CD83⁺ dendritic cells and Foxp3⁺ regulatory T cells in primary lesions and regional lymph nodes are inversely correlated with prognosis of gastric cancer

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Abstract

Background Dendritic cells (DCs) are potent antigen-presenting cells that are central to the regulation, maturation, and maintenance of the cellular immune response against cancer. In contrast, CD4⁺CD25⁺ regulatory T cells (Tregs) play a central role in self-tolerance and suppress antitumor immunity. In this study, we investigated the clinical significance of mature CD83⁺ DCs and Foxp3⁺ Tregs in the primary tumor and regional lymph nodes from the viewpoint of the two opposing players in the immune responses.

Methods We investigated, immunohistochemically, the density of CD83⁺ DCs and Foxp3⁺ Tregs in primary lesions of gastric cancer ($n = 123$), as well as in regional lymph nodes with ($n = 40$) or without metastasis ($n = 40$).
Results Decreased density of CD83⁺ DCs and increased density of Foxp3⁺ Tregs were observed in the primary tumor and metastatic lymph nodes. Density was significantly correlated with certain clinicopathological features. Poor prognosis was observed in patients with a low density of CD83⁺ DCs and a high density of Foxp3⁺ Tregs in primary lesions. For patients with metastatic lymph nodes, the density of CD83⁺ DCs in negative lymph nodes was found to be an independent prognostic factor by multivariate analysis.

Conclusion The density of CD83⁺ DCs and Foxp3⁺ Tregs was inversely correlated with tumor progression and reflected the prognosis of gastric cancer.

Keywords Gastric cancer · Dendritic cells · Regulatory T cells · Prognosis

Introduction

Dendritic cells (DCs), as professional antigen-presenting cells (APCs), play an important role in the antitumor immune response. DCs take up tumor-associated antigen in tumor tissue and migrate to regional lymph nodes to generate cytotoxic T cells. The antigen/pathogen induces the immature DCs to undergo phenotypic and functional changes that culminate in the complete transition from antigen-capturing cells to APCs. DC maturation is intimately linked with their migration from the peripheral tissue to the draining lymphoid organs [1]. CD83 is a 45-kDa transmembranous protein and member of the immunoglobulin super family [2], and is expressed by mature DCs, but not by B cells, natural killer (NK) cells, or monocytes [3], and can be a useful marker of mature DCs [4–6]. Previous studies have shown that a low density of CD83⁺ DCs in various cancer tissues is associated with poor prognosis [7–12]. However, regarding DC activation, it is not clear what kind of immune responses occur in the draining lymph nodes.

In contrast to the activation of immune responses initiated by DCs, the immune response in tumors is suppressed by CD4⁺CD25⁺ regulatory cells (Tregs) [13–16], which play an important role in immunological self-tolerance [17, 18]. Tregs have also been shown to contribute to cancer-related immunosuppression [19, 20]. Recently, it has been reported that Foxp3, forkhead/winged helix transcription

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factor, is a reliable marker of Tregs [21–23], and immunostaining using specific antibody makes it possible to define Tregs more strictly as CD4⁺CD25⁺Foxp3⁺ cells. Increased density of Foxp3⁺ Tregs in cancer tissue has been seen in various cancers, including lung, liver, pancreas, ovary, and head and neck [24–28]. In a recent study, Mizukami et al. [29] reported that the localization pattern of Tregs was associated with clinical behavior in gastric cancer. The density of Foxp3⁺ Tregs in relation to prognosis remain controversial, and there is a question whether infiltrating Tregs, in particular Foxp3⁺ Tregs, are related to clinical outcome [30].

In this study, we focused on the density of CD83⁺ DCs and Foxp3⁺ Tregs in primary tumors and regional lymph nodes, from the viewpoint of two opposing factors in immune responses. We showed that the density of each cell type was inversely correlated with tumor progression and metastasis in the prognosis of patients with gastric cancer.

Patients, materials, and methods

Patients

We enrolled 123 patients (89 men, 34 women; age range 18–86 years, mean 66.9 years), who had undergone gastrectomy in the Fukushima Medical University Hospital between January 2000 and December 2004, and who had histologically proven gastric carcinoma. The follow-up period was from 2000 to 2009. Tumor characteristics were determined according to the *Japanese classification of gastric carcinoma* (JCGC) [31] and are listed in Table 1. The depth of tumor invasion (T category) and extent of lymph node metastasis (N category) were determined by histological diagnosis. There were 62 T1 tumors, 32 T2, 23 T3, and six T4. Lymph nodes were involved in 42 patients (N1 in 23, N2 in 12, and N3 in seven). Consequently, tumor stage was determined as IA in 55 patients, IB in 25, II in 10, IIIA in nine, IIIB in four, and IV in 20. Forty-two patients were lymph node-positive. The positive and negative lymph nodes were examined in 40 patients after excluding two that had all positive lymph nodes. In the 40 lymph-node-negative patients, regional lymph nodes were also examined. Patients with other malignant lesions upon preoperative clinical diagnosis were excluded from the examination. In addition, normal gastric tissue from 30 patients with early gastric cancer, taken far from the cancerous lesion in the same section, was used as a control.

Outcomes in patients with gastric cancer

Relapses occurred in 10 patients who had received curative surgery, because of the recurrence of peritoneal metastasis

($n = 6$), hepatic metastasis ($n = 3$), and lymph node metastasis ($n = 1$). Causes of death in the patients, including those with non-curative operations, were peritoneal metastasis ($n = 9$), hepatic metastasis ($n = 5$), and other diseases ($n = 10$). Patients who underwent non-curative surgery received postoperative chemotherapy using S-1, cisplatin, paclitaxel, or irinotecan, according to the choice of the physician.

Immunohistochemistry

Immunohistochemical reactions were performed using the streptavidin–biotin–peroxidase method. Anti-mouse CD83 monoclonal antibody (mAb) (clone HB15a; Santa Cruz Biotechnology, Heidelberg, Germany) and anti-mouse Foxp3 mAb (clone 236A/E7; Abcam, Cambridge, UK) were used. Tissue specimens including the deepest lesions of the primary tumor and lymph nodes were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 4- μ m thicknesses, deparaffinized, and rehydrated. After the blocking of endogenous peroxidase with methanol containing 0.3% H₂O₂, for antigen retrieval, the sections were autoclaved at 121°C for 10 min in citrate buffer (10 mmol/l sodium citrate, pH 6.0) for staining with anti-Foxp3 mAb. The sections were boiled in a microwave oven for 15 min for staining with anti-CD83 mAb. After blocking with normal horse serum for Foxp3 or normal goat serum for CD83, the sections were reacted overnight with appropriately diluted primary antibodies. The sections were reacted sequentially with biotin-conjugated anti-mouse IgG antibodies (Vector Laboratories, Burlingame, CA, USA) and Vectastain Elite ABC reagent (Vector Laboratories). Immunohistochemical reactions were visualized with freshly prepared 3,3'-diamino-benzidine tetrahydrochloride. Slides were counterstained with hematoxylin and mounted on coverslips.

Quantification methods

Positively stained cells in normal gastric tissue, primary tumors, and lymph nodes were counted with a microscope (Nikon, Tokyo, Japan) at high power (400 $\times$) magnification. The tumor status of primary lesions and lymph nodes was determined by hematoxylin and eosin staining. We assessed each slide at the invasive margin of the tumor. We selected group 1 lymph nodes with and without metastasis classified by JCGC, and the normal gastric tissues in the patients with early gastric cancer were selected more than 1 cm away from carcinoma tissues. In each section, the degree of immune cell infiltration was observed in more than 10 independent high-power microscopic fields. The five areas with the most abundant distribution were selected without any previous knowledge of the patients'

Table 1 Patients' clinical characteristics

Clinicopathological factor ^a	No. of patients (<i>n</i> = 123)
Gender	
Male	89
Female	34
Age (years)	66.9 ± 11.4 ^b
Range	18–86
Depth of tumor invasion	
T1	62
T2	32
T3	23
T4	6
Extent of lymph node metastasis	
N0	81
N1	23
N2	12
N3	7
Histological type	
Tubular (well/moderate)	67
Papillary	5
Poor	27
Signet	17
Mucinous	7
Histological stage	
IA	55
IB	25
II	10
IIIA	9
IIIB	4
IV	20

Well/moderate well-differentiated/moderately differentiated, *poor* poorly differentiated, *signet* signet ring cell

^a Determined according to the *Japanese classification of gastric carcinoma* [31]

^b Mean ± standard deviation

clinical backgrounds. We determined the total number of DCs and Foxp3⁺ Tregs from five areas and then calculated the mean number for analysis of survival. Patients were divided into two groups for each variable, using median values as the cut-off points.

Statistical analysis

Statistical analysis was performed using StatView V.5.0 software (SAS Institute, Cary, NC, USA). Associations among the variables were assessed by the Mann–Whitney *U*-test and Kruskal–Wallis test. Survival curves were calculated using the Kaplan–Meier method and compared with the log-rank test. Both univariate and multivariate

analyses of immune cell infiltration and clinicopathological features were performed using the Cox proportional hazards model. *P* < 0.05 was regarded as significant in all analyses.

Results

Immunohistochemical analysis of CD83⁺ DCs and Foxp3⁺ Tregs in gastric cancer tissues and lymph nodes

CD83⁺ DCs and Foxp3⁺ Tregs were specifically visualized immunohistochemically and quantified in normal gastric tissues, primary gastric cancers, and lymph nodes with or without metastasis (Fig. 1). The density of CD83⁺ DCs decreased with cancer invasion; conversely, that of Foxp3⁺ Tregs increased (Fig. 2a, b). CD83⁺ DC infiltration in primary lesions was counted as 0–8 cells per high-power field (average 1.3), and Foxp3⁺ Tregs as 0–64 (average 15.4). By contrast, CD83⁺ DCs in normal gastric tissue were significantly higher in number than those in cancer tissue (average 3.4 vs. 1.3, *p* < 0.0001). The number of Foxp3⁺ Tregs in primary lesions was significantly lower than that in normal tissue (average 15.4 vs. 2.2, *p* < 0.0001). The numbers of CD83⁺ DCs and Foxp3⁺ Tregs were inversely correlated. The number of CD83⁺ DCs in positive lymph nodes was significantly lower than that in negative nodes in lymph node metastasis-negative [LNM(–)] cases, as well as in LNM(+) cases (average 1.1 vs. 3.5, 4.3, *p* < 0.001). Conversely, the number of Foxp3⁺ Tregs in positive nodes was significantly higher than that in negative nodes in LNM(–) cases, as well as in LNM(+) cases (average 37.6 vs. 16.5, 18.3, *p* < 0.001). There was no significant difference between the number of CD83⁺ DCs and Foxp3⁺ Tregs in the negative lymph nodes of LNM(–) and LNM(+) cases.

Correlation between CD83⁺ DCs, Foxp3⁺ Tregs, and depth of tumor invasion

The density of CD83⁺ DCs and that of Foxp3⁺ Tregs in the primary lesion were compared at different depths of tumor invasion (Fig. 2c, d). The average number of CD83⁺ DCs in normal gastric tissue, and in T1, T2, T3, and T4 tumors was 3.4, 1.8, 1.3, 0.46, and 0.43, respectively. The corresponding number of Foxp3⁺ Tregs was 2.2, 10.6, 22.9, 17.2, and 17.3, respectively. There was a significant difference between normal tissue and T1 tumors for the numbers of CD83⁺ DCs and Foxp3⁺ Tregs (*p* < 0.0001). A significant difference was also noted among T1, 2, 3, and 4 for CD83⁺ DCs and Foxp3⁺ Tregs (*p* < 0.0001).

Fig. 1 Presence of CD83⁺ and Foxp3⁺ cells in gastric cancer. Immunohistochemical study of CD83⁺ and Foxp3⁺ cell recruitment was performed in normal gastric (a–c), cancerous (d–f), and negative (g–i) and positive (j–l) lymph node tissues. H&E stain (a, d, g, and j); CD83⁺ cells (b, e, h, and i); and Foxp3⁺ cells (c, f, i, and l). Arrows indicate positive cells. In normal gastric tissue, the density of CD83⁺ cells was high (b) and that of Foxp3⁺ cells was low (c). In contrast, in cancerous tissue, low dendritic cell (DC) infiltration (e) and a large population of Foxp3⁺ cells (f) were observed. In negative lymph nodes, the density of CD83⁺ cells was high (h) and that of Foxp3⁺ cells was low (i). In contrast, in positive lymph nodes, these densities were inversely correlated (k, l)

Correlation between CD83⁺ DCs, Foxp3⁺ Tregs, and clinicopathological factors

The density of CD83⁺ DCs and that of Foxp3⁺ Tregs in primary lesions were examined in relation to clinicopathological status (Table 2). The numbers of CD83⁺ DCs were significantly lower in patients with histologically undifferentiated-type tumors, positive LNM, hepatic metastasis, peritoneal invasion, positive peritoneal washing cytology, lymphatic vessel invasion, blood vessel invasion, greater depth of invasion, and higher histological stage. The numbers of Foxp3⁺ Tregs were significantly higher in patients with positive LNM, peritoneal invasion, lymphatic vessel invasion, blood vessel invasion, greater depth of invasion, and higher histological stage, but there was no significant difference in numbers of Foxp3⁺ Tregs in relation to histologically undifferentiated tumors, hepatic metastasis, and positive peritoneal washing cytology.

Univariate and multivariate analyses of survival in gastric cancer

We examined our results by univariate and multivariate analyses, using a Cox proportional hazards model. The clinicopathological factors listed in Table 2 were examined for their association with overall survival (OS) and disease-free survival (DFS) after initial surgery. In primary lesions, low numbers of CD83⁺ DCs (Table 3a; OS: $p = 0.0122$, DFS: $p = 0.0016$) and high numbers of Foxp3⁺ Tregs (OS: $p = 0.0222$, DFS: $p = 0.0250$) had a negative influence on OS and DFS in the univariate analysis. In negative lymph nodes in LNM(+) cases, low numbers of CD83⁺ DCs also had a negative influence on OS and DFS in the univariate analysis (Table 3b; OS: $p = 0.0398$, DFS: $p = 0.0266$). In multivariate Cox proportional hazard analysis for clinicopathological variables, the high-CD83⁺ DC group in primary lesions had a better prognosis than the low-CD83⁺

Fig. 2 Density of CD83⁺ DCs and Foxp3⁺ Tregs in gastric cancer and correlation of these densities with depth of cancer invasion. Numbers of CD83⁺ DCs and Foxp3⁺ regulatory T cells (Tregs) in primary lesions and lymph nodes (**a, b**) were examined and the number of these cells was examined in relation to the depth of tumor invasion (**c, d**). The density of CD83⁺ DCs decreased with cancer invasion; conversely, that of Foxp3⁺ Tregs increased in primary lesions, as well as in lymph nodes (**a, b**). The density of CD83⁺ DCs and Foxp3⁺ Tregs in primary lesions was inversely correlated with the depth of tumor invasion (**c, d**). # $p < 0.0001$ by Mann–Whitney test, * $p < 0.0001$ by Kruskal–Wallis test. LNM lymph node metastasis, hpf high-power field

DC group [Table 3a; OS: $p = 0.0374$, hazard ratio (HR) = 0.33, DFS: $p = 0.0118$, HR = 0.57]. The high number of CD83⁺ DCs in negative lymph nodes in LNM(+) cases was identified as a significant prognostic predictor (Table 3b; OS: $p = 0.0296$, HR = 0.11, DFS: $p = 0.0078$, HR = 0.03).

Prognostic significance of CD83⁺ DCs and Foxp3⁺ Tregs in gastric cancer

We examined whether the presence of CD83⁺ DCs and Foxp3⁺ Tregs was correlated with prognosis, by Kaplan–Meier analysis. The high number of infiltrating CD83⁺

DCs contributed greatly to both OS and DFS (Fig. 3a, b). The low-Foxp3⁺ Tregs group showed significantly better OS than the high-Foxp3⁺ Tregs group (Fig. 3c). The low-Foxp3⁺ Tregs group also showed significantly better DFS than the high-Foxp3⁺ Tregs group (Fig. 3d).

When the prognostic significance of CD83⁺ DCs and Foxp3⁺ Tregs was compared among four groups according to the respective parameters (CD83 low/high, Foxp3 low/high), the survival rate in patients with low-CD83/high-Foxp3 group was significantly poorer than that in the other three groups (Fig. 3e). This was also the case with the DFS (Fig. 3f). These results indicate that each cell type is inversely correlated with tumor progression and metastasis

Table 2 Correlation between CD83, Foxp3, and clinicopathological status

	All patients (<i>n</i> = 123)	CD83/hpf	<i>p</i> value	Foxp3/hpf	<i>p</i> value
Gender					
Male	89	1.52 ± 1.2	NS	15.8 ± 12	NS
Female	34	1.27 ± 1.2		14.3 ± 13	
Age (years)					
<60	27	1.05 ± 1.0	NS	15.2 ± 11	NS
≥60	96	1.42 ± 1.2		15.4 ± 13	
Histology					
Differentiated ^a	72	1.66 ± 1.3	0.0003	14.2 ± 12	NS
Undifferentiated ^b	51	0.88 ± 0.9		17.1 ± 12	
Lymph node metastasis					
Negative	81	1.75 ± 1.2	<0.0001	14.2 ± 13	0.0063
Positive	42	0.55 ± 0.6		17.7 ± 10	
Hepatic metastasis					
Absent	116	1.40 ± 1.2	0.0041	15.3 ± 12	NS
Positive	7	0.31 ± 0.4		16.1 ± 8.9	
Peritoneal invasion					
Absent	112	1.43 ± 1.2	0.0007	14.9 ± 12	0.0377
Positive	11	0.35 ± 0.3		20.4 ± 9.6	
Cytology in peritoneal washings					
Negative	115	1.40 ± 1.2	0.0196	15.2 ± 13	NS
Positive	8	0.48 ± 0.43		18.1 ± 6.2	
Lymphatic vessel invasion					
Negative	47	1.94 ± 1.3	<0.0001	11.3 ± 12	<0.0001
Positive	76	0.97 ± 0.9		17.9 ± 12	
Blood vessel invasion					
Negative	54	1.70 ± 1.2	0.0005	10.9 ± 10	<0.0001
Positive	69	1.06 ± 1.1		18.9 ± 12	
Depth of invasion					
T1	62	1.77 ± 1.2	<0.0001	10.7 ± 11	<0.0001
T2–4	61	0.90 ± 0.9		20.2 ± 12	
Histological stage					
I, II	90	1.68 ± 1.2	<0.0001	14.4 ± 13	0.0083
III, IV	33	0.39 ± 0.4		18.0 ± 8.7	

Data are means ± SD

p value by Mann–Whitney *U*-testNS not significant, *hpf* high-power field^a Differentiated; papillary or tubular adenocarcinoma^b Poorly differentiated or undifferentiated adenocarcinoma, signet-ring-cell carcinoma, or mucinous adenocarcinoma

in the prognosis of patients with gastric cancer. When the prognostic significance of CD83⁺ DCs and Foxp3⁺ Tregs in negative lymph nodes in gastric cancer with LNM was examined, the density of CD83 in non-metastatic lymph nodes was found to be associated with prognosis (Fig. 4). The high number of infiltrating DCs contributed greatly to both OS and DFS.

Discussion

Two opposing components of immune responses were examined in primary gastric cancer and regional lymph nodes. CD83⁺ DCs were decreased in proportion to the stage of cancer (i.e., the lower the CD83⁺ DC density, the higher the stage), and a low density was significantly

Table 3 Univariate and multivariate analyses of prognostic factors associated with OS and DFS

Prognostic factors	OS			DFS		
	Univariate (<i>p</i>)	Multivariate (<i>p</i>)	HR (95% CI)	Univariate (<i>p</i>)	Multivariate (<i>p</i>)	HR (95% CI)
(a) All cases						
Gender (male or female)	0.4294			0.4400		
Age (<60 years)	0.5539			0.4727		
Lymphatic vessel invasion	0.0864			0.0206	0.2768	0.27 (0.03–2.87)
Blood vessel invasion	0.1453			0.0212	0.7457	0.75 (0.13–4.34)
Histology (differentiated or undifferentiated)	0.0826			0.0007	0.0296	0.21 (0.05–0.86)
Cytology	<0.0001	<0.0001	0.57 (0.02–0.20)	0.0015	0.1547	0.37 (0.09–1.46)
Hepatic metastasis	0.0424	0.6020	0.60 (0.17–2.20)	0.0039	0.6116	0.72 (0.19–2.57)
pT1 vs. pT2–4	0.0420	1.1940	1.19 (0.27–5.34)	0.0029	0.0350	0.04 (0.002–0.80)
CD83 (high or low)	0.0122	0.0374	0.33 (0.12–0.95)	0.0016	0.0118	0.57 (0.006–0.54)
Foxp3 (high or low)	0.0222	0.2257	2.10 (0.63–6.93)	0.0250	0.5234	0.64 (0.16–2.51)
(b) LNM (+) cases						
Gender (male/female)	0.4864			0.2839		
Age (<60 years)	0.3428	0.0335	12.59 (1.22–130.01)	0.8877		
Lymphatic vessel invasion	–			–		
Blood vessel invasion	0.7588			0.8847		
Histology (differentiated or undifferentiated)	0.5693			0.0476	0.0059	0.004 (0.001–0.20)
Cytology	0.0049	0.0019	0.01 (0.001–0.20)	0.4475		
Hepatic metastasis	0.5693			0.4755		
pT1 vs. pT2–4	0.4472			–		
CD83 LNM (+), node-positive	0.2356			0.2093		
CD83 LNM (+), node-negative	0.0398	0.0296	0.11 (0.01–0.80)	0.0266	0.0078	0.03 (0.002–0.39)
Foxp3 LNM (+), node-positive	0.8072			0.4657		
Foxp3 LNM (+), node-negative	0.5308			0.4121		

OS overall survival, DFS disease-free survival, HR hazard ratio, CI confidence interval, LNM lymph node metastasis

correlated with poor prognosis. Conversely, Foxp3⁺ Tregs were increased in proportion to the stage of cancer (i.e., the higher the Foxp3⁺ Tregs density, the higher the stage), and a high density was significantly correlated with poor prognosis. The density of CD83⁺ DCs and Foxp3⁺ Tregs in metastatic lymph nodes was similar to that in primary lesions, when compared with that in negative lymph nodes. This is, to the best of our knowledge, the first study to show that two opposing factors in the immune response in primary lesions, as well as in metastatic lymph nodes, were inversely related.

Immature DCs with a protein S100 marker, as well as mature DCs with a CD83 marker, have been shown to be correlated with the prognosis of various cancers [32–36]. Tsujitani et al. [32] have reported that infiltrating S100-positive DCs do not prevent the spread of tumor invasion but do prevent nodal involvement. In the present study, the high number of infiltrating DCs contributed to both OS and DFS in negative lymph nodes in gastric cancer with LNM.

Tumor-infiltrating DCs exhibit various phenotypes and functions depending on their maturation status. The expression of S100 on DCs is not specific; therefore, we evaluated maturation-specific tissue infiltration of DCs. We found that the density of CD83⁺ DCs was significantly decreased in T1 tumors (tumor invasion within the sub-mucosal layer), and it decreased further in relation to tumor progression. The density of CD83 in primary lesions was a highly independent prognostic factor by both univariate and multivariate analyses. These data were consistent with earlier studies.

The density of Foxp3⁺ Tregs in gastric cancer was significantly correlated with prognosis in the present study. A significant increase in the density of these cells was found in T1 tumors, and the density of these cells was further increased in relation to tumor progression. Mizukami et al. [29] have reported that the pattern of localization of Foxp3⁺ Tregs, rather than the population of these cells counted in randomly selected areas, is associated with

Fig. 3 Prognostic significance of CD83⁺ DCs and Foxp3⁺ Tregs in gastric cancer. The density of CD83⁺ DCs and Foxp3⁺ Tregs in primary lesions was examined in relation to prognosis by Kaplan–Meier analysis (**a–f**). A high CD83⁺ DC level contributed to both overall survival (OS) (**a**) and disease-free survival (DFS) (**b**). A low Foxp3⁺ Tregs level contributed to OS (**c**) and DFS (**d**). The density of

CD83⁺ DCs and Foxp3⁺ Tregs in primary lesions was divided into four groups: high-CD83/low-Foxp3 ($n = 37$), high-CD83/high-Foxp3 ($n = 30$), low-CD83/low-Foxp3 ($n = 24$), and low-CD83/high-Foxp3 group ($n = 32$). OS and DFS in patients with the low-CD83/high-Foxp3 group were significantly shorter compared with those for the other three groups (**e, f**). p value was determined by log-rank test

clinical behavior in gastric cancer. We examined the degree of immune cell infiltration at the deepest area of tumor invasion. Our study had more stage I patients compared with that of Mizukami et al. (62/123 vs. 31/80). The

difference in these factors might explain the difference in results, but needs to be evaluated in a prospective study.

The density of CD83⁺ DCs is particularly interesting because antigen-pulsed DCs migrate to lymph nodes, in which

Fig. 4 Prognostic significance of CD83⁺ DCs in lymph nodes of gastric cancer. The density of CD83⁺ DCs in negative nodes of metastasis-positive cases was examined in relation to the prognosis of these patients. A high CD83⁺ DC level in the negative nodes of metastasis-positive cases contributed to both OS (a) and DFS (b). *p* value was determined by log-rank test

they activate certain types of T cells, which leads to cytotoxic T-lymphocyte generation. However, no data were available for CD83⁺ DCs or Foxp3⁺ Tregs in the regional lymph nodes of gastrointestinal cancer. We found that the density of CD83⁺ DCs and Foxp3⁺ Tregs in lymph nodes was similar to that in primary tumors. Thus, with metastasis, the density of CD83⁺ DCs decreased and, conversely, that of Foxp3⁺ Tregs increased. This is consistent with the previous findings of Kawaida et al. [37], who have shown that the numbers of CD4⁺CD25^{high} Tregs in the regional lymph nodes in patients with gastric cancer are significantly higher than those in control lymph nodes. In the present study, it was interesting that the density of CD83⁺ DCs in negative lymph nodes in patients with lymph node metastasis was found to be an independent prognostic factor. Although the density of CD83⁺ DCs in negative lymph nodes in patients with LNM was slightly higher than that in the negative lymph nodes of patients without metastasis, the density of CD83⁺ DCs in lymph nodes may be an independent prognostic factor in patients with LNM. A low density of CD83⁺ DCs was correlated with poor prognosis and shorter DFS. This might have resulted from a decreased systemic response in the patients or the possibility of micro-metastasis in the lymph nodes. This is believed to be the first study to show a correlation between the immune response in lymph nodes and the prognosis of gastric cancer. However, further clarification of this correlation, as well as the concept of sentinel lymph nodes, is needed in future studies.

DC maturation is a continuous process that is initiated in the periphery upon antigen encounter and/or by inflammatory cytokines, and is completed during DC-T cell interaction. Numerous factors induce and/or regulate DC maturation, including pathogen-related molecules such as lipopolysaccharide, bacterial DNA, and the balance between pro- and anti-inflammatory signals in the local microenvironment, including tumor necrosis factor, interleukin (IL)-1, IL-6, IL-10, and transforming growth factor (TGF)- β [1]. Cancer cells are known to produce certain cytokines such as IL-6, IL-10, TGF- β , and vascular endothelial growth factor, which do suppress DC maturation.

CD4⁺CD25^{high} Foxp3⁺ T cells also secrete IL-10 and TGF- β , which both mediate suppression of immune responses in the tumor microenvironment [38]. Recently, Sarrs et al. [39] have found that neuropilin-1, which is expressed by most Tregs, but not naïve T-helper cells, promotes prolonged interaction with immature DCs, which suggests the default suppression of immune responses in the absence of danger signals. Terme et al. [40] have also reported that Tregs control DC/NK cell crosstalk in lymph nodes in the steady state by inhibiting CD4⁺ self-reactive T cells. In line with these data, it is reasonable to assume that a high density of Foxp3⁺ Tregs plays an important role, directly or indirectly, in inhibiting the local activation of DCs.

We elucidated two arms of the immune response to cancer: immunoactivation and immunosuppression. For these immune responses, CD83⁺ DCs and Foxp3⁺ Tregs were investigated in primary lesions and regional lymph nodes. Similar trends were found at both sites in association with tumor invasion. For cell-targeted immunotherapy, DCs can be injected directly into the tumor. The elimination of Tregs has been trialed in basic [41, 42] and clinical [43] settings, with some promising results [44]. To further investigate the efficacy of the elimination of Tregs, careful monitoring of the two arms of the immune response will be needed.

References

1. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol*. 2000;18:767–811.
2. Zhou LJ, Schwalling R, Smith HM, Tedder TF. A novel cell-surface molecule expressed by human interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily. *J Immunol*. 1992;149:735–42.
3. Zhou LJ, Tedder TF. Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J Immunol*. 1995;154:3821–35.
4. Bell D, Chomarat P, Broyles D, et al. In breast carcinoma tissue, immature dendritic cells are located in peritumoral areas. *J Exp Med*. 1999;190:1417–25.

5. Zhou L, Tedder TF. CD14⁺ blood monocytes can differentiate into functionally mature CD83⁺ dendritic cells. *Immunology*. 1999;93:2588–92.
6. Thurner M, Radmayr C, Ramoner R, et al. Human renal-cell carcinoma tissue contain dendritic cells. *Int J Cancer*. 1996;68:1–7.
7. Tsukayama S, Omura K, Yoshida K, Tanaka Y, Watanabe G. Prognostic value of CD83 positive mature dendritic cells and their relation to vascular endothelial growth factor in advanced human gastric cancer. *Oncol Rep*. 2005;14:369–75.
8. Saito H, Osaki T, Murakami D, et al. Prediction of sites of recurrence in gastric carcinoma using immunohistochemical parameters. *J Surg Oncol*. 2007;95:123–8.
9. Naito Y, Saito K, Shibata K, et al. CD8⁺ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res*. 1998;58:3491–4.
10. Furihata M, Ono Y, Ichikawa K, Tomita S, Fujimori T, Kubota K. Prognostic significance of CD83 positive, mature dendritic cells in the gallbladder carcinoma. *Oncol Rep*. 2005;14:353–6.
11. Miyagawa S, Soeda J, Takagi S, Miwa S, Ichikawa E, Noike T. Prognostic significance of mature dendritic cells and factors associated with their accumulation in metastatic liver tumors from colorectal cancer. *Hum Pathol*. 2004;35:1392–6.
12. Iwamoto M, Shinohara H, Miyamoto A, et al. Prognostic value of tumor-infiltrating dendritic cells expressing CD83 in human breast carcinomas. *Int J Cancer*. 2003;104:92–7.
13. Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J. Identification and functional characterization of human CD4⁺CD25⁺ T cells with regulatory properties isolated from peripheral blood. *J Exp Med*. 2001;193:1285–94.
14. Ng WF, Duggan PJ, Ponchel F, et al. Human CD4⁺CD25⁺ cells: a naturally occurring population of regulatory T cells. *Blood*. 2001;98:2736–44.
15. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). *J Immunol*. 1995;155:1151–64.
16. Sakaguchi S, Ono M, Setoguchi R, et al. Foxp3⁺CD25⁺CD4⁺ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev*. 2006;212:8–27.
17. DeJaco C, Dultner C, Grubeck B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. *Immunology*. 2005;117:289–300.
18. Beyer M, Schulze JL. Regulatory T cells in cancer. *Blood*. 2006;108:804–11.
19. Viehl C, Moore T, Liyanaga U, et al. Depletion of CD4⁺CD25⁺ regulatory T cells promotes a tumor-specific immune response in pancreas cancer-bearing mice. *Ann Surg Oncol*. 2005;13:1252–8.
20. Rudge G, Barrett SP, Scott B, Driel IR. Infiltration of a mesothelioma by IFN- γ -producing cells and tumor rejection after depletion of regulatory T cells. *J Immunol*. 2007;178:4089–96.
21. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cells development by the transcription factor Foxp3. *Science*. 2003;299:1057–61.
22. Fontenot JD, Gavin MA, Rudensky A. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol*. 2003;10:1330–6.
23. Fontenot JD, Rasmussen JP, Williams M, Dooley L, Farr AG, Rudensky Y, et al. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity*. 2005;22:329–41.
24. Woo EY, Chu CS, Goletz TJ, et al. Regulatory CD4⁺CD25⁺T cells in tumors from patients with early-stage non-small cell lung cancer and last-stage ovarian cancer. *Cancer Res*. 2001;61:4766–72.
25. Badoual C, Hans S, Rodriguez J, et al. Prognostic value of tumor-infiltrating C⁺ T cells subpopulations in head and neck cancers. *Clin Cancer Res*. 2006;12:465–72.
26. Wolf D, Wolf A, Rumpold H, et al. The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. *Clin Cancer Res*. 2005;11:8326–31.
27. Kobayashi N, Hiraoka N, Yamagami W, et al. FOXP3⁺ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res*. 2007;13:902–11.
28. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3⁺ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res*. 2006;12:5423–34.
29. Mizukami Y, Kono K, Kawaguchi Y, et al. Localisation pattern of Foxp3⁺ regulatory T cells is associated with clinical behaviour in gastric cancer. *Br J Cancer*. 2008;98:148–53.
30. Siddiqui SA, Frigola X, Bonne-Annee S, et al. Tumor-infiltrating Foxp3-CD4⁺CD25⁺ T cells predict poor survival in renal cell carcinoma. *Clin Cancer Res*. 2007;13:2075–81.
31. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma, 2nd English edition. *Gastric Cancer*. 1998;1:10–24.
32. Tsujitani S, Kakeji Y, Watanabe A, Kohnoe S, Maehara Y, Sugimachi K. Infiltration of dendritic cells in relation to tumor invasion and lymph node metastasis in human gastric cancer. *Cancer*. 1990;66:2012–6.
33. Dadabayev A, Sandel M, Menon A, et al. Dendritic cells in colorectal cancer correlate with other tumor-infiltrating immune cells. *Cancer Immunol Immunother*. 2004;53:978–86.
34. Miyake M, Taki T, Hitomi S, Hakomori S. Correlation of expression of H/Ley/Leb antigen with survival in patients with carcinoma of the lung. *N Engl J Med*. 1992;327:14–8.
35. Matsuda H, Mori M, Tsujitani S, Ohno S, Kuwano H, Sugimachi K. Immunohistochemical evaluation of squamous cell carcinoma antigen and S-100 protein-positive cells in human malignant esophageal tissues. *Cancer*. 1990;65:2261–5.
36. Schwaab T, Weiss JE, Schned AR, Barth RJ Jr. Dendritic cell infiltration in colon cancer. *J Immunother*. 2001;24:130–7.
37. Kawaida H, Kono K, Takahashi A, et al. Distribution of CD4(+)CD25 high regulatory T-cells in tumor-draining lymph nodes in patients with gastric cancer. *J Surg Oncol*. 2005;124:151–7.
38. Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. A unique subset of CD4⁺CD25^{high}Foxp3⁺ T cells secreting interleukin-10 and transforming growth factor- β 1 mediates suppression in the tumor microenvironment. *Clin Cancer Res*. 2007;13:4345–54.
39. Sarrs M, Andersen KG, Randow F, Mayr L, Betz AG. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. *Immunity*. 2008;28:402–13.
40. Terme M, Chaput N, Combadiere B, Ma A, Ohteki T, Zitvogel L. Regulatory T cells control dendritic cell/NK cell cross-talk in lymph nodes at the steady state by inhibiting CD4⁺ self-reactive T cells. *J Immunol*. 2008;180:4679–86.
41. Fehervari A, Sakaguchi S. Control of Foxp3⁺CD4⁺ regulatory cell activation and function by dendritic cells. *Int Immunol*. 2004;16:1769–80.
42. Yamaguchi T, Hirota K, Nagahama K, et al. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. *Immunity*. 2007;27:145–59.
43. Schmidt J, Eisold S, Büchler MW, Märten A. Dendritic cells reduce number and function of CD4⁺CD25⁺ cells in cytokine-induced killer cells derived from patients with pancreatic carcinoma. *Cancer Immunol Immunother*. 2004;53:1018–26.
44. Van Meirvenne S, Dullaers M, Heirman C, Straetman L, Michiels A, Thielemans K. In vivo depletion of CD4⁺CD25⁺ regulatory T cells enhances the antigen-specific primary and memory CTL response elicited by mature mRNA-electroporated dendritic cells. *Mol Ther*. 2005;12:922–32.