (14)		30 (71)	12 (29)	
CD4+/Foxp3 ratio	Low	47 (52)	37 (79)	10 (21)	0.263	30 (64)	17 (36)	0.266
	High	44 (48)	39 (89)	5 (11)		33 (75)	11 (25)	
CD8+/CD4+ ratio	Low	51 (51)	43 (70)	8 (30)	0.794	38 (75)	13 (25)	0.281
	High	49 (49)	40 (82)	9 (18)		31 (63)	18 (37)	

Abbreviation: Treg, regulatory T cell.
*The patients were divided in two categories with low or high numbers of infiltrating cells (or ratio between subtype of cells) based on the 50th percentile.

had a recurrence, and 2 died of causes unrelated to the primary disease but showed no evidence of disease.

The expression of B7-H1 and B7-DC in the tumors of these patients was determined by immunohistochemistry. Specific examples of B7-H1 and B7-DC staining are shown in Fig. 1. Examination of the entire group of 115 patients revealed the expression of B7-H1 in 22 (19%) tumors, whereas B7-DC was expressed in 34 (29%) cases. The expression of B7-H1 and B7-DC did not correlate ($P = 0.604$). Notably, B7-DC expression was associated with cervical adenocarcinomas and a more advanced stage of cervical cancer (Table 1).

B7-H1 and PD-1 expression in relation to the number and type of TILs. The number and subtype of intraepithelial TIL per square millimeter of tumor within this group of 115 patients has already been quantified (8), and this enabled us to analyze the impact of B7-H1 and B7-DC expression on the number and type of intraepithelial TIL in these tumors. The expression of B7-H1 was associated with higher intraepithelial infiltration by Foxp3+ T cells ($P = 0.022$) but not with CD8+ T cells (Table 2). In contrast, there was no significant association between TIL and B7-DC expressed by tumor cells (Table 2).

Negative regulation of the tumor-specific T-cell response by B7-H1 expressing tumor cells requires the intraepithelial infiltrating T cells to express PD-1. Therefore, PD-1 expression by intraepithelial TIL was tested in a group of patients with a B7-H1–positive tumor, as well as in a group of patients with a B7-H1–negative tumor, which were matched with respect to the number of tumor-infiltrating CD8+ T cells, CD4+ T cells, and regulatory T cells (Table 3). In addition, there was no difference in the CD8/Foxp3 ratio ($P = 0.959$) between these two groups, allowing their comparison with respect to PD-1 expression. The number of single-, double- and triple-positive cells for CD8, Foxp3, and PD-1 was analyzed by triple fluorescent immunohistochemistry. This revealed that, in both groups of patients, more than half of the infiltrating CD8+ T cells and half of the Foxp3+ T cells expressed PD-1 (Table 3). Although, on the whole, the patient group with B7-H1–positive tumors displayed somewhat more intraepithelial PD-1+ T cells, this was not significantly different.

B7-H1 expression confers survival benefit in a subgroup of patients with high numbers of intraepithelial infiltrating regulatory T cells. Retrospective analyses of patients with different types of malignancies showed a link between B7-H1 expression on tumors and poor prognosis (13–17). A similar analysis of the overall survival of patients with cervical cancer did not show such a direct relationship ($P = 0.690$; Fig. 2A). Notably, we

Table 3. PD-1 expression on TILs

Cell type	Median cell number per mm^2 (min-max)		
	B7-H1–positive tumor	B7-H1–negative tumor	*P*
CD8+ T cells	198 (48-505)	160 (9.7-314)	0.337
CD4+ T cells	114 (4.8-317)	73 (15-190)	0.170
Foxp3+ T cells	81 (30-219)	53 (16-101)	0.138
CD8+PD-1- T cells	47.5 (7.5-439)	61 (6.1-101)	0.529
CD8+PD-1+ T cells	107.2 (14-252)	85 (1.2-208)	0.462
CD8+Foxp3+ T cells	3.7 (1.1-5.8)	2.3 (0.0-11)	0.713
CD8+PD-1+Foxp3+ T cells	2.2 (0.0-24)	2.4 (0.0-7.1)	0.815
CD4+PD-1+ T cells	56.1 (2.4-193)	16.2 (2.1-138)	0.248
CD4+Foxp3+ T cells	47.0 (0.0-169)	42.2 (5.8-78)	0.345
CD4+PD-1+Foxp3+ T cells	25.3 (2.4-47)	7.7 (3.7-20)	0.074

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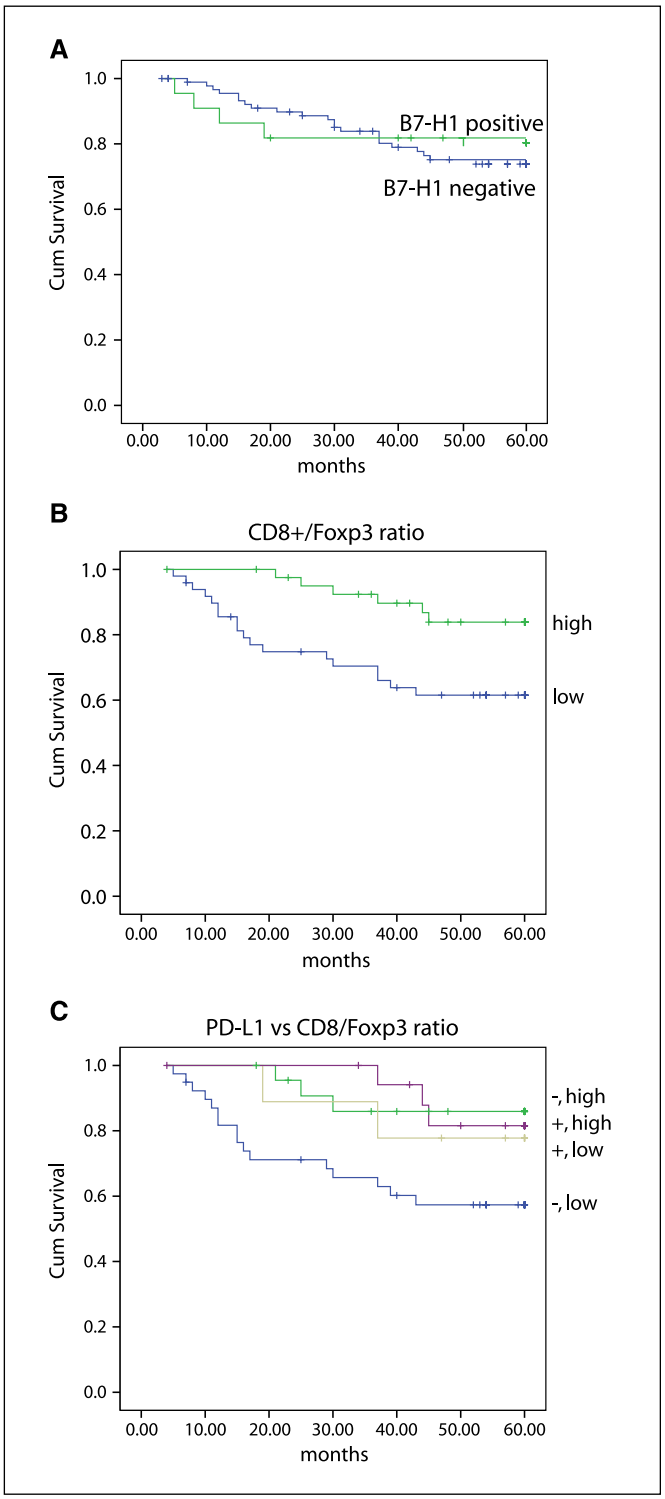

Fig. 2. Kaplan-Meier curves and log-rank test results of 5-y overall survival analyses of patients with cervical cancer based on the expression of B7-H1 on the tumor cell (*A*). Log-rank test result: $P = 0.690$, the ratio between CD8+ and regulatory T cells and the expression of tumor cell surface B7-H1 (PD-L1; *B*) and the ratio between CD8+ and regulatory T cells (*C*). Groups are divided into low (lower 50th percentile) and high (top 50%).

recently reported that the ratio between intraepithelial CD8+ T cells and regulatory (Foxp3+) T cells is an independent prognostic factor for survival in this group of patients with cervical cancer, in which patients with a low CD8/regulatory T cell ratio had the worst survival (ref. 8; Fig. 2B). As a consequence, a potential detrimental effect of B7-H1 expression on survival is more likely to be observed within patients with well-infiltrated tumors. No effect of B7-H1 was seen in the subgroups divided either on the basis of CD8+ T-cell infiltration ($P = 0.584$) or on the number of regulatory T cells ($P = 0.100$; data not shown). In contrast, subdivision based on the CD8+/regulatory T-cell ratio revealed that the overall survival of patients with a B7-H1–positive tumor and a low CD8+/regulatory T-cell ratio was significantly better than in patients with a B7-H1–negative tumor and a low CD8+/regulatory T-cell ratio ($P = 0.033$; Fig. 2C). These data indicate that B7-H1 expression does not have a direct detrimental effect on the overall survival of patients with cervical cancer but on the contrary may improve survival of a small subgroup of cervical cancer patients with tumors relatively heavily infiltrated by regulatory T cells.

PD-1 expression in regulatory T cells and effect of B7-H1 ligation on PD-1–positive regulatory T cells. The association of B7-H1 expression by tumor cells and the enhanced survival of a small subgroup of patients with a low CD8+/regulatory tumor-infiltrating T-cell ratio (Fig. 2C) suggest that the function of regulatory T cells is affected by B7-H1. To test this hypothesis, we made use of a HPV16-specific CD4+Foxp3+ regulatory T-cell clone, which we had isolated from a HPV16+ patient with cervical cancer (7) and which expressed PD-1 at its surface (Fig. 3A), similar to what we observed *in situ* in cervical cancer (Fig. 3B). To determine if B7-H1 ligation has an effect on PD-1 expressing T cells, this CD4+Foxp3+PD-1+ regulatory T-cell clone was stimulated with anti-CD3 in the presence or absence of recombinant B7-H1 protein to test its proliferative capacity. Whereas the proliferation of a PD-1–negative helper T-cell clone was not affected, the PD-1–positive regulatory T-cell clone proliferated less well when B7-H1 was present in the culture (Fig. 3C). To assess whether B7-H1 ligation also inhibited the suppressive capacity of PD-1+ regulatory T cells, the HPV16-specific CD4+Foxp3+PD-1–positive regulatory T cells were stimulated with anti-CD3 in the presence or absence of recombinant B7-H1 protein and then cocultured with CD4+CD25- responder cells (Fig. 3D). Responder cells alone produced high amounts of IFN-γ, but this capacity was suppressed when activated regulatory T cells were added to the culture in a dose-dependent fashion. The presence of recombinant B7-H1 during the activation of the regulatory T cells had a clear negative impact on their suppressive capacity because the IFN-γ production by the responder cells was partly restored (Fig. 3D). These data show that, in principle, PD-1+ regulatory T cells can be incapacitated with respect to their suppressive function when engaged by B7-H1.

Discussion

Following the initial reports that tumor-associated B7-H1 could serve as an immune escape mechanism in a mouse tumor model through downregulation of tumor-specific T-cell responses (19, 20), several retrospective studies in human cancer cohorts showed that B7-H1 expression was associated with clinicopathologic markers for poor prognosis (13, 17) or with lower overall survival (14–16). Our study indicates that most (80%) cervical cancers are B7-H1 negative and that the survival of B7-H1–positive cases is not negatively affected (Figs. 1 and 2). This suggests that tumor-expressed B7-H1 does not play a

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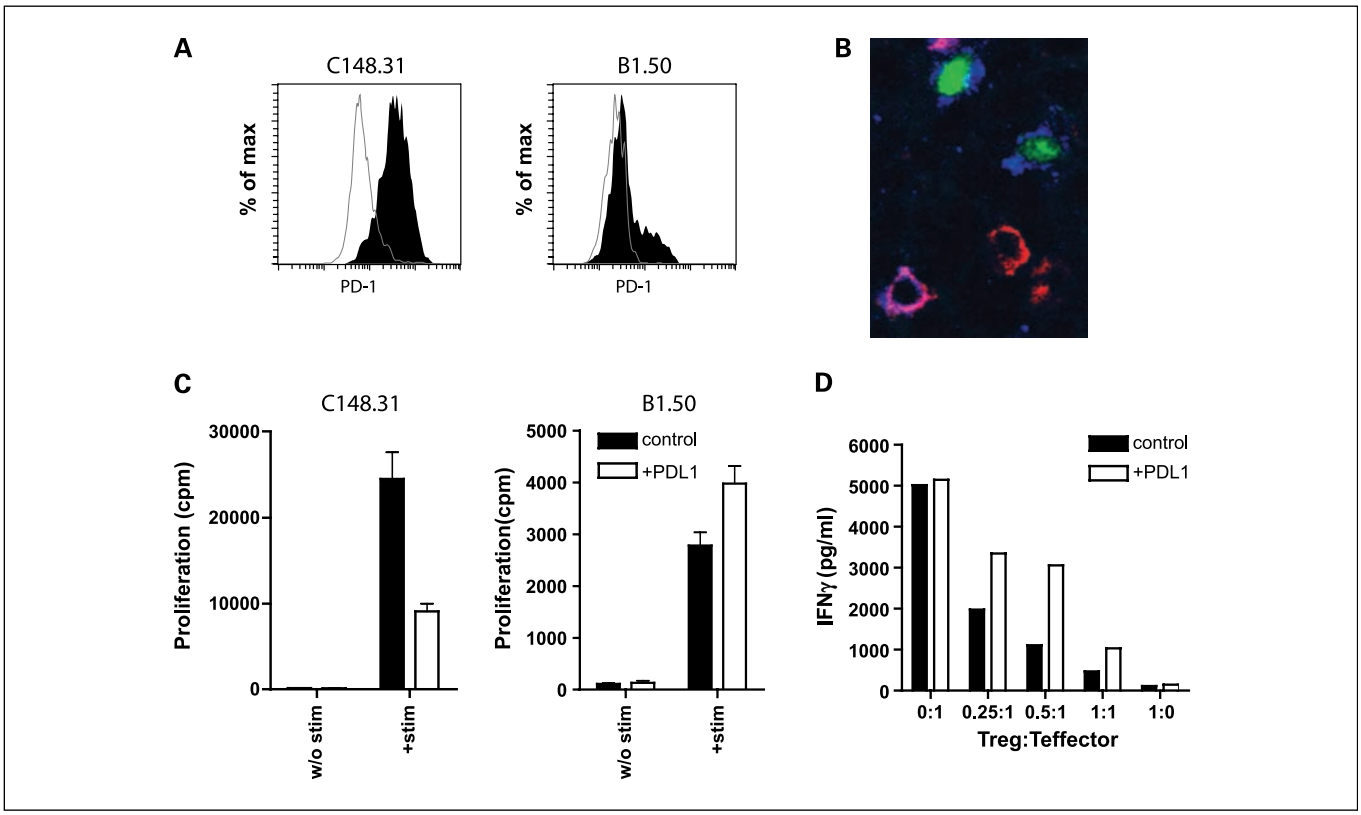

Fig. 3. HPV-specific FoxP3+ regulatory T-cell clone expresses PD-1 and is functionally impaired upon PD-1 ligation. *A,* PD-1 expression (*black areas*) and isotype controls (*gray lines*) of HPV-specific FOXP3+ clone C148.31 and an influenza-specific helper T-cell control clone B1.50. *B,* PD-1 expression observed *in situ* in intraepithelial Foxp3+ T cells in cervical cancer. The different intraepithelial T cells are depicted as CD8+ cells (*red*), PD-1+ cells (*blue*), and Foxp3+ cells (*green*). *C,* proliferation of C148.31 and control clone upon stimulation with plate-bound anti-CD3 and anti-CD28 in the presence or absence of plate-bound B7-H1. *D,* classic suppression assay in which the capacity of the C148.31 regulatory clone to suppress the IFN-γ production of CD4+CD25- cells is tested. C148.31 was pretreated either with plate-bound anti-CD3 and B7-H1 or with plate-bound anti-CD3 only.

significant role in T-cell antitumor immune response in cervical cancer. Rather, our data show that more than half of the tumor-infiltrating CD8+T cells are positive for PD-1, which has previously been shown to be indicative for chronic antigen stimulation and T-cell exhaustion (9).

Although studies reported a negative impact of B7-H1 on the overall survival of patients with either renal or esophageal cancer (15, 16), we did not observe such a direct link between B7-H1 and survival in cervical cancer. A direct effect of B7-H1 on survival likely requires that this survival is associated with CD8+ T-cell infiltration and that these CD8+ T cells express PD-1. Indeed, in esophageal cancer, the infiltration with CD8+ T cells is an independent prognostic factor (21). It will be of interest to determine the proportion of PD-1 expressing CD8+ T cells in renal and esophageal cancer for a better assessment of their role in the observed negative association between B7-H1 and survival in these cancers. The fact that, in cervical cancer, the ratio between CD8+ T cells and coinfiltrating regulatory T cells functions as an independent prognostic factor (8) suggests that the interplay between the two cell types and, as such, the regulation of both cell types is important for survival in cervical cancer. In this view, we made an interesting observation that, among cervical cancer patients whose tumors were infiltrated with relatively higher number of regulatory T cells (low CD8:Foxp3 ratio), tumor-expressed B7-H1 may have conferred a survival benefit (Fig. 2C). Although the underlying mechanism remains to be elucidated, our data provide evidence that it may involve the functional impairment of regulatory T cells because a substantial portion of these cells express the receptor PD-1 *in situ* (Table 3, Fig. 3) and engagement of this receptor through B7-H1 decreases the capacity of an HPV16-specific CD4+Foxp3+ regulatory T-cell clone to suppress the function of effector cells *in vitro* (Fig. 3D). The absence or presence of B7-H1 in the group of patients with a high CD8+/regulatory T-cell ratio did not affect the overall survival (Fig. 2B), suggesting either that B7-H1 does not play a role in patients with a high number of tumor-infiltrating CD8+ T cells and a low number of regulatory T cells or that the stimulatory interaction between B7-H1 and B7.1 on CD8+PD-1- T cells (22) balances the negative interaction between B7-H1 and PD-1+ CD8 T cells within the local tumor environment. It seems, therefore, that B7-H1–mediated impairment of PD-1+ T cells particularly affects the PD-1–expressing regulatory T cells, thereby releasing the brake on the tumor-specific CD8+ T cells. Future experiments using primary tumor-infiltrating T-cell cultures may shed more light on this. Recent studies support the notion that PD-1 expression on regulatory T cells is associated with improved survival (22) and B7-H1–mediated inhibition of proliferation and function of regulatory T cells (23).

From a clinical standpoint, this study represents an unselected series of patients. Given the interest to target PD-1 or B7-H1 for the immunotherapy of cancer, our study suggests that

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treatment with PD-1– or B7-H1–blocking antibodies is a viable option. Although we have studied PD-1 expression in patients with stage IB or stage II disease, PD-1– or B7-H1–targeted immunotherapy is likely to be applied in a more advanced stage of cervical cancer. Although we speculate that PD-1 expression will be even more pronounced in advanced stage of cervical cancer, it will be of interest to determine PD-1 expression on T cells at this stage too. The PD-1–B7-H1 pathway also plays an important role in dendritic cell–T-cell interactions (9). One can easily envisage that B7-H1+ dendritic cell, cross-presenting tumor antigen (e.g., E6 and E7 of HPV), may impair the function of responding PD-1+ tumor–specific T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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