Tumor-infiltrating CD4+ Th17 cells produce IL-17 in tumor microenvironment and promote tumor progression in human gastric cancer

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Received December 2, 2010; Accepted January 27, 2011

DOI: 10.3892/or.2011.1201

Abstract. Recently, a subset of IL-17 producing T cells distinct from Th1 or Th2 cells has been described as key players in inflammation and autoimmune diseases as well as cancer development. In this study, we investigated the expression level of IL-17 and T helper 17 (Th17)-related cytokines in gastric cancer tissues and assessed the association of their expression with angiogenesis and their clinicopathological parameters. Tumor and adjacent normal tissues were obtained from 82 patients with gastric cancer. IL-17, IL-21 and IL-23 mRNA expression levels were quantified by real-time RT-PCR. Th17 infiltration, microvessel density and neutrophil infiltration in tumor tissues were examined by immunohistochemistry and double immunofluorescence histochemistry. Expression of IL-17, IL-21 and IL-23 mRNA was found to be significantly up-regulated in tumor tissues compared with adjacent normal tissues. The expression level of IL-17 mRNA strongly and positively correlated with that of IL-21 mRNA in tumor tissue. The number of vascular endothelial cells and infiltrating neutrophils was significantly larger in tumors expressing a high level of IL-17 mRNA than in tumors expressing a low level of IL-17 mRNA. In tumor tissues most CD4+ cells were stained with anti-IL-17 antibody. The expression level of IL-17 mRNA in gastric tumors was associated with the depth of the tumors, lymph-vascular invasion and lymph node involvement, suggesting that IL-17 obviously was related to tumor progression. IL-17 and IL-21, which regulates IL-17, would be potential therapeutic targets for the treatment of gastric cancer.

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Key words: Th17, IL-17, IL-21, gastric cancer, angiogenesis, inflammation

Introduction

It has been established that cancer can be promoted or exacerbated by inflammation and infection. Chronic inflammation is a major driving force in tumor development (1-3). Interleukin (IL)-17 is considered a proinflammatory cytokine because it has been shown to increase the production of IL-6 and IL-8 in macrophages and fibroblasts (4-6). Recently a new lineage of effector CD4+ T cells characterized by production of IL-17, the T helper 17 (Th17) lineage, was described on the basis of developmental and functional features that are distinct from those of classic Th1 and Th2 lineages (7). The identification of this new subtype of Th17 cell has prompted renewed interest in IL-17 biology. IL-17 plays an important role in inflammation, and is critical in host defense against infectious disease, allergy, and autoimmune diseases such as rheumatoid arthritis and inflammatory bowel diseases, which include Crohn's disease and ulcerative colitis (8,9). Interestingly, IL-17 also has been reported to be up-regulated in *Helicobacter pylori* (Hp) infected gastric mucosa. IL-17 positively regulates the synthesis of IL-8 by gastric mononuclear cells and epithelial cells, which thus emphasizes the role of IL-17 in Hp-driven inflammation (10).

Recently, it has been reported that IL-17 promotes tumor growth through angiogenesis in mice (11). On the other hand, several reports have shown that IL-17 inhibits tumor growth through antitumor immunity in immunocompetent mice (12,13). It remains controversial whether IL-17 promotes or inhibits cancer progression. In humans, IL-17 expression has been reported in several tumor tissues such as ovarian cancer, colon cancer and also gastric cancer (14-16). Most solid tumors contain non-malignant cells, including immune cells and blood vessel cells, which are important in inflammation in the tumor microenvironment. In fact, a high percentage of CD4+ Th17 cells produce IL-17 at sites of ovarian cancer (17). However, in human tumors, the crucial molecular pathways that permit communication between abnormally growing cancer cells and these inflammatory cells remain unknown. In addition, the underlying mechanism of IL-17 at tumor sites in modulating tumor growth is still poorly understood.

Differentiation of Th17 cells from naïve T cells appears to involve signals from transforming growth factor β (TGF-β) and IL-6 (18,19). IL-21 has been reported to play an important role in the initial phase of Th17 differentiation (20,21). Although, in mice, there is a general agreement on the factors required for the generation of Th17 cells as mentioned above, the crucial initiating cytokines in humans for Th17 development remain unclear, and the relationship between IL-21 and IL-17 at tumor sites has not been elucidated.

In this study, we quantitatively investigated expression of IL-17 and IL-21 messenger RNA (mRNA) in gastric cancer tissues. In addition, we assessed the association of IL-17 expression levels with angiogenesis and neutrophil infiltration and its clinicopathological factors to clarify the role of tumor-infiltrating Th17 in tumor growth and progression. We also reviewed the possibility of IL-17 as a therapeutic target for patients with gastric cancer.

Materials and methods

Patients and tissue specimens. Included in the present study was a series of 82 patients (58 men, 24 women) with gastric cancer who underwent gastrectomy at Wakayama Medical University Hospital (WMUH) from 2004 to 2007. None of them received anticancer therapy before surgery. Individuals with concurrence of autoimmune disease, inflammatory bowel disease or viral infection were excluded. Clinicopathological characteristics of these 82 patients are summarized in Table I. Clinical stages of the tumors were determined according to the International Union Against Cancer TNM classification for gastric cancer. Samples of cancer tissues and non-cancerous adjacent tissues were collected from resected specimens of patients. Tumor samples were obtained from the invasive front of resected gastric cancer. Written informed consent was obtained from all patients before their participation in this study. In addition, the local ethics committee of WMUH approved this study.

RNA extraction and DNA synthesis. Total RNA was extracted with an RNeasy mini kit (Qiagen, Hilden, Germany) followed by RNase-Free DNase Set treatment (Qiagen). Complementary DNA was synthesized from 1 μg of total RNA by using the Reverse Transcription System (Promega) according to manufacturer's instructions.

Quantitative real-time RT-PCR. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed with isolated total RNA (1 μg) on the LightCycler system (Roche Molecular Biochemicals, Mannheim, Germany). The following oligonucleotide primers and hybridization probes were used: human IL-17 sense, 5'-CTGGGAAGACC TCATTGG-3' and antisense, 5'-CCTTTTGGGATTGGTA TTGG-3', fluorescein-labeled probe, 5'-TCCTCAGAATTT GGGCATCCTGGATTTC-3', and LC Red 640-labeled probe, 5'-TGGGATTGTGATTCCTGCCTTCACTATGG-3'; human IL-21 sense, 5'-AGGTCAAGATCGCCACAT-3' and antisense, 5'-TTTGCTGACTTTAGTTGGGC-3', fluorescein-labeled probe, 5'-CTGACCACTCACAGTTTGTCTCTACATCTTCTG GA-3', and LC Red 640-labeled probe, 5'-CTGGCAGAAA TTCAGGGACCAAGTCATTCA-3'; human IL-23 sense, 5'-GAGAAGCTGCTAGGATCG-3', and antisense, 5'-TGG TGACCCTCAGGCTGC-3', fluorescein-labeled probe, 5'-GCC TTCTCTGCTCCCTGATAGCCCTGTG-3', and LC Red 640-labeled probe, 5'-GCCAGCTTCATGCCTCCCTACTG GG-3'; human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense, 5'-TGAACGGGAAGCTCACTGG-3' and antisense, 5'-TCCACCACCCTGTTGCTGTA-3', fluorescein-labeled probe, 5'-TCAACAGCGACACCCACTCCT-3', and LC Red 640-labeled probe, 5'-CACCTTTGACGCTGGGGCT-3'. Primers and probes were designed by Nihon Gene Research

Table I. IL-17 mRNA expression and clinicopathological parameters.

Factor	No. of patients	Expression of IL-17 mRNA[a]	P-value
Gender			
Male	59	3.24±0.179	
Female	23	3.65±0.298	0.22
Age (years)			
<65	31	3.78±0.193	
≥65	51	3.11±0.212	0.118
Tumor stage[b]			
T0/T1	15	2.52±0.430	
T2	33	3.13±0.219	
T3	34	3.99±0.186	<0.005[a]
Histological type			
Differentiated	40	3.19±0.247	
Undifferentiated	42	3.52±0.188	0.658
Lymphatic invasion			
Negative	27	2.78±0.303	
Positive	55	3.65±0.164	<0.05[d]
Venous invasion			
Negative	43	2.95±0.226	
Positive	39	3.82±0.184	<0.05[d]
Lymph node metastasis			
Negative	40	2.95±0.234	
Positive	42	3.75±0.186	<0.05[d]
Stage[b]			
0/I	31	2.66±0.254	
II	21	3.63±0.297	
III	19	3.84±0.240	
IV	11	4.00±0.378	<0.05[c]
Tumor size (cm)			
<5	46	3.13±0.202	
≥5	36	3.63±0.232	0.068

[a]Expression of mRNA for IL-17 were corrected with GAPDH housekeeping control amplifications. Values represent mean ± SEM. [b]Stage according to the TNM classification for gastric cancer (UICC). [c]P-value of Kruskal-Wallis test as appropriate. [d]P-value of Mann-Whitney test as appropriate.

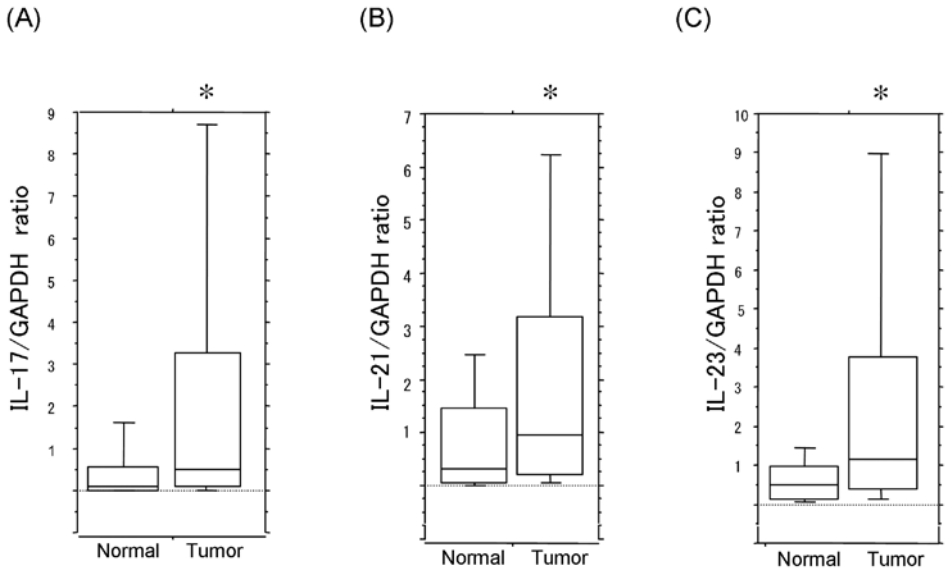

Figure 1. Level of IL-17, IL-21 and IL-23 mRNA expression in tumor and normal tissues of gastric cancer in 82 clinical samples. Expression levels of IL-17 (A), IL-21 (B) and IL-23 (C) mRNA were quantitatively determined by real-time RT-PCR for tumor tissue specimens and non-tumor tissue specimens from gastric cancer. Box plots show the 10th, 25th, 50th (median), 75th and 90th percentile values for log-transformed ratios of mRNA copies to GAPDH copies for IL-17, IL-21 and IL-23. *Significantly different from adjacent normal tissues ($p<0.0005$). Two-tailed p-values are based on the Mann-Whitney test.

Laboratories, Inc. (Miyagi, Japan). After 10 min of initial denaturation at 95˚C, the cycling protocol entailed 40 cycles of denaturation at 95˚C (10 sec), annealing at 62˚C (15 sec) and elongation at 72˚C (8 sec). For GAPDH, the thermocycling protocol was the same, except that annealing was performed at 55˚C (15 sec) and 50 cycles were run. On each run, we quantified all samples according to the LightCycler software program, version 3.8 (Roche Molecular Biochemicals). The levels of mRNA for IL-17, IL-21 and IL-23 were corrected with GAPDH housekeeping control amplifications.

Immunohistochemistry and quantitative microscopy. Sections (4 μm) were prepared from paraffin-embedded blocks derived from gastric tumors. Sections were deparaffinized in xylene and graded alcohols, and rinsed in phosphate-buffered saline. Antigen retrieval from tissue was carried out by autoclaving the tissue in 0.01 M citrate buffer (pH 6.0) at 120˚C for 10 min. The antibodies used included rabbit anti-IL-17 (dilution at 1:100, Santa Cruz Biotechnology); rabbit anti-IL-21 (dilution at 1:100, LifeSpan BioSciences); mouse anti-CD34, specific for endothelial tissue (dilution at 1:50, DakoCytomation, Glostrup, Denmark); and mouse anti-CD66b, specific for neutrophils (dilution at 1:500, BD Pharmingen). The antibodies were incubated overnight at 4˚C. The immunocomplex was visualized by a polymer envision method, EnVision$^+$ Kit (Dako). For quantification of tumor microvessel density and neutrophil infiltration, highly positive areas were initially identified by scanning tumor sections using light microscopy at low power. Areas of infarct-like necrosis and areas immediately adjacent to ulcerations were not considered in counts. Vessel counts were assessed according to the criteria of Weidner *et al* (22). Vessels in five high-power fields (x200 magnification) and neutrophil infiltration in five high-power fields (x400 magnification) were counted. Positive cells were quantified by an image processing application (Win ROOF, version 5.5; Mitani, Tokyo, Japan) and the manual counts were confirmed by a pathologist.

Double immunofluorescence histochemistry. Tissues were stained with primary antibodies: mouse anti-CD4 (dilution at 1:100, Dako) and rabbit anti-IL-17 (dilution at 1:100, Santa Cruz). The CD4 and IL-17 antibodies were detected with Alexa Fluor 488 conjugated goat anti-mouse immunoglobulin G (IgG) (Molecular Probes) and Alexa Fluor 546 conjugated goat anti-rabbit IgG (Molecular Probes). The double-stained sections were analyzed with a confocal laser scanning microscope (LSM5Pascal Exciter, version 4.0; Carl Zeiss, Jena, Germany).

Statistical analysis. The following statistical analyses were used. For data in Figs. 1, 3 and 4, we used the Mann-Whitney test. In Fig. 2, we used the Spearman rank correlation coefficient. In Table I, we used the Mann-Whitney test and the Kruskal-Wallis test. All statistical analyses were performed with StatView 6.0 (SAS Institute Inc) statistical software program. A value of $p<0.05$ was considered statistically significant.

Results

Expression of IL-17, IL-21 and IL-23 mRNA in tumor and non-cancerous tissues. IL-17 was found to be significantly up-regulated in tumor tissue compared with adjacent normal tissue ($p<0.0005$, Fig. 1A). Both IL-21 and IL-23, which are related to IL-17 production, were also significantly up-regulated in tumor tissue ($p<0.0005$, Fig. 1B and C).

Correlation of IL-17 mRNA with IL-21 and IL-23 mRNA in tumor tissues. The expression level of IL-17 mRNA positively correlated with that of IL-21 mRNA in tumor tissues ($r=0.730$,

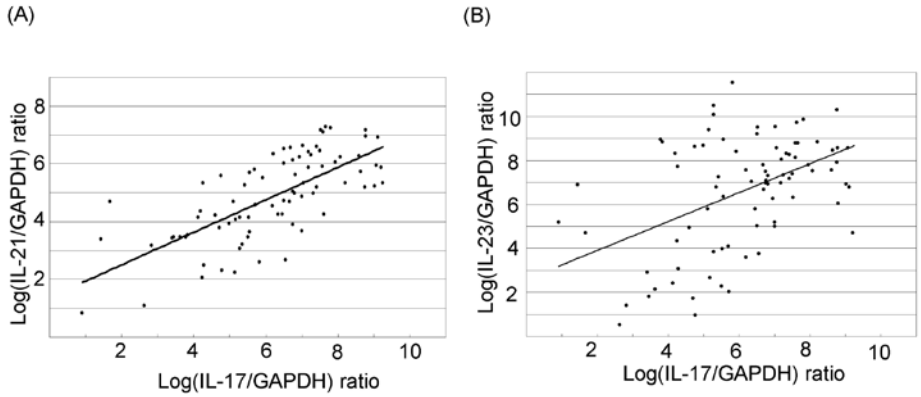

Figure 2. The correlation between expression levels of IL-17 mRNA, IL-21 mRNA and IL-23 mRNA. The correlation between expression levels of IL-17 mRNA and IL-21 mRNA [(A) r=0.730, p<0.0001], and the correlation between expression levels of IL-17 mRNA and IL-23 mRNA [(B) r=0.415, p<0.0005] were examined in tumor tissues. Log-transformed mRNA levels, normalized for GAPDH mRNA, are shown. The relationships are shown along with Spearman's rank-order correlation.

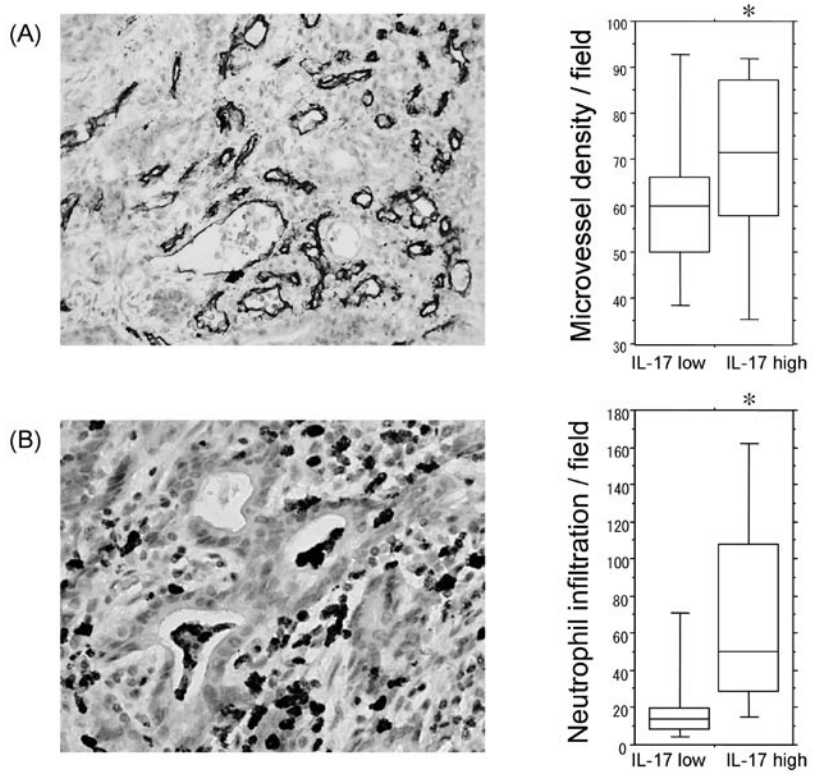

Figure 3. Microvessel density and neutrophils infiltration in tumor tissues. (A) Immunohistochemical staining of tumor tissues with anti-CD34 antibody, specific for endothelial tissues, was performed. Representative pictures of immunohistochemical staining of tumor tissues are shown. Vessels in five power fields (x200 magnification) were counted. (B) Immunohistochemical staining of tumor tissues with anti-CD66b antibody, specific for neutrophils, was performed. Representative pictures of immunohistochemical staining of tumor tissues are shown. Positive cells in five power fields (x400 magnification) were counted. Box plots show the 10th, 25th, 50th (median), 75th and 90th percentile values for the average number. *Significantly different from tumors expressing low levels of IL-17 (p<0.01). Two-tailed p-values are based on the Mann-Whitney test.

p<0.0001, Fig. 2A). On the other hand, there was no correlation between expression levels of IL-17 mRNA and IL-23 mRNA in tumor tissues (r=0.415, p<0.0005, Fig. 2B).

Quantification of tumor microvessel density. To assess the association between microvessel density and IL-17 expression in tumor tissue, we performed immunohistochemical staining with anti-CD34 antibody specific for endothelial cells. High- and low-expression groups were defined by the median value of IL-17 mRNA expression of this study population. The number of vascular endothelial cells was significantly higher in tumors expressing high IL-17 mRNA than in tumors expressing low IL-17 mRNA (p<0.01, Fig. 3A).

Quantification of tumor-infiltrating neutrophils. To assess the association between neutrophil infiltration and IL-17 expres-

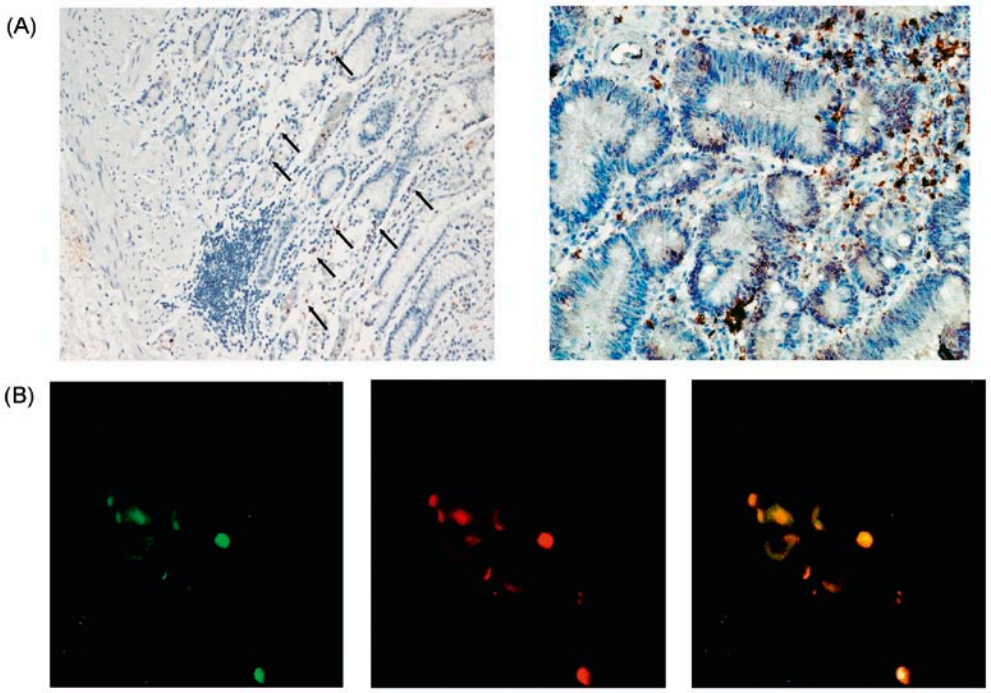

Figure 4. Immunohistochemistry for IL-17 in normal and tumor site. (A) Representative pictures of immunohistochemical staining with anti-IL-17 antibody at the normal (left panel) and tumor (right panel) site of gastric cancer specimens. More IL-17-positive cells were observed in the tumor tissues than in the adjacent normal tissues. The original magnification is x400. (B) Double immunofluorescence histochemistry for CD4 and IL-17 in tumor site. Tissue sections from a tumor were incubated with mouse monoclonal antibody against CD4 together with rabbit polyclonal antibodies against IL-17. The monoclonal antibody was detected with Alexa Fluor 488 conjugated goat anti-mouse IgG (green fluorescence; left panel), and the polyclonal antibodies were detected with Alexa Fluor 546 conjugated goat anti-rabbit IgG (red fluorescence; middle panel). Merged image of the two fluorophores is displayed in yellow (right panel). The original magnificationis x1000.

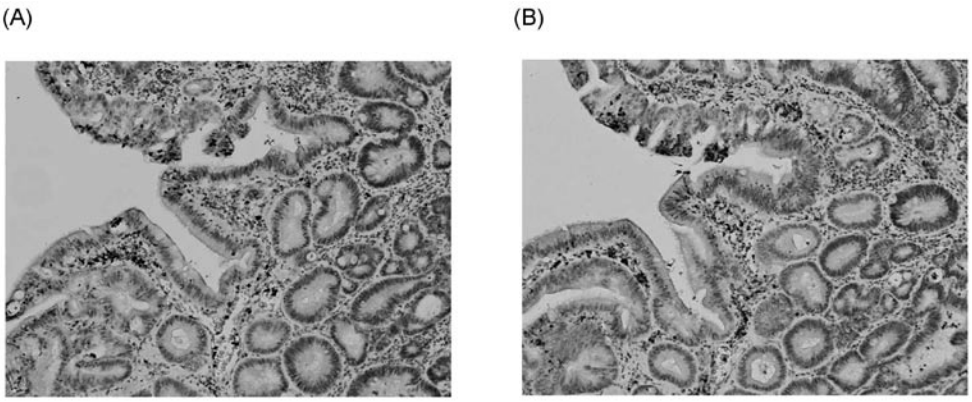

Figure 5. Immunohistochemistry for IL-17 and IL-21 in tumor site. (A) Representative pictures of immunohistochemical staining with anti-IL-17 antibody at the tumor site of gastric cancer specimens. (B) Representative pictures of immunohistochemical staining with anti-IL-21 antibody at the tumor site of gastric cancer specimens. The IL-21-positive cells did not always coincide with the IL-17-positive cells in the two serial sections. Original magnification x100.

sion in tumor tissue, we performed immunohistochemical staining with anti-CD66b antibody specific for neutrophils. High- and low-expression groups were defined by the median value of IL-17 mRNA expression of this study population. The number of infiltrating neutrophils was significantly lager in tumors expressing high IL-17 mRNA than in tumors expressing low IL-17 mRNA ($p<0.001$, Fig. 3B).

Distributions of IL-17-positive cells. To examine the expression of IL-17 in tumor and normal tissue, we performed immunohistochemistry for IL-17. IL-17 immunoreactive cells were rarely detected in non-cancerous adjacent tissues. On the other hand, there were abundant IL-17-expressing cells in tumor tissues; however, none of the tumor cells stained for IL-17 (Fig. 4A).

Identification of IL-17-producing cells in tumors. To identify IL-17-producing cells in tumor tissues, we performed double immunofluorescence histochemistry with anti-IL-17 and anti-CD4 antibodies in tumor tissues. The anti-CD4 primary

antibodies were detected with green fluorescence (Fig. 4B; left panel), and the anti-IL-17 primary antibodies were detected with red fluorescence (Fig. 4B; middle panel). A merged image of the two fluorophores, displayed in yellow, shows that most of the CD4$^+$ cells in tumor tissues are stained with anti-IL-17 antibody (Fig. 4B; right panel). Therefore, Th17 cells that infiltrate tumor tissues produced IL-17.

IL-17 mRNA expression and clinicopathological characteristics. To evaluate the biological significance of IL-17 expression in tumor tissues in patients with gastric cancer, we investigated the association of mRNA expression levels of IL-17 with clinicopathological factors (Table I). IL-17 mRNA expression levels in gastric tumors were higher in positive groups of venous invasion, lymphatic invasion and lymph node metastases than in negative groups. IL-17 mRNA expression levels in gastric tumors increased according to the depth of invasion and the stage of disease. On the other hand, no significant association was recognized between the expression levels of IL-17 mRNA and histological type and tumor size.

Discussion

In this study, we quantitatively analyzed the expression of IL-17 mRNA in gastric tumor tissues. IL-17 was originally identified as a proinflammatory cytokine that induces neutrophils (4) and previous studies also have shown that inflammation is linked to cancer development and progression. Therefore, it is reasonable to speculate that proinflammatory Th17 cells may accumulate and produce IL-17 in the tumor microenvironment. Indeed, we showed in the present study that the expression level of IL-17 mRNA in tumor tissues was significantly higher compared with that in adjacent normal tissues. In addition, we examined IL-17 expression in tumor and normal tissues by immunohistochemistry, and showed that, in contrast to normal glands, malignant glands were surrounded by dense inflammatory cells including many IL-17-producing cells. This result was consistent with the data of IL-17 mRNA expression. Conventionally, it has been reported that IL-17 is predominantly produced by CD4$^+$ helper T cells, which have been termed 'Th17' (5). However, recent studies have shown that T cells other than CD4$^+$ T cells, such as γδT cells and CD8$^+$ T cells, produce IL-17 in mouse models (23,24). In the present study, we performed double immunofluorescence histochemistry using anti-IL-17 and CD4 antibodies in tumor tissues to identify IL-17-producing cells in human gastric cancer tissues. We showed that most of the CD4$^+$ cells infiltrating tumors were also stained with anti-IL-17 antibody, suggesting that IL-17 was predominantly produced by conventional Th17 cells in human gastric cancer tissues. Consistent with this observation, it has been reported that there is a high percentage of Th17 cells in tumor-derived T cell populations in 5 of 10 ovarian tumors, and those Th17 cells secreted IL-17 (17).

Differentiation of Th17 cells from naïve T cells is dependent on signals from TGF-β, IL-6, IL-21 and IL-23 in mice (21). Importantly, IL-21 is essential for the generation of Th17 cells and also amplifies Th17 cell differentiation (20). IL-23 does not act on naïve T cells, but instead acts on T cells that are already committed to the Th17 lineage. IL-23 enhances the production of IL-17 and stabilizes the Th17 phenotype (25,26). In humans, IL-21 likewise is required for differentiation of Th17 cells, and IL-23 enhances IL-17 secretion from those cells (27,28). However, there have been few studies to investigate the expression of IL-17-related cytokines such as IL-21 and IL-23, which play a crucial role in the generation of Th17 cells and the production of IL-17 in the tumor tissues of cancer patients. Our data showed that mRNA expression of both IL-21 and IL-23 was higher in gastric tumor tissues than in non-cancerous adjacent tissues. Importantly, in tumor tissues, the IL-21 mRNA expression level showed a strong correlation with the IL-17 mRNA expression level, but the IL-23 mRNA expression level did not show as strong a correlation with the IL-17 mRNA expression level. We also examined other cytokines such as TGF-β and IL-6, but we did not find a correlation as strong as that of IL-17 with IL-21 (data not shown). In addition, we performed immunohistochemical staining for IL-21 in tumor tissues and non-cancerous adjacent tissues to identify IL-21-producing cells. As with IL-17, there were many IL-21-positive cells around malignant glands, although the IL-21-positive cells did not always coincide with the IL-17-positive cells (Fig. 5). These results suggest that IL-21 may be crucial for the generation of Th17 cells and may play a pivotal role in the regulation of IL-17 secretion in the tumor microenvironment.

IL-17 is expressed in a considerable proportion of patients with ovarian cancer and its expression is associated with tumor angiogenesis (14). IL-17 up-regulates production of a variety of proangiogenic factors, such as vascular endothelial growth factor (VEGF), prostaglandin E1 (PGE1) and PGE2, and macrophage inflammatory protein-2 (MIP-2), by fibroblasts as well as tumor cells; IL-17 also promotes angiogenesis through stimulation of vascular endothelial cell migration and cord formation, resulting in tumor progression (11). The microvessel density and other angiogenic factors such as VEGF and PGE1, which are related to tumor development and progression, are predictive of patient survival in patients with gastric cancer. Therefore, it is important to examine the association of the expression level of IL-17 mRNA and angiogenesis in tumor tissues for the purpose of clarifying the biological significance of IL-17 in the tumor microenvironment. Our results revealed that tumor tissues with high expression levels of IL-17 mRNA had significantly higher microvessel density than those with low expression levels. Our findings strongly suggested that IL-17 released from tumor-infiltrating Th17 cells promoted angiogenesis by the production of proangiogenic chemokines in human gastric cancer. That process may lead to tumor progression.

From the standpoint of inflammation, IL-17 has the ability to stimulate IL-8 production in both epithelial cells and macrophages raising the possibility that this cytokine may play an important role in the recruitment of inflammatory cells in the tumor microenvironment. Neutrophils, acting alone or in concert with macrophages, also have been linked to tumor progression (2). Tumor-associated neutrophils promote tumor progression by a variety of mechanisms, including stimulation of angiogenesis and invasion (29). It has been shown in several tumor transplant models that tumor-associated neutrophils can stimulate tumor angiogenesis through production of

proangiogenic factors, including VEGF, IL-8 and certain proteases such as matrix metalloproteinases and elastases, and that these neutrophils can facilitate tumor progression. In this study, immunostaining for neutrophils revealed that tumor tissues with high expression levels of IL-17 mRNA had more infiltrating neutrophils than tissues with low expression levels. This observation suggested that IL-17 from Th17 recruited neutrophils into the tumor microenvironment, and that these tumor-associated neutrophils may contribute to the invasive potential.

There are conflicting data on a possible role in carcinogenesis in mouse models. Forced overexpression of IL-17 ectopically in tumor cells can either suppress tumor progression through enhanced antitumor immunity (12,30) or promote it through an increase in inflammation and angiogenesis (11). The role of endogenous IL-17 in tumorigenesis has been assessed with IL-17 knockout mice (13). Although the results are still controversial, they give useful information for clarifying whether IL-17 promotes or inhibits tumor progression in human cancer and for investigating the association of locally expressed IL-17 and clinicopathological characteristics in patients with gastric cancer. In this study, we focused on the clinical significance of IL-17 in the tumor microenvironment. To our knowledge, no prior reports exist on the correlations between locally expressed IL-17 and clinicopathological factors of cancer patients from this point of view. Although Zhang *et al* reported that IL-17 was significantly increased in serum and tumor-draining lymph nodes in patients with advanced gastric cancer (16), locally expressed IL-17 would have quite a different role in tumor progression. This study is the first to report the clinical significance of locally expressed IL-17 in gastric cancer. Clinicopathological analysis revealed that patients with high IL-17 mRNA expression level showed a deeper invasion of tumors into the wall, more lymph-vascular invasion and more positive lymph node involvement than those with low expression. IL-17 at the tumor sites may thus play a vital role in proliferation and progression of gastric cancer.

In conclusion, our findings showed that $CD4^+$ Th17 cells were generated around the tumor, and that Th17 cells infiltrated the tumor and secreted IL-17, leading to tumor progression through angiogenesis and neutrophil infiltration in patients with gastric cancer. One of the key cytokines to regulate the production of IL-17 in the tumor microenvironment was thought to be IL-21. It was suggested that IL-17 or IL-21 locally expressed in the tumor would be a potential therapeutic target for treatment of patients with advanced gastric cancer.

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