Superficially-located enlarged lymphoid follicles characterize nodular gastritis

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Summary

Aims: Nodular gastritis is a form of chronic *Helicobacter pylori* gastritis affecting the gastric antrum and characterized endoscopically by the presence of small nodular lesions resembling gooseflesh. It is generally accepted that hyperplasia of lymphoid follicles histologically characterizes nodular gastritis; however, quantitative analysis in support of this hypothesis has not been reported. Our goal was to determine whether nodular gastritis is characterized by lymphoid follicle hyperplasia.

Methods: The number, size, and location of lymphoid follicles in nodular gastritis were determined and those properties compared to samples of atrophic gastritis. The percentages of high endothelial venule (HEV)-like vessels were also evaluated.

Results: The number of lymphoid follicles was comparable between nodular and atrophic gastritis; however, follicle size in nodular gastritis was significantly greater than that seen in atrophic gastritis. Moreover, lymphoid follicles in nodular gastritis were positioned more superficially than were those in atrophic gastritis. The percentage of MECA-79+ HEV-like vessels was greater in areas with gooseflesh-like lesions in nodular versus atrophic gastritis.

Conclusions: Superficially-located hyperplastic lymphoid follicles characterize nodular gastritis, and these follicles correspond to gooseflesh-like nodular lesions observed
endoscopically. These observations suggest that MECA-79+ HEV-like vessels could play at least a partial role in the pathogenesis of nodular gastritis.

*Key words: Helicobacter pylori, nodular gastritis, lymphoid follicle hyperplasia, high endothelial venule (HEV)-like vessel*
INTRODUCTION

Nodular gastritis is a form of chronic *Helicobacter pylori* gastritis characterized endoscopically by the presence of small nodular or granular lesions resembling gooseflesh distributed predominantly in the gastric antrum.\(^1\) Nodular gastritis occurs frequently in children, but there is increasing evidence that nodular gastritis also occurs in adults, particularly in pre-menopausal women.\(^2-5\) Intriguingly, a recent report by Kamada *et al.* demonstrated that, particularly in young women, there is a possible association between nodular gastritis and diffuse-type gastric carcinoma, including signet-ring cell carcinoma.\(^6\) Signet-ring cell carcinoma in advanced stages shows a higher rate of lymph node metastasis and peritoneal dissemination than do other histological types of gastric carcinoma.\(^7\) Thus, it is important to understand the pathogenesis of nodular gastritis and its impact on the development of signet-ring cell carcinoma.

It is generally accepted that histological findings of nodular gastritis include lymphoid hyperplasia, namely hyperplasia of lymphoid follicles.\(^2,3,8\) Shiotani *et al.* noted that biopsy specimens with nodular gastritis tend to contain larger and more superficially-located lymphoid follicles compared to those with atrophic gastritis and hypothesized that follicle size and location are likely related to development of nodular
gastritis. However, those investigators did not perform quantitative histological analysis of the disease.

Lymphoid follicles, a component of mucosa-associated lymphoid tissue (MALT), are believed to be absent from normal stomach. Instead they emerge from chronic antigenic stimulation exerted by *H. pylori* in the gastric mucosa and therefore reflect a specific immune response to this microorganism. Formation of MALT, or lymphoid neogenesis, is thus required for subsequent lymphoid hyperplasia and is elaborated by a multistep process marked by sequential adhesive interactions between circulating lymphocytes and specialized endothelial cells comprising high endothelial venule (HEV)-like vessels.

Two adhesion molecules expressed on the luminal surface of HEV-like vessels play a critical role in the initial step of lymphocyte recruitment: peripheral lymph node addressin (PNAd) and mucosal addressin cell adhesion molecule 1 (MAdCAM-1). PNAd, a set of sialomucins recognized by the monoclonal antibody MECA-79, is constitutively displayed on HEVs in secondary lymphoid organs. Although absent under normal conditions, PNAd is also displayed on HEV-like vessels induced in chronic inflammatory gastrointestinal diseases including chronic *H. pylori* gastritis, ulcerative colitis and
autoimmune pancreatitis. The MECA-79 epitope has been shown to be 6-sulfo \(N\)-acetyllactosamine attached to extended core 1 \(O\)-glycans,

\[
\text{Gal}\beta_1\rightarrow4(\text{sulfo}\rightarrow6)\text{GlcNAc}\beta_1\rightarrow3\text{Gal}\beta_1\rightarrow3\text{GalNAc}\alpha_1\rightarrow\text{Ser}/\text{Thr},
\]

which overlaps with the L-selectin recognition determinant 6-sulfo sialyl Lewis X (sLeX), sialic acid\(\alpha_2\rightarrow3\text{Gal}\beta_1\rightarrow4[\text{Fuc}\alpha_1\rightarrow3(\text{sulfo}\rightarrow6)]\text{GlcNAc}\beta_1\rightarrow3\text{Gal}\beta_1\rightarrow3\text{GalNAc}\alpha_1\rightarrow\text{Ser}/\text{Thr}.\]

MAdCAM-1 belongs to the immunoglobulin superfamily and is constitutively displayed on HEVs in Peyer's patches and mesenteric lymph nodes and on flattened venular endothelial cells in the lamina propria of the intestine, but not seen in the stomach under physiological conditions. MAdCAM-1 is an exclusive ligand for integrin \(\alpha 4\beta 7\) expressed on memory lymphocytes and functions not only in lymphocyte firm attachment but also in L-selectin-independent lymphocyte rolling in intestinal MALT. Moreover, MAdCAM-1 can be posttranslationally decorated with 6-sulfo sLeX at its mucin-like domain and also supports L-selectin-dependent lymphocyte rolling. MAdCAM-1 is thus a dual-function molecule that can serve as a vascular addressin for both L-selectin and \(\alpha 4\beta 7\) integrin. Ohara et al. has reported that the percentage of MAdCAM-1\(^+\) HEV-like vessels in the lamina propria of nodular gastritis is greater than that in control \(H. pylori\)-positive gastric mucosa. It is also critical to know whether PNAd expression parallels that of MAdCAM-1, since both
molecules may synergize in mucosal lymphocyte homing.\textsuperscript{27,28}

In the present study, we determined the number, size, and location of lymphoid follicles, as well as the percentages of MECA-79\textsuperscript{+} and MAdCAM-1\textsuperscript{+} HEV-like vessels in nodular gastritis, and compared these properties with those seen in atrophic gastritis and normal gastric mucosa. We found that lymphoid follicles formed in nodular gastritis were more enlarged and superficially located than were those in atrophic gastritis. Hyperplastic lymphoid follicles in nodular gastritis were closely associated with an increased percentage of MECA-79\textsuperscript{+} HEV-like vessels rather than MAdCAM-1\textsuperscript{+} HEV-like vessels. These findings indicate that an increase in the percentage of MECA-79\textsuperscript{+} HEV-like vessels and subsequent formation of superficially-located enlarged lymphoid follicles can serve as histological hallmarks of nodular gastritis.
MATERIALS AND METHODS

Tissue samples

The analysis of human stomach tissues was approved by the Ethics Committee of Shinshu University School of Medicine (protocol numbers 2259 and 2260, approved on April 1st, 2013). Formalin-fixed, paraffin-embedded (FFPE) tissue blocks of gastric biopsy specimens with an endoscopic diagnosis of either nodular \((n = 99)\) or atrophic \((n = 65)\) gastritis were retrieved from the pathological archives of Shinshu University Hospital and its affiliated hospitals. Nodular gastritis was defined endoscopically as an antrum-dominant gastritis with a gooseflesh appearance (Fig. 1A, B), and atrophic gastritis was diagnosed when gastric rugae were flattened and a submucosal vascular pattern was visible in the distended stomach (Fig. 1C). Normal gastric mucosa samples \((n = 31)\) served as controls. In accordance with the updated Sydney system, at least five biopsy specimens were taken from each patient: one each from the lesser (A1) and greater curvature (A2) of the antrum; one from the incisura angularis (IA); and one each from the lesser (B1) and the greater curvature (B2) of the corpus. Patients’ clinical characteristics are summarized in Table 1. In all cases, \(H. pylori\) infection status was determined by a combined histological evaluation and bacterial culture. Of note, one nodular gastritis patient had diffuse-type gastric carcinoma (signet-ring cell carcinoma).
For the integrin \(\alpha 4\beta 7\cdot\text{IgG}\) heterodimeric chimera in situ binding assay described below, we obtained fresh gastric mucosa from nodular gastritis patients with informed consent. Fresh tissues were embedded in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan) and frozen at -80°C. Frozen tissues were sectioned at 6 \(\mu\)m, fixed in acetone for 5 min, and briefly air-dried.

**Establishment of an anti-human MAdCAM-1 monoclonal antibody**

Anti-human MAdCAM-1 monoclonal antibody was generated by ITM Co Ltd (Matsumoto, Japan) basically as described.\(^3\) Briefly, BALB/c mice were immunized with Chinese hamster ovary (CHO) cells stably expressing human MAdCAM-1 (CHO/MAdCAM-1).\(^2\) Two weeks later, lymphocytes obtained from iliac lymph nodes were fused with SP2/0 mouse myeloma cells in the presence of 50% polyethylene glycol (PEG) solution, and cell hybrids were selected for one week in GIT medium (Wako, Osaka, Japan) containing a HAT supplement. After changing the medium to GIT medium containing an HT supplement, cells were cultured another week, and conditioned medium was then screened by immunohistochemistry using FFPE human mesenteric lymph node tissue sections. After several rounds of limited dilution cloning, four hybridoma clones were obtained. We then
used the IsoQuick Kit for Mouse Monoclonal Isotyping (Sigma-Aldrich, St. Louis, MO) to determine that the isotype of all monoclonal antibodies was mouse IgG1κ. Based on its specific reactivity with HEVs in mesenteric lymph nodes (Fig. 2A), clone #15-8G-61 was chosen and used throughout the study. Antibody specificity was confirmed by flow cytometry (Fig. 2B) and Western blot analysis (Fig. 2C).

**Immunohistochemistry**

The following monoclonal antibodies served as primary antibodies: QBEND10, which recognizes human CD34, a marker of vascular endothelial cells (mouse IgG; Immunotech, Luminy, France); MECA-79, which recognizes 6-sulfo N-acetyllactosamine attached to extended core 1 O-glycans (rat IgM; BD Pharmingen, San Diego, CA); and #15-8G-61, which recognizes human MAdCAM-1 (described above).

Immunohistochemistry for CD34 and MAdCAM-1 was undertaken using the EnVision system (Dako) and that for MECA-79 was carried out using an indirect method described previously.

**Quantitative evaluation of lymphoid follicles**
We defined a lymphoid follicle as a dense nodular lymphocyte aggregate with or without germinal centers. Using hematoxylin and eosin (H&E)-stained tissue sections, we 1) counted the number of lymphoid follicles in each biopsy specimen and 2) measured the maximal diameter (including mantle zone) of each lymphoid follicle. When possible, we also 3) analyzed occupancy and 4) determined the location of lymphoid follicles in the gastric mucosa with nodular gastritis and atrophic gastritis, as illustrated in Fig. 3. All measurements were carried out at x40 magnification using a BX-51 microscope (Olympus, Tokyo, Japan) and Micro-Ruler for Microscope (Kenis, Osaka, Japan). Histological evaluation was done in a blinded fashion by a single observer (TO) after training by an experienced pathologist (MK).

**Quantification of CD34⁺, MAdCAM-1⁺ and MECA-79⁺ vessels**

The number of respective CD34⁺, MAdCAM-1⁺ and MECA-79⁺ vessels in the entire area of each biopsy specimen was determined at x200 magnification under a BX-51 microscope. The number of MAdCAM-1⁺ or MECA-79⁺ vessels was divided by the number of CD34⁺ vessels to calculate percentages of MAdCAM-1⁺ or MECA-79⁺ vessels, respectively.
Integrin \( \alpha 4 \beta 7 \cdot \text{IgG} \) heterodimeric chimera in situ binding assay

An integrin \( \alpha 4 \beta 7 \cdot \text{IgG} \) heterodimeric chimera was obtained from the culture medium of human embryonic kidney (HEK) 293T cells transiently cotransfected with pcDNA3.1/Hygro-\( \alpha 4 \cdot \text{IgG-FLAG} \) and pcDNA3.1/Hygro-\( \beta 7 \cdot \text{IgG-HA} \) at a 1:1 ratio. Culture media was concentrated ~10-fold using Centriprep YM30 (Millipore, Billerica, MA), as described previously.\(^{32}\) After blocking with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HyClone, South Logan, UT) for 20 min, tissues sections were incubated for 30 min with the integrin \( \alpha 4 \beta 7 \cdot \text{IgG} \) heterodimeric chimera in the same media supplemented with 20 mM HEPES (pH 7.0) and 1 mM MnCl\(_2\). After washing with DMEM, tissues were incubated with anti-FLAG monoclonal antibody M2 (Sigma-Aldrich) diluted 1:100 for 30 min followed by a 30-min incubation with Alexa Fluor 488-labeled goat anti-mouse IgG (Invitrogen, Carlsbad, CA). Tissues were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and observed under an AX-80 fluorescence microscope (Olympus). Negative control experiments were done using DMEM supplemented with 5 mM EDTA throughout the procedure to chelate divalent cations.
Statistical analysis

Differences among groups were statistically analyzed by the Kruskal-Wallis test with Dunn’s post-test using GraphPad Prism 6 software (GraphPad Software, San Diego, CA). $P$ values less than 0.05 were considered significant.
RESULTS

Nodular gastritis and atrophic gastritis tissues exhibit a comparable number of lymphoid follicles

To conduct quantitative analysis to evaluate whether nodular gastritis is characterized by lymphoid follicle hyperplasia, we first determined the number of lymphoid follicles in nodular gastritis and atrophic gastritis. In control normal gastric mucosa, only minimal lymphocyte infiltrate was observed in the lamina propria, and formation of lymphoid follicles was scarcely detected (Fig. 4A). However, in both nodular (Fig. 4B) and atrophic (Fig. 4C) gastritis, we frequently observed formation of lymphoid follicles. As shown in Fig. 5A, the number of lymphoid follicles in both nodular and atrophic gastritis was significantly greater than that seen in normal gastric mucosa in all five biopsy sites; however, this number did not differ significantly between the two types of gastritis.

Lymphoid follicles in nodular gastritis are larger than those in atrophic gastritis

Next, we determined the size of lymphoid follicles in nodular and atrophic gastritis and found that follicles formed in nodular gastritis were larger than those seen in atrophic gastritis (see Fig. 4B, C). Analysis of a total of 195 cases, including normal gastric mucosa, showed that
lymphoid follicle diameter in nodular gastritis was significantly greater than that seen in
atrophic gastritis, with particularly high statistical significance ($p < 0.001$) at biopsy sites A1, A2, IA, and B1 (Fig. 5B). Follicle diameter in atrophic gastritis was comparable to that seen in normal gastric mucosa, except at biopsy site IA, where inflammation is generally more prominent than it is in other biopsy sites.\textsuperscript{33} We then compared occupancy, defined as the ratio of follicle diameter to mucosal thickness (see Fig. 3, left panel), between nodular and atrophic gastritis. As shown in Fig. 6A, except for biopsy site B2, the follicle occupancy ratio in nodular gastritis was never less than 0.64 and was greater than that in atrophic gastritis (which did not exceed 0.51) with high statistical significance ($p < 0.001$). These findings indicate that appearance of enlarged hyperplastic lymphoid follicles is a histological characteristic of nodular gastritis.

**Lymphoid follicles are located more superficially in nodular compared to atrophic gastritis**

We then compared the location of lymphoid follicles between nodular and atrophic gastritis. As indicated schematically in Fig. 3 (right panel), the smaller the A/B ratio, the more superficially located is a lymphoid follicle. Lymphoid follicles in nodular gastritis were
located more superficially than those observed in atrophic gastritis except at biopsy site B2
(Fig. 6B), suggesting that superficial position of lymphoid follicles is an additional indicator
of nodular gastritis.

**MAdCAM-1⁺ HEV-like vessels are induced in both nodular and atrophic gastritis**

We next focused on HEV-like vessels observed in nodular and atrophic gastritis. In both
cases, a portion of CD34⁺ vessels were morphologically similar to HEVs in secondary
lymphoid organs (Fig. 7, top rows), and these HEV-like vessels were also positive for
MAdCAM-1. Such MAdCAM-1⁺ HEV-like vessels were scarcely detected in normal
gastric mucosa (Fig. 7, middle rows). MAdCAM-1⁺ HEV-like vessels may function in
recruitment of memory lymphocytes, because they were bound by an integrin α4β7•IgG
heterodimeric chimera in a divalent cation-dependent fashion (Fig. 8). Interestingly, as
shown in Fig. 9A, while the percentage of MAdCAM-1⁺ HEV-like vessels in both nodular
and atrophic gastritis exceeded that seen in normal gastric mucosa with high statistical
significance (p < 0.001), the percentage did not differ significantly between the two gastritis
conditions except at biopsy site IA, where, as noted, inflammation is generally more
prominent than it is at other biopsy sites.³³ This finding suggests that MAdCAM-1 is
induced on venular endothelial cells in the gastric lamina propria regardless of the type of chronic gastritis, and thus this protein may not directly contribute to the unique pathogenesis of nodular gastritis.

**MECA-79⁺ HEV-like vessels are preferentially induced in areas of nodular gastritis**

As was the case with MAdCAM-1⁺ HEV-like vessels, a portion of CD34⁺ vessels was positive for MECA-79 in both types of gastritis, while MECA-79⁺ HEV-like vessels were hardly detected in normal gastric mucosa (Fig. 7, bottom rows). Interestingly, unlike MAdCAM-1⁺ HEV-like vessels, the percentage of MECA-79⁺ HEV-like vessels in nodular gastritis was greater than that seen in both normal gastric mucosa and atrophic gastritis with high statistical significance ($p < 0.001$) at biopsy sites A1, A2 and IA (Fig. 9B), which precisely match the appearance of gooseflesh seen in endoscopic observation of nodular gastritis.
DISCUSSION

In the present study, we demonstrate that superficially-located, enlarged hyperplastic lymphoid follicles histologically characterize nodular gastritis, and that these follicles correspond to nodular and/or granular lesions resembling gooseflesh, which are observed endoscopically. It is accepted that the characteristic histological finding of nodular gastritis is hyperplasia of lymphoid follicles;\(^2,3,8\) however, quantitative analysis of nodular gastritis focusing on lymphoid follicles has not been reported. To the best of our knowledge, this is the first such analysis employing a substantial number of nodular gastritis samples.

Here we show that the percentage of MAdCAM-1\(^+\) HEV-like vessels in both nodular and atrophic gastritis was significantly greater than that seen in normal gastric mucosa, while this percentage did not differ significantly between the two types of gastritis, suggesting that MAdCAM-1 protein does not preferentially contribute to the pathogenesis of nodular gastritis. This finding is inconsistent with a report by Ohara et al. who demonstrated that the percentage of MAdCAM-1\(^+\) vessels in nodular gastritis was significantly greater than that seen in \textit{H. pylori}-positive controls.\(^4\) This lack of agreement could be due to the fact that the \textit{H. pylori}-positive controls used in that study may have had only a mild chronic inflammation or to differences in sample size.
We also found that the percentage of MECA-79+ HEV-like vessels in nodular gastritis was greater than that in atrophic gastritis with high statistical significance at the biopsy sites A1, A2 and IA, where endoscopic gooseflesh appearance is evident. This finding agrees with previous reports demonstrating that samples obtained from the gastric corpus show far less inflammation and fewer lymphoid follicles compared to antral biopsies.\textsuperscript{11,34}

We also demonstrated that MAdCAM-1 proteins expressed on HEV-like vessels are functional, since an integrin $\alpha 4\beta 7\cdot$IgG heterodimeric chimera bound to these vessels in a divalent cation-dependent manner. We also performed L-selectin$\cdot$IgM chimera \textit{in situ} binding assay but did not detect signals in the samples tested (data not shown). In our previous study of chronic \textit{H. pylori} gastritis, only a fraction of HEV-like vessels were bound by the L-selectin$\cdot$IgM chimera.\textsuperscript{20} These results suggest that interaction between L-selectin and 6-sulfo sLeX contributes in only a limited manner to lymphocyte recruitment in gastric MALT, and that the interaction of integrin $\alpha 4\beta 7$ in its low-affinity state with MAdCAM-1 is important primarily for the “rolling” step of lymphocyte recruitment.

Kamada \textit{et al.} have reported a possible association between nodular gastritis and diffuse-type gastric carcinoma, including signet-ring cell carcinoma.\textsuperscript{6} Indeed, among 99
nodular gastritis cases examined here, one patient also had signet-ring cell carcinoma. It is now evident that development of diffuse-type carcinoma is closely related to *H. pylori* infection; however, earlier it was thought that this carcinoma had little relation to *H. pylori* infection because atrophic changes seen in these patients were not severe in the area adjacent to the tumor. Since atrophic changes are usually inconspicuous in nodular gastritis, we speculate that diffuse-type gastric carcinoma may develop during progression from nodular to atrophic gastritis. Further studies are required to clarify nodular gastritis-associated pathogenesis of diffuse-type gastric carcinoma.
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**Figure legends**

**Fig. 1**  Representative endoscopic appearance of nodular gastritis (A, B) and atrophic gastritis (C).  (A) Multiple small nodular or granular lesions resembling gooseflesh are observed in the gastric antrum.  (B) The gooseflesh appearance is highlighted after spraying tissue with a solution containing indigo carmine stain.  (C) Gastric rugae are flattened and submucosal vascular patterns are visible in the distended stomach.

**Fig. 2**  Specificity of human MAdCAM-1 monoclonal antibody (clone #15-8G-61) as assessed by immunohistochemistry (A), flow cytometry (B) and Western blot analysis (C).  (A) HEVs in human mesenteric lymph nodes were specifically decorated by #15-8G-61.  Bar, 100 μm.  (B) #15-8G-61 specifically recognizes CHO cells stably expressing human MAdCAM-1 (CHO/MAdCAM-1) (gray histogram).  White dotted histogram represents background staining to parental CHO cells.  (C) Western blot analysis demonstrates a single band at ~82 kDa, corresponding to a glycosylated species of human MAdCAM-1.

**Fig. 3**  Schematic representation showing how occupancy (left panel) and location (right panel) of lymphoid follicles were assessed.  (Left panel) Occupancy is reported as the A/B
ratio, where \( A \) is follicle diameter perpendicular to muscularis mucosae and \( B \) is mucosal thickness (distance between the apical surface of foveolar epithelium to the upper face of muscularis mucosae). (Right panel) Location is quantified by the ratio \( A/B \), where \( A \) is the distance between the apical surface of foveolar epithelium and the center of lymphoid follicle, and \( B \) is mucosal thickness. In terms of location, the smaller the \( A/B \) value, the more superficially a lymphoid follicle is located. FE, foveolar epithelium; LF, lymphoid follicle; MM, muscularis mucosae.

Fig. 4  Representative histology of normal gastric mucosa (A), nodular gastritis (B), and atrophic gastritis (C). Lymphoid follicles form in the gastric lamina propria of nodular gastritis and atrophic gastritis. Note that the size of the lymphoid follicle seen in nodular gastritis is greater than that seen in atrophic gastritis. Hematoxylin and eosin (H&E) stain. Bar, 200 \( \mu \)m.

Fig. 5  Comparison of the number (A) and diameter (B) of lymphoid follicles among samples of nodular gastritis, atrophic gastritis, and normal gastric mucosa. Five biopsy sites are indicated as A1, A2, IA, B1 and B2, respectively, based on the updated Sydney system.
Data are expressed as mean ± SEM. *, *p < 0.05; **, *p < 0.001; NS, not significant.

**Fig. 6** Comparison of occupancy (A) and location (B) of lymphoid follicles in nodular gastritis and atrophic gastritis. Five biopsy sites are indicated as A1, A2, IA, B1 and B2, respectively, based on the updated Sydney system. Data are expressed as mean ± SEM. **, *p < 0.01; ***, *p < 0.001; NS, not significant.

**Fig. 7** HEV-like vessels observed in nodular gastritis and atrophic gastritis. HEV-like vessels were immunostained for CD34, MAdCAM-1 or MECA-79. Normal gastric mucosa lacks HEV-like vessels. Bar, 100 μm.

**Fig. 8** Integrin α4β7•IgG heterodimeric chimera in situ binding assay. Frozen stomach tissues with nodular gastritis were probed with an integrin α4β7•IgG heterodimeric chimera in the absence (left panel) or presence (right panel) of 5 mM EDTA to chelate divalent cations. Green fluorescence signals represent integrin α4β7•IgG heterodimeric chimera binding. Bar, 50 μm.
**Fig. 9** Comparison of the percentage of MAdCAM-1$^+$ (A) and MECA-79$^+$ (B) HEV-like vessels among samples of nodular gastritis, atrophic gastritis, and normal gastric mucosa.

Five biopsy sites are indicated as A1, A2, IA, B1 and B2, respectively, based on the updated Sydney system. Data are expressed as mean ± SEM. **, $p < 0.01$; ***, $p < 0.001$; NS, not significant.
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(A) Follicle occupancy

- A1
- A2
- IA
- B1
- B2

*** indicates statistical significance.
NS indicates no significant difference.

(B) Location of follicles

- A1
- A2
- IA
- B1
- B2

*** indicates statistical significance.
NS indicates no significant difference.