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CD169-positive macrophages in regional lymph nodes are associated with a favorable prognosis in patients with colorectal carcinoma

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CD169 (sialoadhesin) is a sialic acid receptor that is expressed on specific macrophages such as lymph node sinus macrophages. Animal studies have suggested that CD169$^+$ macrophages have a pro-inflammatory property, however, the role of these cells in human diseases has not been clarified. In our *in vitro* experiments with human macrophages, pro-inflammatory cytokines, such as type 1 interferon, induced strong expression of CD169, suggesting that CD169 might be a specific marker of inflammatory macrophages. To examine the role of CD169 in antitumor immunity, we examined the expression of CD169 in regional lymph nodes (RLNs) and its association with overall survival in colorectal carcinoma (CRC). In a clinicopathological analysis on 83 CRC patients, paraffin-embedded specimens were evaluated for CD169 expression of RLN macrophages by immunohistochemistry. We found, for the first time, a high density of CD169$^+$ macrophages was significantly associated with longer overall survival; multivariate analysis showed that the ratio of CD169$^+$ cells to CD68$^+$ cells was an independent prognostic factor. The majority of CD169$^+$ macrophages were in direct contact with CD8$^+$ T cells expressing CD43, a major ligand of CD169. We also found that the density of CD169$^+$ macrophages had a positive correlation with the number of CD8$^+$ cytotoxic T cells infiltrating tumor tissues. These data suggest that CD169$^+$ macrophages in RLNs promote CD8$^+$ T-cell-mediated antitumor immunity and are associated with a better prognosis for CRC patients. CD169$^+$ macrophages in RLNs could be a useful marker for assessing clinical prognosis and monitoring antitumor immunity in patients with CRC. (*Cancer Sci* 2013; 104: 1237–1244)

Regional lymph nodes (RLNs) draining human malignant tumors are believed to be the first major components of the immune system to have contact with tumor cells or their products.[1] Lymph nodes (LNs) contain various types of macrophages, such as subcapsular or medullary sinus macrophages, medullary cord macrophages, and tingible body macrophages. In particular, sinus macrophages are thought to be involved in lymph-borne tumor antigen capture and activation of antigen-specific lymphocyte responses,[2,3] which suggests that sinus macrophages in RLNs are crucial components of the antitumor immune response in cancer patients.

CD169, also called sialoadhesin, is the foremost member of the sialic acid-binding lectin (Siglec) superfamily and is one of the extremely useful markers on sinus macrophages.[3–5] It binds sialylated glycoproteins including CD43 (sialophorin) and MUC1 and is involved in cell–cell adhesion as well as cell–pathogen interactions.[5–8] CD169 expression is found in splenic marginal metallophilic macrophages and in certain tissue macrophages in bone marrow, colon, liver, and lung, as well as in sinus macrophages.[5,9] Monocytes/macrophages may also be induced to express CD169 under many inflammatory conditions such as systemic lupus erythematosus,[10] systemic sclerosis,[11] glomerulonephritis,[12,13] atherosclerosis,[14,15] and acute cellular rejection after transplantation of the intestine.[16] Previous experiments with CD169-deficient mice suggested that CD169 may exacerbate an activity in experimental autoimmune encephalomyelitis through inhibition of regulatory T cell expansion.[17] These findings suggest that CD169$^+$ macrophages have pro-inflammatory functions and are phenotypically differentiated into classically activated (M1) macrophages.

On the basis of these findings, we hypothesized that CD169$^+$ RLN macrophages may be involved in the development of antitumor immunity in human malignant tumors. Previous CD169 studies were mainly carried out with rodents; only a few studies described human macrophage CD169 expression.[4,5,9] To examine the role of CD169 in human antitumor immunity, we investigated the correlation between antitumor immunity and CD169$^+$ RLN macrophages by using pathological specimens obtained from patients with colorectal carcinoma (CRC).

Materials and Methods

Macrophage culture. Peripheral blood mononuclear cells were obtained from healthy volunteer donors, who gave written informed consent for participation in this study. The cells were suspended in RPMI-1640 medium (Wako, Tokyo, Japan) supplemented with 10% FBS and penicillin–streptomycin (Wako), seeded in polystyrene culture plates, and incubated for 2 h at 37°C. Non-adherent cells were removed by gentle washing with PBS. The remaining monocytes were cultured with granulocyte macrophage-colony stimulating factor (GM-CSF) (5 ng/mL; Wako) for 5 days to induce immature macrophages.

Cell ELISA assay. We evaluated expression of CD169 in human macrophages with the aid of a cell ELISA, as described previously.[18,19] Monocytes were cultured in a 96-well plate (5×10^5 cells per well) with 5 ng/mL GM-CSF for 5 days to induce macrophage differentiation. These monocyte-derived macrophages were stimulated with the optimal concentration of: interferon (IFN)-α (50 ng/mL), IFN-β (20 ng/mL), IFN-γ (20 ng/mL), interleukin (IL)-4 (20 ng/mL), IL-6 (20 ng/mL), or IL-10 (20 ng/mL) (all from PeproTech, Rocky Hill, NJ, USA); LPS (100 ng/mL; Sigma-Aldrich, St. Louis, MO, USA); or PBS (control) for 24 h.[13,15,19] After cells were treated with an anti-CD169 antibody (clone HSn 7D2; Santa Cruz

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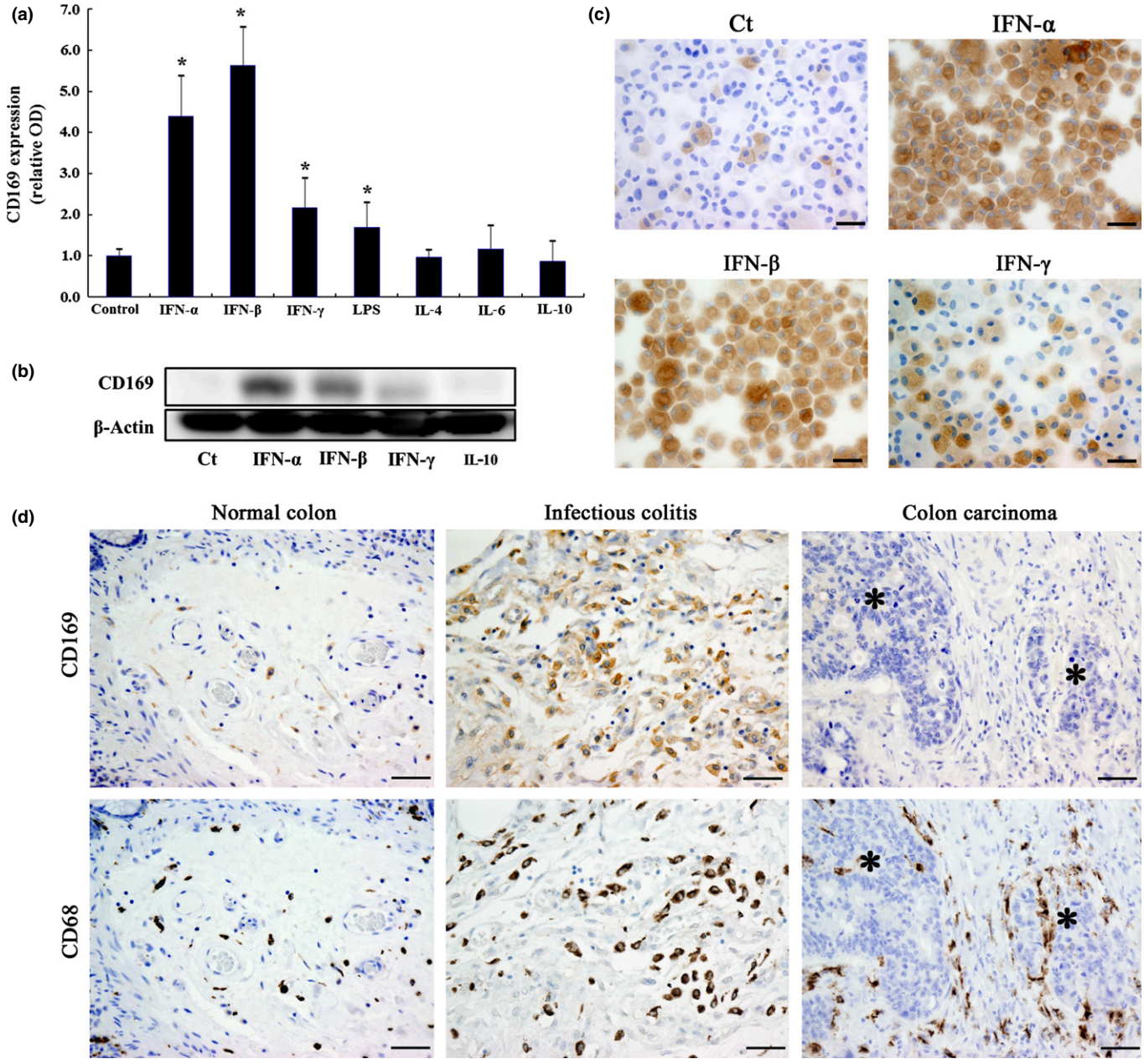

Fig. 1. CD169 expression in human macrophages. (a) CD169 expression in cultured macrophages stimulated with interferons (IFN), LPS, interleukin (IL)-4, IL-6, or IL-10 was evaluated using cell ELISA. *$P < 0.01$. CD169 expression in cultured macrophages was also examined by means of immunoblot analysis (b) and immunocytochemistry (c). (d) Immunohistochemical analysis was carried out to detect CD169 and CD68 antigens in normal colon, infectious colitis, and colorectal carcinoma tissues. Asterisks indicate tumor nests. Ct, control; OD, optical density. Scale bar = 50 μm.

Biotechnology, Santa Cruz, CA, USA) or isotype-matched control antibody (RM-4),[18,19] samples were incubated with HRP-labeled goat anti-mouse antibody (Nichirei, Tokyo, Japan). Plates were read by using a microplate reader at 450 nm to detect signals.

Western blot analysis. We used Western blotting to determine the amount of CD169 protein. Human monocyte-derived macrophages were solubilized with Triton X-100, and the protein concentration was determined by using BCA Protein Assay Reagent (Pierce, Rockford, IL, USA). The protein (20 μg) was run on 10% SDS-polyacrylamide gel and transferred to a PVDF transfer membrane (Millipore, Bedford, MA, USA). The membranes were incubated with anti-CD169 or anti-β-actin (Santa Cruz Biotechnology) antibodies with gentle agitation for 18 h at 4°C and were then visualized by using HRP-conjugated anti-mouse IgG antibody with ECL Western Blotting Detection Reagent (GE Healthcare, Chalfont St Giles, Buckinghamshire, UK). Signals were detected with an LAS-4000 image analyzer (Fujifilm, Tokyo, Japan).

Patients. In this study, we evaluated tumor and RLN specimens resected from 83 patients with CRC (43 men, 35 women; median age, 64 years, range: 29–90 years) and colon specimens resected from three patients with infectious colitis complicated by diverticulitis who had undergone operations at Kumamoto University Hospital (Kumamoto, Japan) from 2000 to 2006. All patients gave informed written consent for

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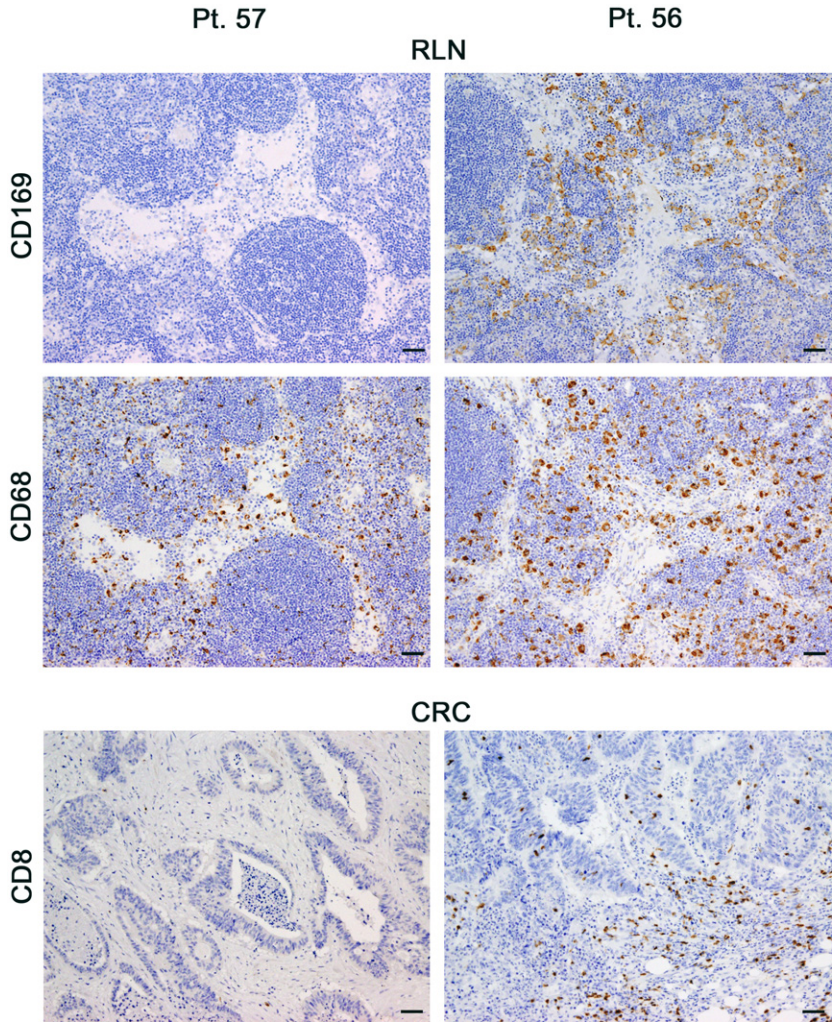

Fig. 2. Immunohistochemical analysis of $CD169^+$ and $CD68^+$ macrophages in regional lymph nodes (RLN) and $CD8^+$ cells in colorectal carcinoma (CRC) tissues. Results are shown for patient no. 57 (Pt. 57, left panels) and patient no. 56 (Pt. 56, right panels). Scale bar = 50 μm.

participation in the study, in accordance with protocols approved by the Kumamoto University Hospital Review Board.

Immunostaining and triple immunostaining. Tissue samples from CRC or RLNs were routinely fixed in 10% neutral buffered formalin and were embedded in paraffin. Monocyte-derived cultured macrophages were fixed with 2% paraformaldehyde on 8-well chamber slides (Sigma-Aldrich). Antigen retrieval was carried out as follows: sections were immersed in EDTA solution (pH 8.0), and samples were heated with a microwave for staining of CD169 and CD43 (clone DF-T1; Dako, Glostrup, Denmark) or with a pressure cooker for staining of CD68 (clone PG-M1; Dako) and CD8 (clone C8/144B; Nichirei). An isotype-matched mouse IgG (Dako) was used as a negative control. After reaction of the primary antibodies, samples were incubated with HRP-labeled goat anti-mouse antibody (Nichirei). Immunoreactions were visualized by using the diaminobenzidine substrate system (Nichirei). The numbers of $CD68^+$ and $CD169^+$ cells per mm^2 in sinus areas in RLNs without metastatic cancer cells and $CD8^+$ cells per mm^2 in intratumoral areas were counted in high power fields (0.028 mm^2 per field) by two independent pathologists (K.O. and Y.K.) who were blinded to information about the samples. The count data assessed by K.O. or Y.K. were averaged as described previously.[19]

Triple immunostaining was carried out as described previously.[20] An alkaline phosphatase-conjugated antibody (Nichirei) was used as the secondary antibody, and the reaction was visualized by using Fast Red TR Salt (for CD43 staining) or Fast Blue BB Salt (for CD8 staining) (both from Sigma-Aldrich).

Statistical analysis. Statistical analysis was carried out with JMP 10 (SAS Institute, Chicago, IL, USA). All data from *in vitro* studies represent the results of two or three independent experiments. Data were expressed as means ± SD. The Mann–Whitney *U*-test was used for two-group comparisons. Associations between different categorical variables were assessed by using ANOVA. The cumulative survival rate was compared between two groups using the log–rank test and generalized Wilcoxon test. The simultaneous relationship between multiple prognostic factors for survival was assessed with the Cox proportional hazards model with stepwise backward reduction. Multivariate analysis included adjustment for age, tumor size, clinical stage, lymphatic invasion, LN metastasis, the ratio of $CD169^+$ cells to $CD68^+$ cells in RLNs and the number of $CD8^+$ cells in tumor tissue. A *P*-value of <0.05 was considered statistically significant.

Results

CD169 expression was high in IFN-stimulated cultured macrophages. We examined CD169 expression in human macrophages under various conditions. Cell ELISA revealed elevated CD169 expression in M1 macrophages stimulated

with IFN or LPS; in particular, type 1 IFN (IFN-α and IFN-β)-stimulated macrophages showed strong CD169 expression (Fig. 1a). The increased CD169 expression after IFN treatment was also detected by immunoblot analysis and immunocytochemistry (Fig. 1b,c). This CD169 increase, however, was not detected in IL-4-, IL-6-, or IL-10-treated macrophages (Fig. 1a,b).

We next evaluated $CD169^+$ cell distribution in colon tissue by means of immunohistochemistry. Many macrophages infiltrating infectious colitis specimens were positive for CD169 as well as CD68. In contrast, $CD68^+$ macrophages infiltrating tumor tissue were completely negative for CD169 (Fig. 1d).

Number of $CD169^+$ sinus macrophages in RLNs decreased in CRC patients with LN metastasis. We next used immunohistochemistry to investigate the expression of CD169 and CD68 in samples of RLNs, obtained from CRC patients, that contained no metastatic carcinoma cells. The number of $CD169^+$ sinus macrophages varied for each case, although they were almost positive for the pan-macrophage marker CD68 (Fig. 2).

We then analyzed the correlation between clinicopathological features and the number of $CD169^+$ sinus macrophages in 83 patients with CRC. We classified two groups as CD169-low cases or CD169-high cases based on the median and compared clinical factors in each group. The number of $CD169^+$ cells per mm^2 and the ratio of $CD169^+$ cells to $CD68^+$ cells were not associated with age, sex, clinical stage, lymphatic invasion, or vascular invasion (Table 1). In contrast, the low density of $CD169^+$ cells was significantly correlated with cases with tumor enlargement, advanced T stage, or LN metastasis (Table 1). The number of $CD68^+$ sinus macrophages, however, was not associated with these clinical factors.

High density of $CD169^+$ sinus macrophages associated with good prognosis in CRC patients. The number of $CD169^+$ cells per mm^2 and the ratio of $CD169^+$ macrophages to $CD68^+$ sinus macrophages were significantly correlated with a favorable overall survival in a univariate analysis ($P = 0.0092$ and $P = 0.0012$, respectively, log–rank test) (Fig. 3a,b, Table 2). Multivariate analysis indicated that the ratio of $CD169^+$ cells to $CD68^+$ cells was an independent prognostic factor (Table 2). No correlation existed between the number of $CD68^+$ RLN macrophages and overall survival (Fig. 3c). As expected, LN metastasis in CRC patients was associated with a poor prognosis, as shown in previous studies (Fig. 3d).[21] The number of $CD169^+$ macrophages was significantly correlated with overall survival in cases with no LN metastasis ($n = 55$; $P = 0.0236$, log–rank test), but this correlation was not observed in LN metastasis-positive cases ($n = 28$; $P = 0.1836$, log–rank test) (Fig. 3e,f). The clinical stage (<stage III), tumor size <50 mm, no lymphatic invasion, and no LN metastasis were also associated with longer overall survival in the univariate analysis (Table 2). Multivariate analysis showed a significant correlation between age, tumor size, lymphatic invasion, or clinical stage and overall survival.

Density of $CD169^+$ sinus macrophages correlated positively with $CD8^+$ T cell infiltration in tumor tissue. Because M1 macrophage activation of the $CD8^+$ T-cell-mediated immune response is generally accepted,[22] we speculated that $CD169^+$ sinus macrophages interacted with $CD8^+$ T cells and induced T-cell-mediated antitumor immunity. To investigate the details of interactions between macrophages and T cells *in vivo*, we carried out triple immunostaining with mAbs for CD169, CD8, and CD43, which is a CD169 ligand.[7] Immunostaining

Table 1. Clinicopathological features and the number of macrophages in regional lymph nodes (RLNs) from 83 patients with colorectal carcinoma

Clinicopathological feature	*n*	$CD169^+$ cells/mm^2 in RLNs			$CD68^+$ cells/mm^2 in RLNs			$CD169^+$ cells/$CD68^+$ cells in RLNs		
		<150	≥150	*P*-value	<300	≥300	*P*-value	<0.6	≥0.6	*P*-value
Age, years										
<65	43	24	19	NS	20	23	NS	27	16	NS
≥65	40	21	19		21	19		20	20	
Sex										
Male	48	26	22	NS	22	26	NS	25	23	NS
Female	35	19	16		19	16		22	13	
Tumor size, mm										
<50	41	18	23	NS	17	24	NS	17	24	0.023
≥50	39	24	15		21	18		27	12	
Stage										
I, II	49	24	25	NS	25	24	NS	23	26	NS
III, IV	34	21	13		15	18		24	10	
T classification										
T1, T2	19	5	14	0.012	7	12	NS	6	13	0.034
T3, T4	64	40	24		34	30		40	24	
Lymphatic invasion										
Negative	63	34	29	NS	32	31	NS	33	30	NS
Positive	20	11	9		9	11		13	7	
Vascular invasion										
Negative	35	18	17	NS	18	17	NS	18	17	NS
Positive	48	27	21		23	25		28	20	
LN metastasis										
Negative	55	26	29	0.030	26	29	NS	26	29	0.023
Positive	28	19	9		15	13		21	7	
$CD8^+$ cells/mm^2 in tumor										
<160	42	30	12	<0.001	21	21	NS	32	10	<0.001
≥160	41	15	26		21	20		15	26	

Underlining indicates statistically significant results. LN, lymph node; NS, not significant.

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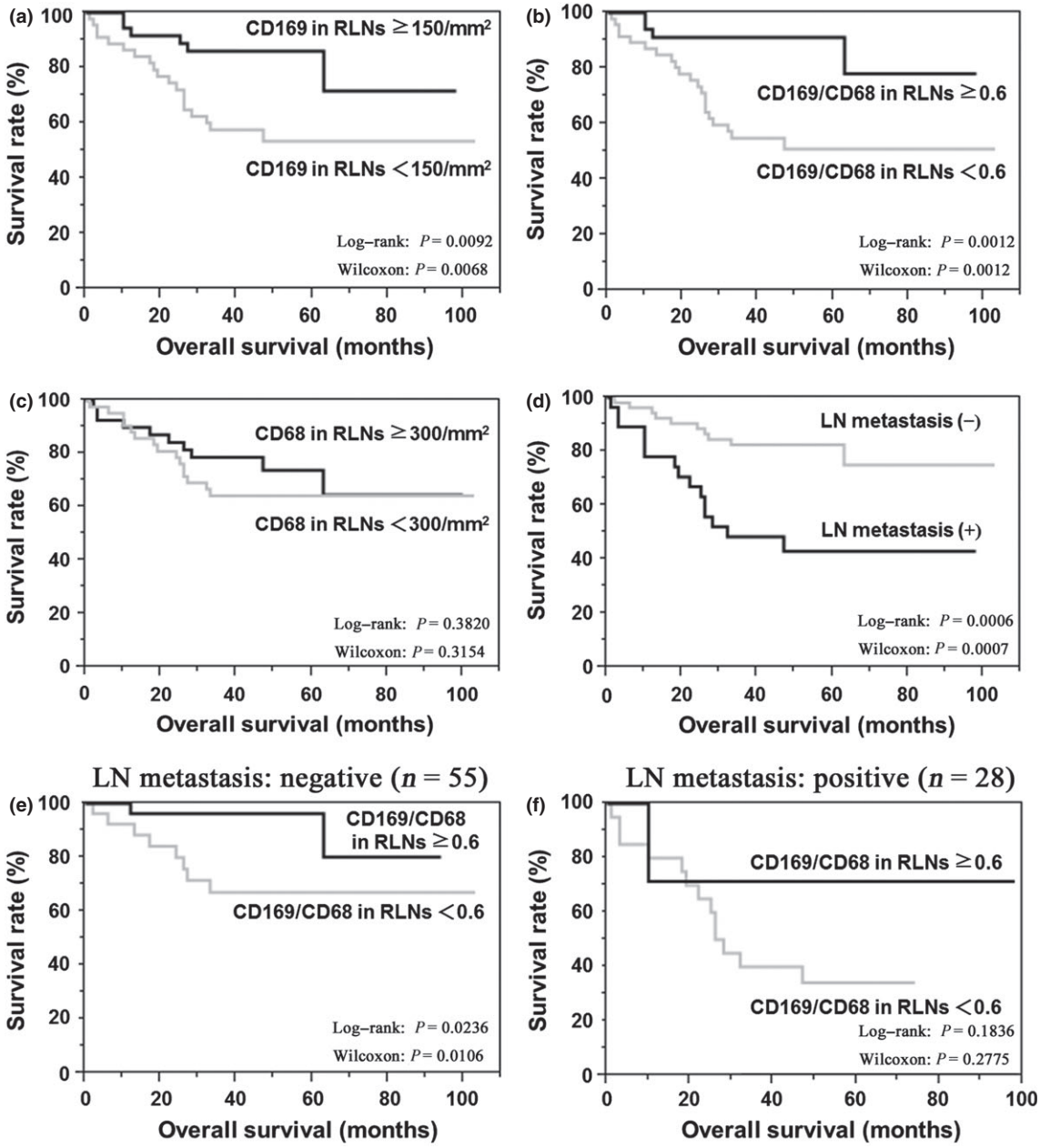

Fig. 3. Overall survival curves for patients as related to $CD68^+$ and $CD169^+$ macrophages in regional lymph nodes (RLNs). Kaplan–Meier survival curves for patients with colorectal carcinoma as related to the number of $CD169^+$ macrophages (a), the ratio of $CD169^+$ macrophages to $CD68^+$ macrophages (b), the number of $CD68^+$ macrophages (c), lymph node (LN) metastasis (d), the ratio of $CD169^+$ macrophages to $CD68^+$ macrophages in patients without LN metastasis (e), and the ratio of $CD169^+$ macrophages to $CD68^+$ macrophages in patients with LN metastasis (f).

showed that many $CD169^+$ cells had direct contact with $CD8^+$ $CD43^+$ double-positive T cells in the sinus area of RLNs (Fig. 4a).

We next analyzed the association between $CD169^+$ RLN macrophages and $CD8^+$ T cells infiltrating tumor tissues from CRC patients. The number of $CD8^+$ T cells in tumor nests and tumor stroma increased significantly when many $CD169^+$ RLN sinus macrophages were present (Fig. 2). Statistical analysis showed that the number of $CD169^+$ cells and the ratio of $CD169^+$ cells to $CD68^+$ cells correlated positively with the number of $CD8^+$ cells in tumor nest and tumor stroma (Table 1, Fig. 4b). The CRC patients with abundant $CD8^+$ T cell infiltration also had a favorable overall survival (Fig. 4c, Table 2).

Discussion

Although a number of previous studies of $CD169^+$ macrophages led to advances in understanding their distribution among various macrophages in rodents, only a few described regulation of CD169 expression in human macrophages.[4,5,9] In the present study, we showed, by means of *in vitro* experiments, high expression of CD169 in IFN-stimulated M1-type human macrophages, and we suggest that CD169 expression may be seen in exudative macrophages in human tissues. We observed CD169 expression in LN sinus macrophages and resident macrophages in the submucosa of the colon; however, our preliminary observations also showed expression of CD163, which is a specific marker of alternatively activated (M2)

Table 2. Univariate and multivariate Cox regression analyses of potential prognostic factors for overall survival in patients with colorectal carcinoma (n = 83)

Clinicopathological feature	n	Univariate analysis P-value		Multivariate analysis		
		Log–rank	Wilcoxon	HR	95% CI	P-value
Age, years						
<65	43	0.9500	0.7100	10.17	3.35–33.67	<0.0010
≥65	40					
Sex						
Female	35	0.4800	0.3900	ND	ND	ND
Male	48					
Tumor size, mm						
<50	41	0.0100	0.0047	4.46	1.59–14.42	0.0036
≥50	39					
Stage						
I, II	49	<0.001	<0.0010	25.12	5.45–128.46	<0.0010
III, IV	34					
T classification						
T1, T2	19	0.0520	0.0660	ND	ND	ND
T3, T4	64					
Lymphatic invasion						
Negative	63	<0.0010	<0.0010	3.99	1.52–10.8	0.0054
Positive	20					
Vascular invasion						
Negative	35	0.3100	0.1600	ND	ND	ND
Positive	48					
LN metastasis						
Negative	55	<0.0010	<0.0010	0.41	0.12–1.46	0.1650
Positive	28					
$CD169^+$ cells/mm^2 in RLNs						
<150	45	0.0092	0.0068	ND	ND	ND
≥150	38					
$CD68^+$ cells/mm^2 in RLNs						
<300	41	0.3800	0.3100	ND	ND	ND
≥300	42					
$CD169^+$ cells/$CD68^+$ cells in RLNs						
<0.6	47	0.0012	0.0010	0.23	0.06–0.69	0.0074
≥0.6	36					
$CD8^+$ cells/mm^2 in tumor						
<160	42	0.0120	0.0090	0.32	0.11–0.83	0.0180
≥160	41					

Underline indicates statistically significant results. CI, confidence interval; HR, hazard ratio; LN, lymph node; ND, not done; RLN, regional lymph node.

macrophages,[18–20] in these cells. Therefore, we believe that CD169 is not a marker restricted to M1 macrophages but is rather a marker of IFN-induced inflammatory macrophages. That Th1-type lymphocytes secrete IFNs is well known, so CD169 expression may be associated with inflammatory conditions in the sinus areas of LNs.

We also showed that the higher density of $CD169^+$ RLN sinus macrophages was correlated with increased numbers of cytotoxic $CD8^+$ T cells infiltrating tumor tissue and with a favorable overall survival of CRC patients. Because our present study and many previous studies[23–26] indicated a relationship between infiltrating $CD8^+$ T cells and clinical prognosis in CRC, we suggest that $CD169^+$ RLN macrophages are closely related to activation of $CD8^+$ T-cell-mediated antitumor immunity. Because the close association between infiltrating $CD8^+$ T cells and clinical prognosis was reported not only for CRC but also for other malignant tumors, including endometrial carcinoma, urothelial carcinoma, gastric cancer, breast cancer, and melanoma,[27–33] similar mechanisms related to $CD169^+$ cells and T cells may occur in RLNs.

Recently, Asano and colleagues found that $CD169^+$ LN macrophages engulfed tumor cell antigens and caused the proliferation of antigen-specific cytotoxic T cells in a tumor transplantation mouse model.[34] Barral and colleagues showed that $CD169^+$ macrophages acted as antigen-presenting cells that control invariant natural killer T cell activation in murine LNs.[35] Xiong and colleagues showed that increased CD169 expression on monocytes was related to the $CD4^+$ or $CD8^+$ T cell proliferation, and CD169 knockdown by siRNA could attenuate T cell proliferation.[15] Our study showed that $CD169^+$ macrophages had direct contact with $CD8^+$ $CD43^+$ T cells in sinus areas of RLNs, and this finding indicated that CD169–CD43 ligation in the RLN mediated cell–cell interaction between sinus macrophages and $CD8^+$ T cells. Although the detailed mechanism behind CD169–CD43 ligation and consequent $CD8^+$ T cell activation is unclear, these data suggested that $CD169^+$ macrophages played an important role in antitumor immunity in patients with CRC by presenting tumor antigens to cytotoxic lymphocytes. To address this problem, we are in the process of investigating CD169-mediated $CD8^+$ T cell proliferation by means of *in vitro* experiments.

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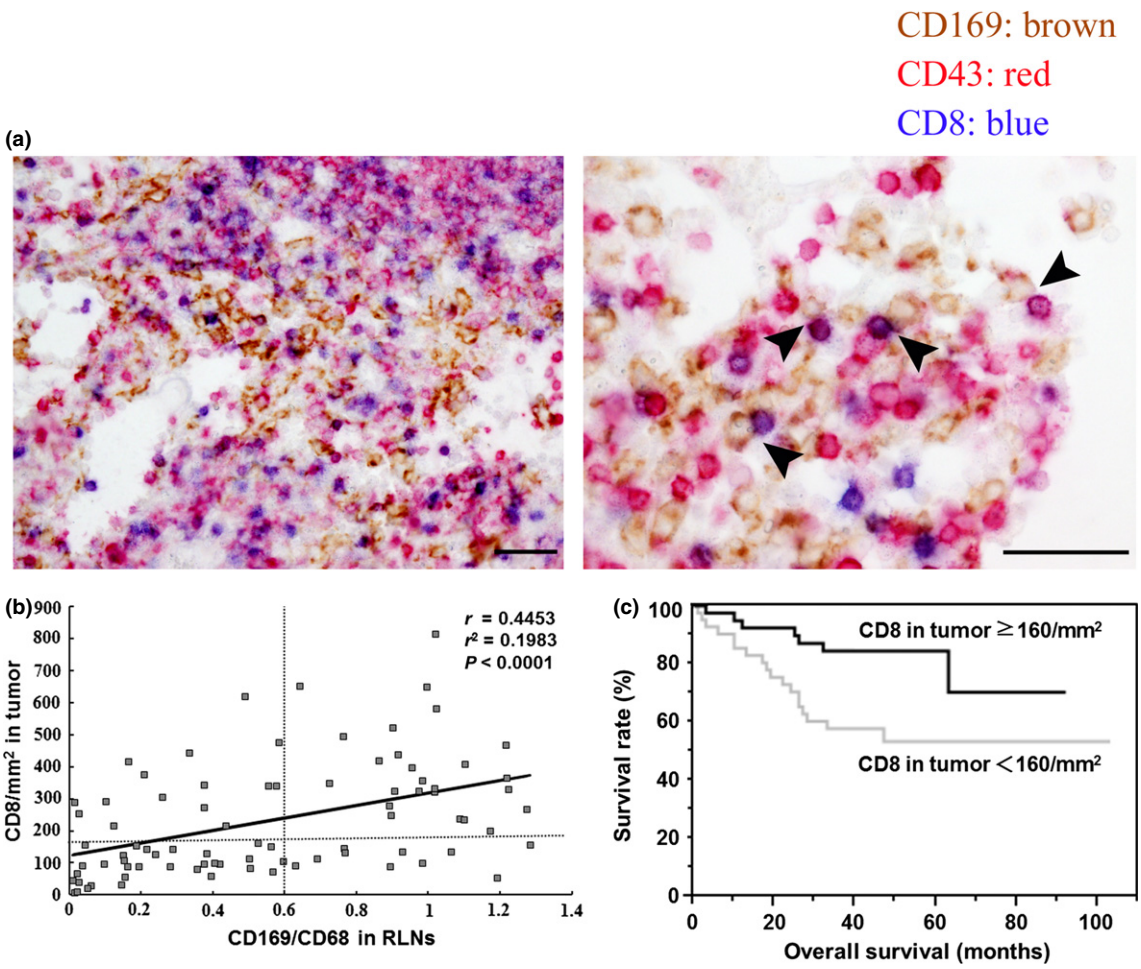

Fig. 4. Interaction between CD169$^+$ macrophages and CD8$^+$ T cells in regional lymph nodes (RLNs) of patients with colorectal carcinoma. (a) Triple immunostaining with CD169 (brown), CD43 (red), and CD8 (blue). Arrowheads indicate direct contact between CD169$^+$ cells and CD8$^+$ CD43$^+$ cells. Scale bar = 50 μm. (b) Correlation between the number of CD8$^+$ T cells in tumor tissues and the ratio of CD169$^+$ macrophages to CD68$^+$ macrophages in RLNs. (c) Overall survival curves for patients with CD8$^+$ T cells in tumor tissues.

In conclusion, we showed the significance of CD169$^+$ sinus macrophages in RLNs obtained from patients with CRC. A higher number of CD169$^+$ macrophages and an increased ratio of CD169$^+$ macrophages to CD68$^+$ macrophages in RLNs were significantly associated with better clinical prognosis; moreover, the ratio of CD169$^+$ macrophages to CD68$^+$ macrophages was an independent risk factor for overall survival. Because the ratio of CD169$^+$ macrophages to CD68$^+$ macrophages was also related to the number of CD8$^+$ T cells infiltrating tumor tissues, we believe that CD169$^+$ macrophages are involved in activation of cytotoxic T cells in RLNs (Fig. S1). Evaluation of the CD169 antigen in RLNs may aid estimation of clinical prognosis and monitoring of the antitumor immune response in patients with CRC.

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Disclosure Statement

The authors have no conflict of interest.

References

1 Lores B, García-Estevez JM, Arias C. Lymph nodes and human tumors (review). *Int J Mol Med* 1998; **1**: 729–33.
2 Hickok DF, Miller L, Harris L. Regional hyperplastic lymph nodes in breast cancer: the role of lymphocytes and nodal macrophages. An immunological study with a five-year follow-up. *Surgery* 1977; **82**: 710–15.
3 Gray EE, Cyster JG. Lymph node macrophages. *J Innate Immun* 2012; **4**: 424–36.
4 Martens JH, Kzhyshkowska J, Falkowski-Hansen M *et al.* Differential expression of a gene signature for scavenger/lectin receptors by endothelial cells and macrophages in human lymph node sinuses, the primary sites of regional metastasis. *J Pathol* 2006; **208**: 574–89.
5 Martinez-Pomares L, Gordon S. CD169+ macrophages at the crossroads of antigen presentation. *Trends Immunol* 2012; **33**: 66–70.
6 Nath D, Hartnell A, Happerfield L *et al.* Macrophage-tumour cell interactions: identification of MUC1 on breast cancer cells as a potential counter-receptor

for the macrophage-restricted receptor, sialoadhesin. *Immunology* 1999; **98**: 213–19.
7 van den Berg TK, Nath D, Ziltener HJ *et al.* Cutting edge: CD43 functions as a T cell counterreceptor for the macrophage adhesion receptor sialoadhesin (Siglec-1). *J Immunol* 2001; **166**: 3637–40.
8 Klaas M, Crocker PR. Sialoadhesin in recognition of self and non-self. *Semin Immunopathol* 2012; **34**: 353–64.
9 Hartnell A, Steel J, Turley H, Jones M, Jackson DG, Crocker PR. Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory macrophage populations. *Blood* 2001; **97**: 288–96.
10 Biesen R, Demir C, Barkhudarova F *et al.* Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. *Arthritis Rheum* 2008; **58**: 1136–45.
11 York MR, Nagai T, Mangini AJ, Lemaire R, van Seventer JM, Lafyatis R. A macrophage marker, Siglec-1, is increased on circulating monocytes in patients with systemic sclerosis and induced by type I interferons and toll-like receptor agonists. *Arthritis Rheum* 2007; **56**: 1010–20.
12 Ikezumi Y, Suzuki T, Hayafuji S *et al.* The sialoadhesin (CD169) expressing a macrophage subset in human proliferative glomerulonephritis. *Nephrol Dial Transplant* 2005; **20**: 2704–13.
13 Nagase R, Kajitani N, Shikata K *et al.* Phenotypic change of macrophages in the progression of diabetic nephropathy; sialoadhesin-positive activated macrophages are increased in diabetic kidney. *Clin Exp Nephrol* 2012; **16**: 739–48.
14 Xiong YS, Zhou YH, Rong GH *et al.* Siglec-1 on monocytes is a potential risk marker for monitoring disease severity in coronary artery disease. *Clin Biochem* 2009; **42**: 1057–63.
15 Xiong YS, Wu AL, Lin QS *et al.* Contribution of monocytes Siglec-1 in stimulating T cells proliferation and activation in atherosclerosis. *Atherosclerosis* 2012; **224**: 58–65.
16 Ashokkumar C, Gabriellan A, Ningappa M, Mazariegos G, Sun Q, Sindhi R. Increased monocyte expression of sialoadhesin during acute cellular rejection and other enteritides after intestine transplantation in children. *Transplantation* 2012; **93**: 561–4.
17 Wu C, Rauch U, Korpos E *et al.* Sialoadhesin-positive macrophages bind regulatory T cells, negatively controlling their expansion and autoimmune disease progression. *J Immunol* 2009; **182**: 6508–16.
18 Komohara Y, Hirahara J, Horikawa T *et al.* AM-3K, an anti-macrophage antibody, recognizes CD163, a molecule associated with an anti-inflammatory macrophage phenotype. *J Histochem Cytochem* 2006; **54**: 763–71.
19 Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. *J Pathol* 2008; **216**: 15–24.
20 Ohnishi K, Komohara Y, Sakashita N, Iyama K, Murayama T, Takeya M. Macrophages in Langerhans cell histiocytosis are differentiated toward M2 phenotype: their possible involvement in pathological processes. *Pathol Int* 2010; **60**: 27–34.
21 Compton CC, Fielding LP, Burgart LJ *et al.* Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000; **124**: 979–94.
22 Heusinkveld M, van der Burg SH. Identification and manipulation of tumor associated macrophages in human cancers. *J Transl Med* 2011; **9**: 216.
23 Naito Y, Saito K, Shiiba 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.
24 Guidoboni M, Gafà R, Viel A *et al.* Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *Am J Pathol* 2001; **159**: 297–304.
25 Galon J, Costes A, Sanchez-Cabo F *et al.* Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960–4.
26 Pagès F, Kirilovsky A, Mlecnik B *et al. In situ* cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol* 2009; **27**: 5944–51.
27 Kondratiev S, Sabo E, Yakirevich E, Lavie O, Resnick MB. Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. *Clin Cancer Res* 2004; **10**: 4450–6.
28 Sharma P, Shen Y, Wen S *et al.* CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci U S A* 2007; **104**: 3967–72.
29 Lee HE, Chae SW, Lee YJ *et al.* Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. *Br J Cancer* 2008; **99**: 1704–11.
30 Baker K, Lachapelle J, Zlobec I, Bismar TA, Terracciano L, Foulkes WD. Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon both oestrogen receptor status and histological grade. *Histopathology* 2011; **58**: 1107–16.
31 Martinet L, Garrido I, Filleron T *et al.* Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. *Cancer Res* 2011; **71**: 5678–87.
32 Liu Y, Komohara Y, Domenick N *et al.* Expression of antigen processing and presenting molecules in brain metastasis of breast cancer. *Cancer Immunol Immunother* 2012; **61**: 789–801.
33 Erdag G, Schaefer JT, Smolkin ME *et al.* Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res* 2012; **72**: 1070–80.
34 Asano K, Nabeyama A, Miyake Y *et al.* CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* 2011; **34**: 85–95.
35 Barral P, Polzella P, Bruckbauer A *et al.* CD169(+) macrophages present lipid antigens to mediate early activation of iNKT cells in lymph nodes. *Nat Immunol* 2010; **11**: 303–12.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Schema of the interaction between tumor immunity and lymph node macrophages.

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