Notch2 signaling is required for proper mast cell distribution and mucosal immunity in the intestine

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Notch receptor-mediated signaling is involved in the developmental process and functional modulation of lymphocytes, as well as in mast cell differentiation. Here, we investigated whether Notch signaling is required for antipathogen host defense regulated by mast cells. Mast cells were rarely found in the small intestine of wild-type C57BL/6 mice but accumulated abnormally in the lamina propria of the small-intestinal mucosa of the *Notch2*-conditional knockout mice in naive status. When transplanted into mast cell–deficient W^{sh}/W^{sh} mice, *Notch2*-null bone marrow-derived mast cells were rarely found within the epithelial layer but abnormally localized to the lamina propria, whereas control bone marrow-derived mast cells were mainly found within the epithelial layer. After the infection of *Notch2* knockout and control mice with L3 larvae of *Strongyloides venezuelensis*, the abundant number of mast cells was rapidly mobilized to the epithelial layer in the control mice. In contrast, mast cells were massively accumulated in the lamina propria of the small intestinal mucosa in *Notch2*-conditional knockout mice, accompanied by impaired eradication of *Strongyloides venezuelensis*. These findings indicate that cell-autonomous Notch2 signaling in mast cells is required for proper localization of intestinal mast cells and further imply a critical role of Notch signaling in the host-pathogen interface in the small intestine. (*Blood*. 2011;117(1):128-134)

Introduction

Mast cells are important in a wide variety of physiologic and pathologic processes, including protective immune responses to parasites and allergic disorders.[1,2] In intestinal parasite infection, mast cells play a central role in the immune response.[3] During the induction phase of parasite-induced inflammation, mast cells move from the submucosa to the tip of the villi, accompanying the serial changes in the protease expression pattern. Initially, they are positive for mouse mast cell protease-5 (mMCP-5) but negative for mMCP-1 and mMCP-2; eventually, they become positive for mMCP-1 and mMCP-2 but negative for mMCP-5, demonstrating convergence from connective tissue–type mast cells (CTMCs) to mature mucosal-type mast cells (MTMCs).[4] The parasite-infected mice consequently experience jejunal mast cell hyperplasia,[5] and the serum concentration of mMCP-1, an activation marker of small intestinal mast cells, is increased by > 1000-fold compared with that in the naive status.[5]

In the mammalian immune system, we and other groups have demonstrated that Notch signaling is involved in the commitment and differentiation of T cells, the development of splenic marginal zone B cells, and the differentiation and functional modulation of mature T cells, including T-helper type I (Th1)/Th2 polarization[6,7] and differentiation of CD8-positive cytotoxic T cells.[8] Regarding the Notch signaling in mast cells, bone marrow-derived mast cells (BMMCs) highly express Jagged1[9] and Notch2[10] among the Notch ligands and the receptors, respectively. We have previously shown that signaling through the Notch2 receptor induces mast cell development from myeloid progenitors by transcriptional up-regulation of hairy and enhancer of split homolog-1 (Hes-1) and transacting T cell–specific transcription factor GATA-3 (GATA3).[11] Induction of antigen-presenting potential of mast cells by Notch signaling is also demonstrated.[12] A question yet to be solved is how Notch signaling affects mast cell properties in vivo.

In this report, we examined the effect of Notch2 signaling in in vivo mast cells using *Notch2*-conditional knockout mice.[13] We show that Notch2 signaling is specifically required for intraepithelial localization of intestinal mast cells and antiparasite immunity. In contrast, Notch2 is dispensable for either distribution or development of CTMCs.

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Methods

Mice

The generation of *Notch2*$^{flox/flox}$ mice was described previously.[13] Mx-*Cre* transgenic mice[14] were crossed with *Notch2*$^{flox/flox}$ mice (*N2*-MxcKO mice) and the progeny were injected with polyinosinic-polycytidylic acid (pIpC; Sigma-Aldrich) 7 times every other day from 3 days after birth (25 μg/g body weight) or 3 times between 4 and 6 weeks of age (20 μg/g body weight). *N2*-MxcKO mice were further crossed with C57BL/6-Ly5.1 mice (a kind gift from Dr H. Nakauchi, University of Tokyo) to generate Ly5.1-*N2*-MxcKO mice. *Notch2* deletion in bone marrow was examined by polymerase chain reaction and 3% agarose gel electrophoresis[13] (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). *W*^{sh}/*W*^{sh} mice were purchased from The Jackson Laboratory. All experiments were done with approval from the University of Tsukuba Institutional Review Board.

Staining

Sections, fixed with Carnoid fluid, were stained with 0.5% toluidine blue (Sigma-Aldrich), pH 0.3, followed by eosin. Small intestine was embedded in optimal cutting temperature (OCT) compound (TissueTek) and cut with cryostat (Leica CM1850). The section was fixed with 4% paraformaldehyde, washed with phosphate-buffered saline (PBS), blocked in 10% horse serum and 0.1% Triton-PBS, and then stained with either 1:100 goat anti-Jagged1 antibody (C-20; Santa Cruz Biotechnology), goat anti-Delta1 antibody (Genzyme Tech), or control goat immunoglobulin G (IgG; Santa Cruz Biotechnology) overnight at 4°C. The sections were washed with PBS and stained with anti–goat Alexa 594 (Invitrogen). Sections were analyzed by fluorescence microscope (Zeiss; Axioplan2), original magnification ×200.

BMMCs

Bone marrow cells from each mouse strain were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 50 ng/mL stem cell factor (SCF; PeproTech), and 10 ng/mL interleukin-3 (IL-3; PeproTech) for 4 weeks. Generation of BMMCs was confirmed by staining with lineage markers, c-Kit and IgE, as previously described.[11] Briefly, the cells were incubated with purified IgE (BD Biosciences) after blocking the Fcγ receptors with purified anti-CD16/32 antibody (BD Biosciences), stained with anti-IgE–fluorescein isothiocyanate (FITC; BD Biosciences), anti–Gr-1–phycoerythrin (PE), anti-Mac1–PE (eBioscience), and anti–c-Kit–allophycocyanin (APC; eBioscience), and then analyzed by FACSCalibur (BD Biosciences).

Peritoneal mast cells

Five milliliters ice-cold PBS was injected into the peritoneal cavity, and then 3 mL PBS was recovered. c-Kit and IgE receptor (FcεRI) expression was used to define the cells as peritoneal mast cells. Ly5.1 and Notch2 were stained with anti-Ly5.1–PE (BD Biosciences) or biotinylated anti-Notch2 antibody (clone HMN2-35)[8] followed by streptavidin PE (eBioscience), respectively.

Bone marrow transplantation

C57BL/6 mice and *W*^{sh}/*W*^{sh} mice were lethally irradiated with a total dose of 9.5 Gy and then transplanted with 1×10^7 whole bone marrow cells from either *N2*-MxcKO-Ly5.1 mice or *Notch2*$^{flox/flox}$-Ly5.1 mice from the tail vein. Tissues of transplanted mice were assessed at 3 to 4 months after transplantation. Donor-cell engraftment was assessed by fluorescence-activated cell sorting (FACS) analysis of peripheral blood, which was stained by anti-Ly5.2–FITC (BD Biosciences) and anti-Ly5.1–PE.

S venezuelensis infection

Mice were infected by subcutaneous injection of third-stage infective larvae of *Strongyloides venezuelensis*. The degree of infection was monitored by counting the number of eggs per gram of feces. Mast cells were counted and presented as the number per 10 villus crypt units. BMMCs were washed with PBS twice and then cultured with 10 ng/mL IL-4 and 10 ng/mL IL-10 for 3 days. These Th2-conditioned BMMCs were injected at day 3 and day 6 of experiments.[15] In contrast to the bone marrow transplantation, mice were not irradiated before BMMC injection.

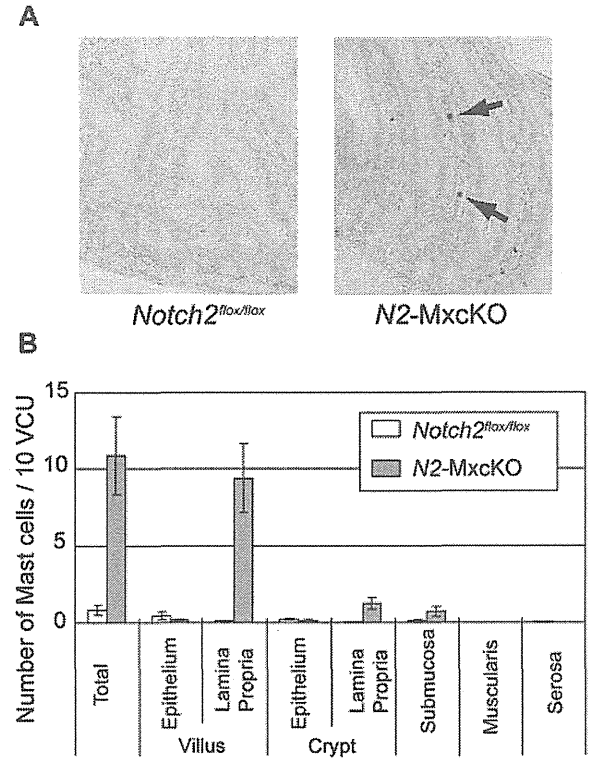


Figure 1. Mature mast cells were abnormally accumulated in the lamina propria of the small intestine of *Notch2*-deficient mice. (A) Sections of the small intestine of *N2*-MxcKO or littermate control *Notch2*$^{flox/flox}$ mice. Toluidine blue staining, followed by eosin. Original magnification ×200. (B) The numbers of mast cells per 10 villus crypt units (vcus) distributing to various layers of the small intestine. Data are presented as means ± SEM; *Notch2*$^{flox/flox}$ (n = 10) versus *N2*-MxcKO (n = 8); $P = .000461$ (total), $P = .000261$ (villus, lamina propria), $P = .001918$ (crypt, lamina propria), $P = .046874$ (submucosa).

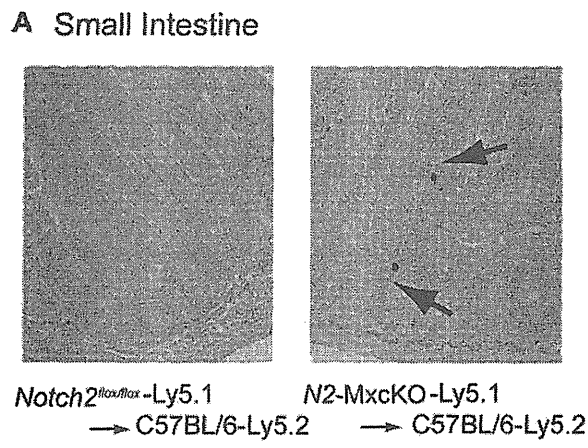
Statistical analysis

The data for the number of mast cells and the *S venezuelensis* infection data were analyzed by the *t* test. *P* values < .05 were considered significant.

Results

Notch signaling affects the number and localization of mast cells in the small intestine

We have previously reported that Notch2 regulates mast cell differentiation in vitro.[11] To examine whether Notch2 controls the differentiation or development of MTMCs in vivo, we examined intestinal mast cells by toluidine blue staining in C57BL/6 mice carrying the *Notch2*$^{flox/flox}$ allele with or without the Mx1-*Cre* transgene (*N2*-MxcKO mice or *Notch2*$^{flox/flox}$ mice, respectively) after pIpC treatment.[13] Mast cells were only sparsely detected in the small intestine of *Notch2*$^{flox/flox}$ mice, mainly within the epithelium. However, the total number of mast cells in the small intestine of *N2*-MxcKO mice was unanticipatedly greater than that of *Notch2*$^{flox/flox}$ mice. Furthermore, those mast cells were mainly



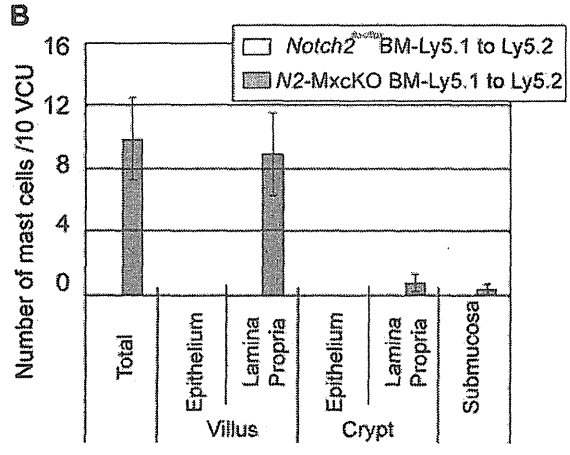


Figure 2. Localization of intestinal mast cells is abnormal in wild-type mice transplanted with *N2*-MxcKO-Ly5.1 bone marrow cells, reminiscent of that in *N2*-MxcKO mice. (A) Bone marrow cells from either *N2*-MxcKO- Ly5.1 mice or littermate *Notch2*$^{flox/flox}$- Ly5.1 mice were transplanted into lethally irradiated (9.5 Gy) C57BL/6-Ly5.2 mice. Toluidine blue staining, followed by eosin. Original magnification ×200. (B) The numbers of mast cells per 10 vcus distributing to various layers of the small intestine. Data are presented as means ± SEM; Mast cells in C57BL/6-Ly5.2 mice transplanted with *Notch2*$^{flox/flox}$-Ly5.1 (n = 3) versus *N2*-MxcKO-Ly5.1 (n = 3). $P = .020594$ (total) and $P = .030123$ (villus, lamina propria).

localized to the lamina propria, and very few mast cells were found within the epithelium (Figure 1A-B).

Localization of MTMCs is abnormal in wild-type mice transplanted with *N2*-MxcKO bone marrow cells, reminiscent of that in *N2*-MxcKO mice

Because the Mx-*Cre*–based conditional knockout system deletes target genes not only in the bone marrow cells but also, albeit partially, in the intestinal cells,[14] there was a possibility that *Notch2* deletion in the intestinal cells was responsible for the distinct distribution pattern or increased number of mast cells in *N2*-MxcKO mice compared with control mice. To exclude this possibility, we transplanted *Notch2*-null bone marrow cells carrying the Ly5.1 marker to irradiated wild-type C57BL/6-Ly5.2 mice. A chimerism of donor-derived Ly5.1-positive fraction accounted for more than 70% in the peripheral blood (data not shown). The recipients of bone marrow cells from *Notch2*$^{flox/flox}$ mice showed that the intestinal mast cell distribution was virtually the same as that in wild-type mice, whereas the recipients of *Notch2*-null bone marrow cells showed an increase in mast cells mainly in the lamina propria in an indistinguishable manner from the *N2*-MxcKO mice (Figure 2A-B). This result indicates that deletion of *Notch2* in bone marrow-derived cells alters the distribution pattern and increases the number of mast cells in the small intestine.

Notch-ligand expression in the small intestine

Notch signaling is known to be activated through Notch ligand-receptor binding.[16] We examined the expression pattern of Notch ligands in the small intestine with antibodies against Notch ligands Jagged1 and Delta1 and found that the epithelial layer was clearly stained with anti-Jagged1 but not with anti-Delta1 antibody (Figure 3). The staining with the anti-Jagged1 antibody was confined to the surface of epithelial cells, especially at their basal side rather than the apical side (Figure 3). The Jagged1 expression pattern suggests a possibility that Jagged1-Notch2 interaction between the basal side of the epithelial cells and mast cells has an important role for mast cell migration from the lamina propria across the basement membrane toward the epithelium (Figure 3). Furthermore, the ligand-receptor binding itself might contribute to mast cell–epithelial cell adhesion to some extent, based on our observation that *Notch2*-expressing BMMCs attached to the Jagged1-expressing Chinese hamster ovary (CHO) cells, while *Notch2*-null BMMCs did not (supplemental Figure 2).

Notch2 is dispensable for the CTMC development and distribution

We next investigated the roles of Notch2 in the development of CTMCs. The localization and the number of CTMCs in the skin and peritoneal cavity were not significantly different between *N2*-MxcKO and littermate *Notch2*$^{flox/flox}$ mice more than 4 weeks after the treatment with pIpC (data not shown). This observation might simply indicate that the Mx-*Cre* system was inefficient in the tissue-resident mast cells, as a great majority of peritoneal

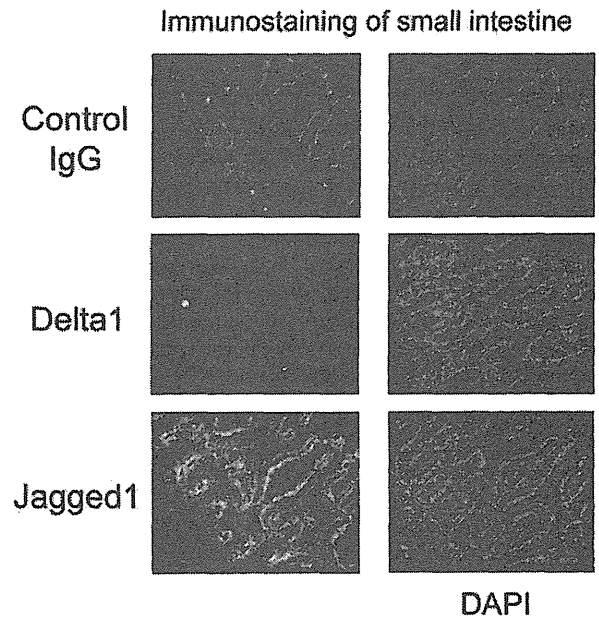


Figure 3. Jagged1 is strongly expressed on the surface of the epithelial cells, especially at their basal side. A section of small intestine prepared using cryostat was stained with goat anti-Jagged1 and goat anti-Delta1 antibodies followed by anti–goat Alexa594. Original magnification ×200.

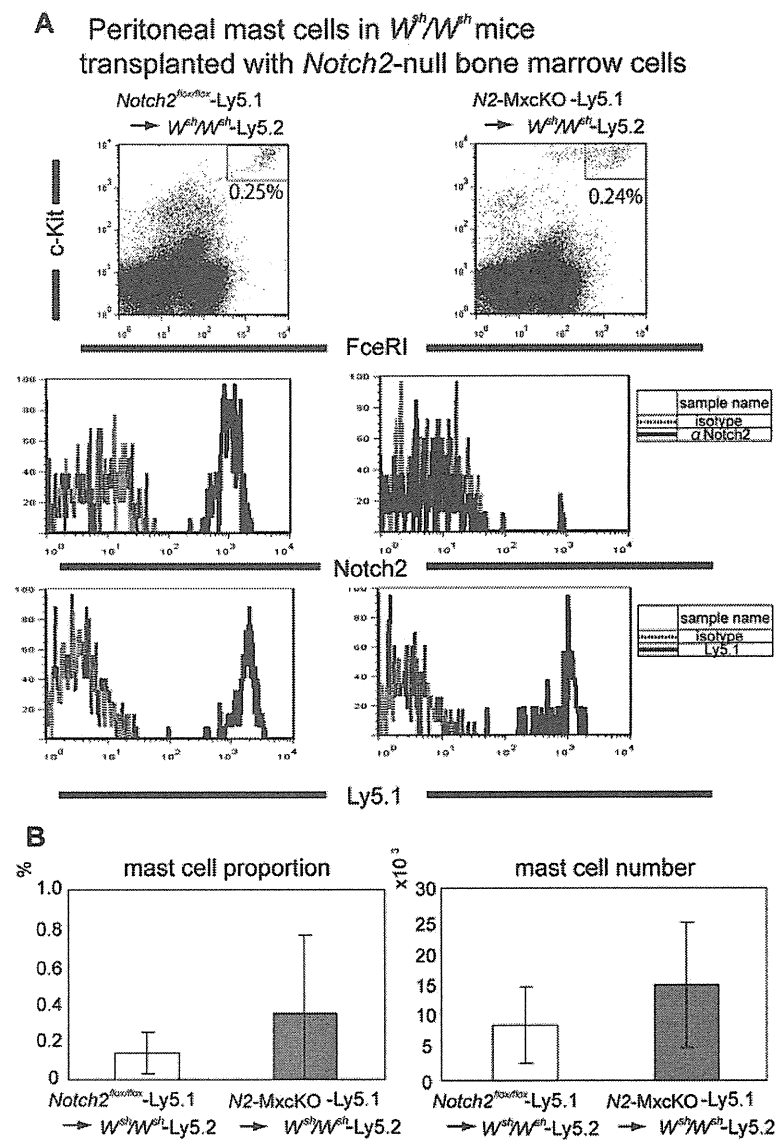


Figure 4. Notch2 is not required for peritoneal mast cell development. (A) Bone marrow cells from *N2*-MxcKO-Ly5.1 mice or control *Notch2*$^{flox/flox}$-Ly5.1 mice were transplanted into lethally irradiated *W*^{sh}/*W*^{sh} mice. Peritoneal mast cells were stained with anti–c-Kit–APC, IgE, and biotinylated anti-Notch2 antibody (HMN2-35), followed by anti-IgE–FITC and streptavidin-PE, or they were stained with anti–c-Kit–APC, IgE, and anti-Ly 5.1–PE, followed by anti-IgE–FITC; they were then analyzed by FACSCalibur (BD Biosciences). (B) The proportion (left) and the absolute number (right) of peritoneal mast cells were not significantly different between *W*^{sh}/*W*^{sh} mice transplanted with *Notch2*-WT bone marrow cells and those transplanted with *Notch2*-null bone marrow cells. $P = .210642$ (mast cell proportion) and $P = .196045$ (mast cell number).

mast cells of pIpC-treated *N2*-MxcKO mice still expressed Notch2 (data not shown). Therefore, to clarify the requirement of *Notch2* in the CTMC development, we examined peritoneal mast cells in mast cell-deficient *W*^{sh}/*W*^{sh} mice after transplantion of *Notch2*-null bone marrow cells carrying the Ly5.1 marker. In this system, mast cells exclusively develop from transplanted bone marrow progenitors, in which the *Cre* recombinase under the Mx-promoter is quite effective [14] (supplemental Figure 1). In this experiment, we found that the proportion and absolute number of peritoneal mast cells was not significantly different between those developed from the *N2*-MxcKO-Ly5.1 bone marrow cells and those developed from littermate *Notch2*$^{flox/flox}$-Ly5.1 bone marrow cells (Figure 4A-B). Notch2 was not expressed in the peritoneal mast cells derived from *N2*-MxcKO-Ly5.1 bone marrow cells but was expressed in those derived from littermate *Notch2*$^{flox/flox}$-Ly5.1 bone marrow cells (Figure 4A middle), indicating that *Notch2* was deleted efficiently. These results suggest that Notch2 is dispensable for the development and distribution of CTMCs.

Cell-autonomous Notch2 signaling in mast cells is important for mast cell migration across the basement membrane in the small intestine

We then asked a question whether aberrant mast cell migration in the small intestine in *N2*-MxcKO mice is dependent on Notch2 signaling in mast cells per se. We intravenously infused *Notch2*-null or control BMMCs into nonirradiated *W*^{sh}/*W*^{sh} mice after *S venezuelensis* infection, because it is reported that BMMCs could only transiently reconstitute intestinal mast cells in mast-cell deficient mice if these recipient mice are in naive status.[17] In tissue sections, we found that the distribution of mast cells in the small intestine was different between control BMMCs-reconstituted mice and *Notch2*-null BMMCs-reconstituted mice; control BMMCs were mainly migrated into the epithelial layer, while a majority of *Notch2*-null BMMCs remained in the lamina propria. This observation indicates that mast cell-autonomous Notch2 expression contributes to mast cell migration across the basement membrane from lamina propria into the epithelial layer (Figure 5A-B). Even in the control BMMC-infused mice, however, a substantial proportion of

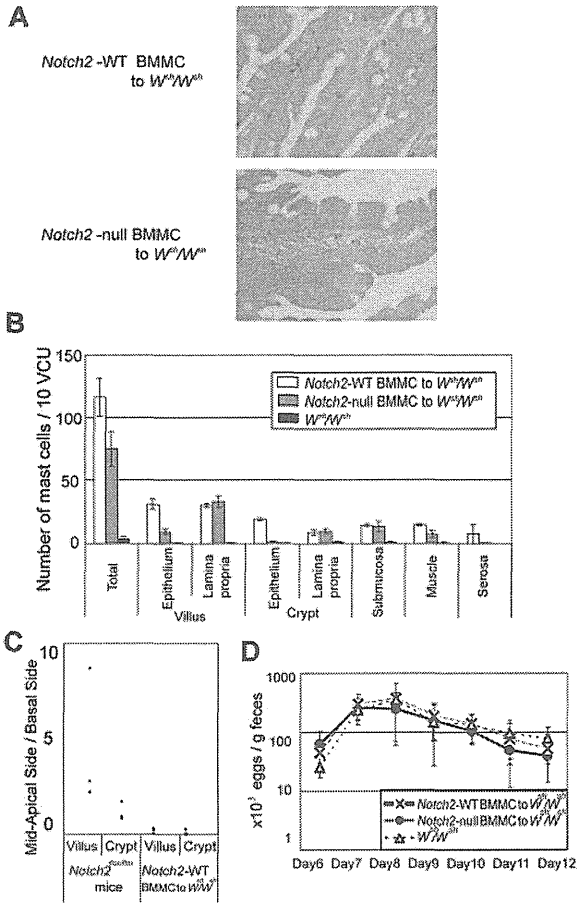


Figure 5. Mast cell–autonomous Notch2 expression is required for mast cell migration toward the epithelium. *Wsh/Wsh* mice infected with *S venezuelensis* were intravenously infused with Th2-conditioned *Notch2*-null or control BMMCs on days 3 and 6 of infection. (A) *Notch2*-null BMMCs poorly migrated toward the epithelium compared with control BMMCs. Toluidine blue staining followed by eosin staining. Original magnification ×200. (Top) Control BMMCs; (Bottom) *Notch2*-null BMMCs. (B) The number of mast cells per 10 vcus in the small intestine on day 12 after *S venezuelensis* infection in *Wsh/Wsh* mice, without BMMC infusion, with control BMMC infusion, and with *Notch2*-null BMMC infusion. Data are presented as means ± SEM; n = 3 (control BMMC infusion) and n = 4 (*Notch2*-null BMMC infusion), $P = .004080$ (villus, epithelium) and $P = .000020$ (crypt, epithelium). Note that mast cells in *Wsh/Wsh* mice infused with *Notch2*-null BMMCs abnormally resided in the lamina propria, whereas most of those in *Wsh/Wsh* mice infused with control BMMCs had intraepithelially migrated. (C) Mast cell number in mid to apical side of the epithelial layer was divided with that in the basal side of the epithelial layer. (D) Time course of *S venezuelensis* egg numbers in the stool. The number of excreted eggs was not significantly different between *Wsh/Wsh* mice infused with *Notch2*-null and control BMMCs. Data are presented as means ± SEM.

mast cells still remained in the lamina propria, submucosa, and smooth muscle layers, and the distribution of mast cells within the epithelium was confined to the basement membrane side of the epithelial layer (Figure 5B-C). This mast cell localization pattern was different from that in the *Notch2*$^{flox/flox}$ mice with *S venezuelensis* infection, in which mast cells were present mainly at the mid to apical side of the epithelial layer (Figure 5C). The numbers of *S venezuelensis* eggs in the stool were virtually the same in the *S venezuelensis*–infected *Wsh/Wsh* mice infused with *Notch2*-null and control BMMCs and in the *S venezuelensis*–infected *Wsh/Wsh* mice without any BMMC infusion throughout the period after infection (Figure 5D).

Taken together, the BMMC-*Wsh/Wsh* transplantation model demonstrated that Notch2 in the mast cells indeed determines their intraepithelial migration from lamina propria; nevertheless, this model was not adequate to examine the physiologic mast cell distribution pattern and subsequent parasite expulsion that depends on mast cells.

Notch2 signaling regulates antiparasite immunity of mast cells in the intestine

The BMMC-*Wsh/Wsh* reconstitution model could not completely reflect physiologic mast cell distribution pattern in the small intestine. Therefore, to further assess the effect of Notch2 signaling on the mucosal immune response of intestinal mast cells under a pathologic condition, *N2*-MxcKO or control *Notch2*$^{flox/flox}$ mice were infected with *S venezuelensis*. Total mast cell number was increased in *Notch2*$^{flox/flox}$ mice much more than in *N2*-MxcKO mice, especially in the epithelium in both crypts and villi 8 days after infection (Figure 6A-B). Thirteen days after infection, mast cells in the epithelium in *Notch2*$^{flox/flox}$ mice were still more abundant than those in *N2*-MxcKO mice (Figure 6C-D), while mast cell accumulation in the lamina propria in *N2*-MxcKO mice was more prominent in both villi and crypt than that in the earlier stage of infection (Figure 6A,C). In particular, dense aggregation of mast cells was prominent in the lamina propria of *N2*-MxcKO mice at the tip of the villi (Figure 6D). As a consequence, the total number of mast cells in the intestine of *N2*-MxcKO mice became equivalent to those of *Notch2*$^{flox/flox}$ mice 13 days after infection (Figure 6C,E). The number of *S venezuelensis* eggs in the stool was gradually decreased during day 8 to 10 in control *Notch2*$^{flox/flox}$ mice but not in *N2*-MxcKO mice (Figure 6F). Furthermore, the worms were still observed in *N2*-MxcKO mice but not in *Notch2*$^{flox/flox}$ mice 12 days after infection (Figure 6G). These data suggest that Notch2 deficiency alters the distinct distribution pattern of mast cells in the small intestine, which is responsible for the defective eradication of *S venezuelensis*.

Discussion

There is a growing body of evidence that Notch signaling modulates cellular migration and adhesion in endothelial, neural, and lymphoid lineage cells, as well as cancer cells.[18] We have shown that Notch2 signaling induces the development of mast cells.[11] However, it has remained unclear whether Notch2 signaling is involved in the distribution of mast cells in the intestinal mucosa or connective tissues or in controlling the functions of mast cells against microorganisms. Here, we investigated the role of Notch2 signaling in mast cells in terms of their distribution and functions using cell-specific *Notch2*-deficient mice. We found that in *N2*-MxcKO mice, mast cells were abnormally accumulated in the lamina propria of the small intestine, suggesting that *Notch2*-null mast cells have some defect in the migration toward the epithelium. Furthermore, *N2*-MxcKO mice failed to eradicate *S venezuelensis* and exhibited a distinct mast cell migration pattern in the intestine compared with control mice, suggesting that mast cells regulate the host-microbial interface in the intestine through Notch2 signaling.

Mast cell number was rather increased in the intestinal mucosa of *N2*-MxcKO mice compared with control mice in naive status. Mast cell progenitors were supposed to reside in the submucosa and gradually move toward the villi, accompanied by their differentiation into mature mast cells. Based on our observation in

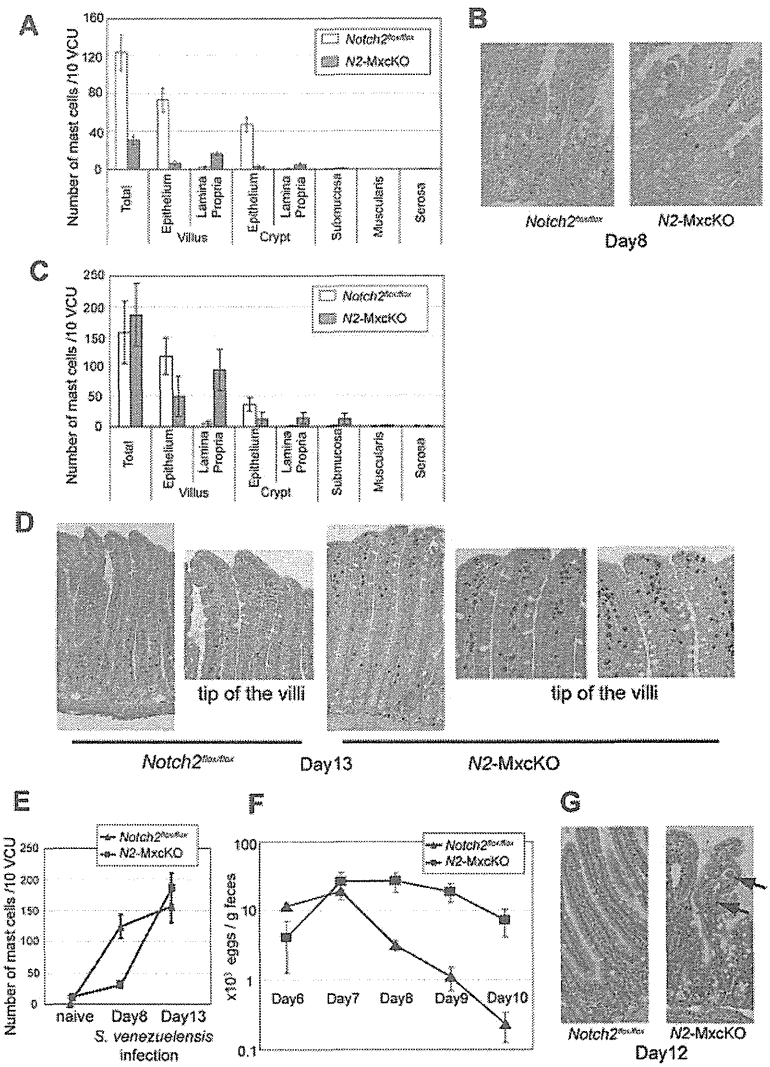


Figure 6. Notch2 is essential for antiparasite immunity of mast cells in the intestine. *N2*-MxcKO or control *Notch2*$^{flox/flox}$ mice were subcutaneously injected with third-stage infective larvae of *S venezuelensis*. (A) The number of mast cells per 10 vcus in the small intestine on day 8 after *S venezuelensis* infection. Data are presented as means ± SEM. The number of mast cells was much less in *N2*-MxcKO mice; n = 3, $P = .008592$ (total), $P = .005695$ (villus, epithelium), $P = .000715$ (villus, lamina propria), $P = .005245$ (crypt, epithelium), and $P = .045466$ (crypt, lamina propria). Note that mast cells in *N2*-MxcKO mice were abnormally clustered in the lamina propria, whereas most of those in the control *Notch2*$^{flox/flox}$ mice were intraepithelially migrated. (B) Toluidine blue staining followed by eosin staining of the small intestine on day 8; original magnification ×200. (C) The number of mast cells per 10 vcus in the small intestine on day 13 after *S venezuelensis* infection. Data are presented as means ± SEM; n = 3, $P = .026076$ (villus, epithelium), $P = .00194$ (villus, lamina propria), $P = .021177$ (crypt, epithelium), and $P = .019324$ (crypt, lamina propria), $P = .047445$ (submucosa). (D) Toluidine blue staining followed by eosin staining of the small intestine on day 13. Original magnification ×200. (E) The total number of mast cells per 10 vcus on day 0, day 8, and day 13 of infection. The total number of mast cells was significantly lower in *N2*-MxcKO mice at the early phase (day 8) and almost equal at the later phase (day 13) to that of control mice. Data are presented as means ± SEM; n = 10 and 8 (day 0, *Notch2*$^{flox/flox}$ and *N2*-MxcKO); n = 3 and 3 (day 8, *Notch2*$^{flox/flox}$ and *N2*-MxcKO); n = 4 and 4 (day 13, *Notch2*$^{flox/flox}$ and *N2*-MxcKO). (F) Time course of egg number in the stool. The number of excreted eggs was significantly greater in *N2*-MxcKO mice compared with those in *Notch2*$^{flox/flox}$ mice. Data are represented as means ± SEM; n = 4; $P = .0291$ (day 8) and $P = .0219$ (day 9). (G) Hematoxylin-eosin staining of the small intestine on day 12. Original magnification ×200. Arrows indicate worms. Worms were still observed in the villi in the jejunum of *N2*-MxcKO, but not of *Notch2*$^{flox/flox}$ mice.

an *S venezuelensis*–infection model, mast cells increase in number in the epithelium in control *Notch2*$^{flox/flox}$ mice, while they abnormally aggregate in lamina propria in *N2*-MxcKO mice, especially in the later stage of infection. This suggests that mast cell migration from lamina propria toward the epithelium across the basement membrane is impaired in *N2*-MxcKO mice. Consequently, mast cell turnover might be prolonged in *N2*-MxcKO mice. Given that the mechanism of mast cell migration from lamina propria toward the epithelium is common in naive status and infection status, such migration defect may also explain the mast cell increase in *N2*-MxcKO mice in naive status that we observed.

The defect of mast cell migration toward intraepithelium of the small intestine in *N2*-MxcKO mice is very similar to that in integrin β6-deficient mice,[19] in which activation of transforming growth factor (TGF)–β signaling is impaired.[20] A crosstalk between Notch signaling and TGF-β signaling might occur in intestinal mast cells as well as the cases of other cell types.[21] Alternatively, Notch signaling might directly regulate a downstream target of TGF-β1 in intestinal mast cell migration (eg, the induction of integrin αE expression).[19,22] Integrin αE, forming an integrin αEβ7 complex on mast cells, binds to E-cadherin on epithelial cells and is involved in mast cell localization in the epithelium.[22] The expression level of integrin αEβ7, measured by flow cytometric analysis, however, was not affected by Notch-ligand stimulation in BMMCs (unpublished data).

In the previous paper we showed that Notch signaling facilitates mast cell lineage development at the expense of granulocyte/macrophage development from both common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs) in vitro.[11] Mast cells, however, were not depleted in *N2*-MxcKO mice in naive status in vivo, but rather slightly increased in the small intestine of *N2*-MxcKO mice. This clearly indicates that Notch2 signaling is dispensable for steady-state mast cell generation in vivo. However, the dynamic increase of mast cells during the early phase of intestinal parasite infection was markedly impaired in *N2*-MxcKO mice. The mechanisms underlying the Notch2 signaling requirement only in parasite-infected mice remain to be clarified. Nevertheless, rapidly increasing intestinal mast cells have to be supplied by mast cell progenitors. The pathways and mechanisms responsible for mast cell progenitor recruitment and trafficking are likely to be dynamic and susceptible to modification during inflammation.[1] Such a modulation of the mast cell generation pathway during intestinal infection might underlie the requirement of *Notch2* only during parasite infection. This is similar to

IL-3–deficient mice. IL-3 is essential for mast cell differentiation in vitro; however, IL-3–deficient mice have the normal number of mast cells at the steady state, whereas mast cell hyperplasia is impaired upon intestinal parasite infection.[23]

Our data showed that parasite expulsion was impaired in *N2*-MxcKO mice. We could not exclude the possibility that the *Notch2* deletion in immune cells other than mast cells modulate the response against the nematode infection. If we could show that Th2-conditioned wild-type BMMCs successfully eradicate *S venezuelensis* in W^{sh}/W^{sh} mice and that *Notch2*-null BMMCs do not, it would be clearer that Notch2 signaling in mast cells per se but not in other immune cells should be critically important for defense against *S venezuelensis* infection. The failure of rescue experiments may be caused by the abnormal mast cell distribution pattern of wild-type BMMCs in W^{sh}/W^{sh} mice. Nevertheless, the result of this experiment supported the previous finding that the proper epithelial migration of mast cells is required for efficient expulsion of *S venezuelensis*[24] and thus provides an insight that the impaired *S venezuelensis* expulsion in *N2*-MxcKO mice is attributed to the mast cell-autonomous deletion of *Notch2*.

In conclusion, our data clearly indicate that Notch2 receptor signaling is specifically required for proper intestinal mast cell distribution in a cell-autonomous manner. Furthermore, involvement of Notch2 signaling in mucosal immunity was proven, particularly for eradication of infected parasites, although whether this is due to the Notch2 signaling in mast cells is yet to be elucidated.

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Authorship

Contribution: M.S.-Y. designed and performed the research, analyzed the data, and wrote the paper; T.S., Y. Miyake, and Y. Morishita performed the research; T.I.S., H.M., and H.Y. contributed new reagents; E.N.-Y., K.K., M.F., S.O., and M.K. provided vital discussion; K.Y. designed the research; and S.C. designed the research, analyzed the data, and wrote the paper.

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Case report

Zoonotic filariasis caused by *Onchocerca dewittei japonica* in a resident of Hiroshima Prefecture, Honshu, Japan

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ABSTRACT

A female of *Onchocerca* sp. was found to be the probable causative agent of a subcutaneous nodule in the left knee of a 70-year-old man in a rural area of Hiroshima Prefecture, Honshu, the main island of Japan. We compared the characteristics of the agent with the features of the four previously suspected species found in cattle and horses in various parts of the world, as well as *O. lupi* and *O. jakutensis* that were suspected or proved, respectively, in zoonotic cases in Europe. In addition, the morphologic characteristics of this parasite were compared with those of the four *Onchocerca* species found in wild animals in Japan. Based on such characteristics as the large triangle ridges, the considerable distance between any two adjacent ridges, and the absence of inner cuticular striae in the longitudinal sections, we found the causative agent in the present case to be identical to the female of *Onchocerca dewittei japonica*. All five previous cases of zoonotic onchocerciasis in Japan had been found in Oita, Kyushu, the main southern island. This human case caused by *O. dewittei japonica* suggests that zoonotic onchocerciasis is liable to occur in rural areas in Japan where wild boar, *Simulium* vectors, and humans overlap.

1. Introduction

Zoonotic filariasis is an infection found in humans which is caused by filarioids of animals [1]. Numerous human cases caused by members of the genus *Dirofilaria* have been found throughout the world [2]. In contrast, human cases caused by *Onchocerca* species parasitic in animals are rare; the first of 15 cases known to date was reported in Ukraine in 1965. Five human cases in Europe, five in Japan, four in North America, and one on the Arabian Peninsula have been discovered worldwide [3–18].

In zoonotic onchocerciasis, the causative agents suspected are *O. gutturosa* Neumann, 1910 from cattle and *O. cervicalis* Railliet and Henry, 1910 from horses [3–8,13]. Much more recently, in Japan, *Onchocerca dewittei japonica* Uni et al., 2001;[19] from wild boar was identified in four of the most recent five cases in Oita, Kyushu, the main southern island of Japan [9,12,14,15]. In retrospect, *O. lupi* found from dogs was suspected to be responsible for subconjunctival infections [3,11] in which the causative agent could not be unambiguously determined in Europe [20]. Finally, *Onchocerca* of the red deer, *O. jakutensis* (Guvanov, 1964) was identified in a patient in Austria [17].

Here we present a new case of a zoonotic onchocerciasis found from a patient living in a rural area of Hiroshima Prefecture in Honshu, the main island of Japan.

2. Case study

The patient, a 70-year-old man living in Fuchu City, Hiroshima Prefecture, found a nodule on the left knee in the beginning of the year 2009 and reported feeling pain in the nodule in May 2009. The nodule, 2 cm in diameter, was surgically removed from the subcutaneous connective tissue at the knee at the Shobara Red Cross Hospital in Hiroshima Prefecture in July 2009. The mass excised (1×2 cm) was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin, a routine process. The sections were stained with hematoxylin and eosin. Histologic sections examined: S3-1, S3-2, S3-4, and S7-9.

A coiled worm was found in the nodule and several longitudinal, oblique, and transverse sections of the main part of the body (midbody) of the worm were examined with a microscope (Fig. 1; 1–5). The worm

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was a female with its uteri in which microfilariae or embryos were not seen (Fig. 1; 1 and 4). In addition, a section of the thin anterior part, 83 μm wide, with the esophagus and a section of the posterior part of the worm, 125 μm wide, were found. Thus, this nodule was occupied by one female adult.

In the longitudinal sections of the worm (Fig. 1; 2), the external transverse ridges were salient on the cuticle and the distance between two adjacent ridges in the main body is shown in Table 1. Our examination revealed two adjoining ridges of one side indicated by two arrows; an arrowhead indicated a small overlapping ridge of the other side (Fig. 1; 2). The distance between the ridges was therefore measured on the ridges of the one side. The ridges formed a sharp triangle (Fig. 1; 3). The cuticle was divided into two main parts of equal thickness and no inner striae were found on the middle line (Fig. 1; 3).

In the transverse sections of the main body, the cuticle was composed of four layers, the muscle cells were 42–52 in number, and the two lateral chords were large. No inner projections of the cuticle were found at the lateral chords. The transverse sections of the midbody were round and lateral thickening of the cuticle was not seen (Fig. 1; 4). The differences of the thickness of the cuticle can be seen at the ridge in Fig. 1; 5. The difference (30 μm) between the thick cuticle (55 μm) and the thin cuticle (25 μm) corresponded to the height of the ridges of *O. dewittei japonica* (Table 1).

Regarding the histologic reaction of the host, the worm was surrounded with eosinophilic exudate in the center of the granuloma while away from the center, macrophages, neutrophils, eosinophils, and lymphocytes had infiltrated the granulomatous tissue (Fig. 1; 1). Macrophages and eosinophils intensively accumulated around some sections of disintegrating parts of the worm (Fig. 1; 5) but neutrophils were very scarce.

The patient, a farmer, lives in a rural area near mountains inhabited by wild boar. He has never been outside Japan and had not visited Oita, Kyushu, within the past several years. He has not raised pets such as

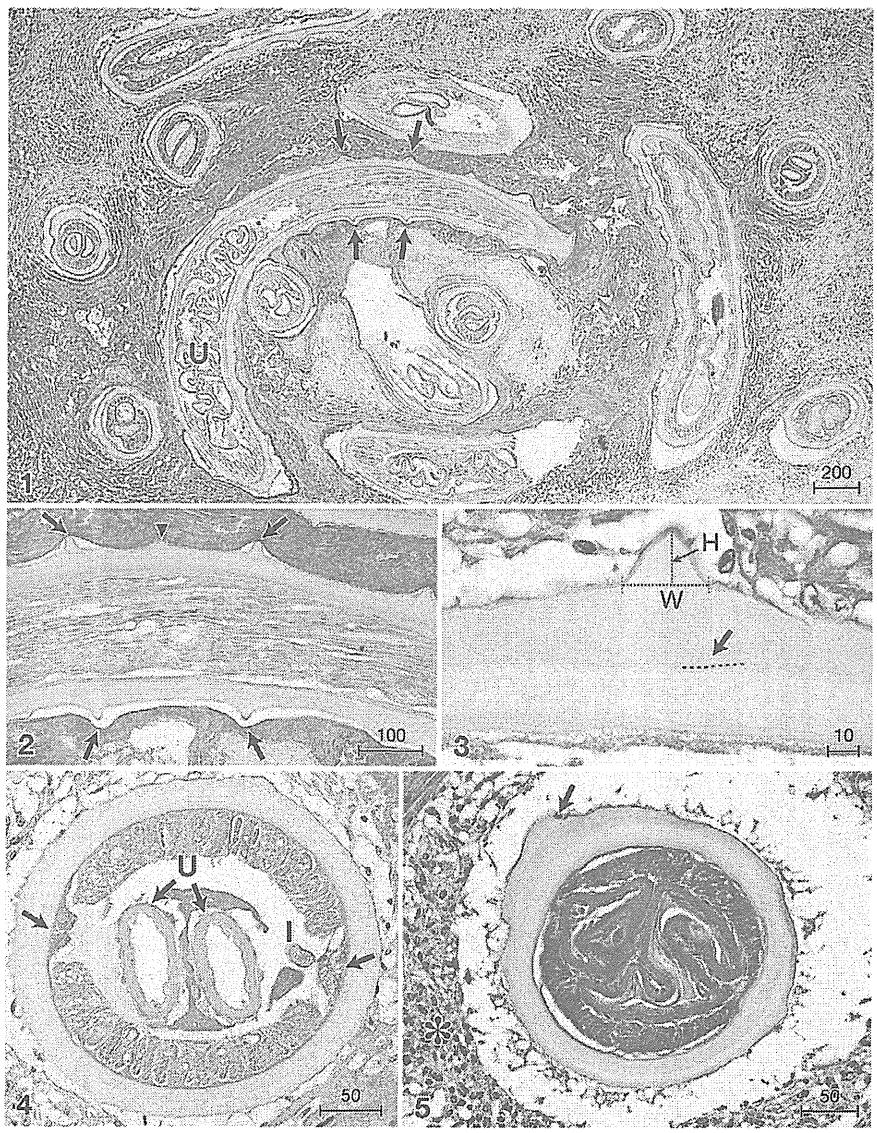

Fig. 1. Histologic sections of a female *Onchocerca dewittei japonica*, found in a nodule excised from the left knee of a 70-year-old man. The sections are stained with hematoxylin and eosin. Bars, micrometers. 1. A coiled female with transverse ridges (arrows) on the cuticle and the uteri (U) in the pseudocoelom. 2. The salient transverse ridges (arrows) of one side and a small ridge (an arrowhead) of the other side (semicircular ridges are overlapping at the lateral field, see text). 3. The triangle ridge in the longitudinal section: height (H) and width (W), and the lack of the inner striae at the dotted line with an arrow in the cuticle. 4. The transverse section at the midbody with thick cuticle, muscle cells, and two large lateral chords (arrows). Two empty uteri (U) and intestine (I) in the pseudocoelom. 5. In the transverse section, a thick portion of the cuticle indicates the ridge (an arrow). The section is intensively surrounded by macrophages (*).

Table 1
Comparison of histologic characteristics of *Onchocerca* sp. found from a human nodule with females of *Onchocerca* species known in Japan.

	Onchocerca sp. (present study)	*O. gutturosa*	*O. lienalis*	*O. skrjabini*	*O. eberhardi*	*O. suzukii*	*O. dewittei japonica*
Body width at midbody	270–310	170–250**, 140–225***, 200–300*****	180–200**,180–220****, 150–220*****	170–300	60–170	228–430	180–310
Distance between 2 adjacent ridges	210–280	50–75**, 70–80***, 87–166*****	30–40**, 25–45****, 60*****	48–55	25–40	No ridges	185–290
Shape of ridges (H/W)* in longitudinal sections	Triangle (13–25/23–28)	Rounded ridges (5/12**, 4–5/5–10***, 10–13/26*****)	Small, rounded ridges (3/15–23*****)	Small ridges (6/12)	Small, rounded ridges (3/8)	No ridges	Triangle (8–23/23–30)
Thickness of cuticle without ridges	30–40	30**, 25–35***	12–17****, 10*****	20–30	28–32	15–50	10–32
Number of inner striae between 2 adjacent ridges	None	3**, 4–8***, 2–4*****	2**, ****, *****	3–4	2	None	None
Size of lateral chords (H/W)* in transverse sections	13–25/42–55	13/54****	16–21/52–63****	15–25/50–88	10/38	5–37/125–132	10–18/45–63
Number of muscle cells per quadrant	8–15	2–4****	5–7******	2–3	1–2	8–13	8–22
Height of muscle cells	38–43	40****	31****	25–40	15–18	30–38	45–50
Diameter of intestine	15–18	20–34****	26****	38–68	15–20	20–48	15–17
Host(s)	Human	Cattle	Cattle	Deer and serows	Deer	Serows	Wild boar
Histologic sections and references	Sections (S3-1, S3-2, S3-4, S7-9) from a human	**[5]; ***[22]; ****[23]; *****[24]	**[5]; ****[23]; ***** [24]; ******[14]	Sections (YG2-31) from a serow	Sections (S57-F3) from a deer	Sections (YG2-35) from a serow	Sections (B57-1) from a wild boar

Dimensions in micrometers.
*H/W: height/width.

dogs and cats. Immunologic deficiency was not found in his laboratory examination at the hospital.

3. Discussion

In the longitudinal sections, the presence of the transverse ridges on the cuticle of the worm appeared to be typical of a female of the species among the genus *Onchocerca*: 28 species and one subspecies (*O. dewittei japonica*) with the ridges on the cuticle of the female worms and three species without the ridges [21].

The present causative agent can be distinguished from *O. gutturosa* and *O. lienalis*, the two species found in cattle. In these species, the ridges (evident in the posterior part in *O. lienalis*) are rounded, not triangular; the distance between adjacent ridges is smaller; and the females possess inner striae in the cuticle (Table 1), [5,22–24]. In addition, the female of *O. lienalis* has fine, irregular longitudinal striations on the outer surface [23]. The females of *O. cervicalis* and *O. reticulata* from horses have the inner striae in the cuticle and shorter distance between adjoining ridges than that of the causative agent [22]. These four *Onchocerca* species from domestic animals can therefore be excluded from consideration as the causative agent in this case.

The present agent was distinguished from *O. lupi* and *O. jakutensis* in such characteristics as the distance between ridges, the shapes of the ridges, and the inner striae [20,25].

To compare the causative agent with the *Onchocerca* species already found from wild ungulates in Japan, we used portions of the collections of the histologic sections made from females of *Onchocerca* species in the Department of Medical Zoology, Osaka City University Medical School (Table 1) and published descriptions of these species. In Table 1, *O. skrjabini* Rukhlyadev, 1964, taken from a serow, is distinguished from the present human-case causative agent, owing to great differences in such principal characteristics as the distance between adjacent ridges, shape of the ridges, and the inner striae in the cuticle [26,27]. *Onchocerca eberhardi* Uni et al., 2007 taken from a sika deer differs from the causative agent in the diameter of the midbody as well as such characteristics as the distance between the ridges, the shape of ridges, and the inner striae [21]. In *O. suzukii* Yagi et al., 1994 taken from a serow, the transverse ridges are absent on the cuticle [27].

On the contrary, *O. dewittei japonica* taken from a wild boar was found to be identical to the present human-case causative agent in the distance between adjacent ridges, the shape of the ridges, the lack of inner striae in the cuticle, and other dimensions such as the body width, number of muscle cells, and size of lateral chords (Table 1) [19]. Therefore, comparison of the causative agent of the human case with the *Onchocerca* species, either outside Japan or present in Japan, indicated that the agent was a female adult of *O. dewittei japonica*.

Detailed examinations of the histologic sections of *O. dewittei japonica* found both in a human (the present study) and from wild boar now allow us to identify retrospectively this species as the causative agent of the first human case in Japan [7,8] which had been suspected as *O. gutturosa* or *O. cervicalis*. Uni et al. [19] described the ridges of *O. dewittei japonica* female as semicircular, with double thickness of the cuticle on the transverse sections. Thus, the difference in the cuticular thickness has a specific value to suggest the presence of salient ridges in transverse sections. In the slightly oblique section shown in their Fig. 3 of the first human case [7], it is our consideration that the difference (24 μm) between the thick cuticle (36 μm) (with the transverse ridge) and the thin cuticle (12 μm) (without ridges) corresponds to the height (8–23 μm) of ridges on *O. dewittei japonica* rather than that (4–13 μm) of those on *O. gutturosa* (Table 1). The difference in the cuticular thickness therefore indicates that the causative agent in the first human case found was *O. dewittei japonica* also, but this species had not yet discovered from wild boar at that time.

Wolbachia bacterial endosymbionts were found in *O. dewittei japonica* [28, Casiraghi et al., ongoing work]. It is indicated that (1) *Wolbachia* stimulate neutrophil infiltration in onchocercomata caused by *Wolbachia*-positive filariae and that (2) eosinophils accumulate to kill the parasites after elimination of the *Wolbachia* by antibiotics [29,30]. Histologic examination showed that, rather than neutrophils, macrophages and eosinophils were abundant around the sections of the worm. The endosymbionts appear to have been destroyed in the altered parts of

the worm; the neutrophils appear to have already been replaced with eosinophils in the course of the death of the parasite in the immunological environment.

Until now, all four known cases of zoonotic onchocerciasis caused by *O. dewittei japonica* parasites found in wild boar (plus probably an earlier case, examined in retrospect) were limited only to Oita, Kyushu. However, the present findings indicate that zoonotic onchocerciasis has occurred in at least one other island of Japan as well. The prevalence of *O. dewittei japonica* in wild boar, as measured by the presence of microfilariae in skin snips, was high in and near Hiroshima Prefecture: 78% (31/40) of wild boar were found to harbor this filarioid in Shimane Prefecture adjoining Hiroshima Prefecture, examined between 2005 and 2006, and was close to the prevalence of the parasites (40/45, or 89%) in wild boar in Oita, Kyushu, in 2003. In Wakayama Prefecture, located in the west-central part of Honshu, 77% (23/30) of wild boar harbored this filarioid in 2007. Thus, as in Kyushu, almost all wild boar over one year old in the western part of Honshu examined, were found to harbor *O. dewittei japonica* (Uni et al., unpublished data).

Having obtained experimentally the infective larvae of *O. dewittei japonica* from several kinds of black flies [31], and having identified as *O. dewittei japonica* the larvae from black flies caught in Oita, Fukuda et al. suggested that *Simulium bidentatum* is a vector in the transmission of the zoonotic onchocerciasis caused by *O. dewittei japonica* in Oita [32]. The black fly inhabits Honshu and Shikoku as well as Kyushu and bites both animals and humans. In the present case, DNA sequences of the mitochondrial *CO1* gene obtained from the causative agent, embedded in paraffin for histologic examination, showed high similarities to those of *O. dewittei japonica* [33, Fukuda et al., in preparation].

In Japan, the habitat of the wild boar (estimated population: several hundred thousands) has recently broadened in the western part of Honshu, Shikoku, and Awaji-shima Island as well as Kyushu, because the annual snowfall has decreased, rice fields left unused by migration of segments of the work forces away from rural areas are favorable habitat for wild boar, and the population of hunters has largely decreased [34]. Therefore, the present human-host case caused by *O. dewittei japonica* suggests that zoonotic onchocerciasis is now liable to occur in other rural areas in Japan, or wherever wild boar and humans are in close proximity and the *Simulium* vectors are known, as well as in Oita.

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寄生虫の標的臓器別症状からすすめる実地診療
—疑い，問診・診断から治療まで—

腹部症状(腹痛，下痢，下血など)

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はじめに●

いわゆる「感染性胃腸炎」の中に占める寄生虫感染症の割合は低いが，寄生虫の側から考えると，消化管はもっとも広く採用されている寄生部位である．回虫，蟯虫，鞭虫，赤痢アメーバやクリプトスポリジウムなどすべて消化管寄生虫である．消化管に一定数以上の虫がいれば何らかの腹部症状が現れる．

症状あるいは訴えが直接的かつ特徴的なときは，それだけで寄生虫を言い当てることも可能なことがある．例えば「排便時にお尻から虫が出てきた．引っ張ったら千切れた」とか「お尻からミミズのような虫が出てきた」などというものである．前者は広節(日本海)裂頭条虫，後者は回虫の可能性が高い．しかしながら，通常の診療では「これはひょっとして寄生虫か」と途中で疑い出すことのほうが多いであろう．本項では，どういうときに寄生虫のことを思い出すべきかに焦点を当てる．

なお，マラリアでも悪心，嘔吐，下痢，腹痛などの腹部症状を訴えることがあるが，これは「発熱」の項目で詳述されるので本項では述べない．

寄生虫の種類●

寄生虫の種類自体は膨大な数に上るが，外来で遭遇するであろう消化管関連の寄生虫に絞ればそれほど多いものではない．消化管および肝臓に病変をもたらす寄生虫の種類を**表1**にまとめた．以下に述べるとおり症状は寄生部位に依存するので，主な寄生部位を知っておくと整理しやすい．

注意すべきは，ヒト体内では成熟できずに幼虫が腸管外を移動する，いわゆる幼虫移行症のカテゴリーに含まれる寄生虫も消化管に障害を引き起こす可能性があることである(後述)．

寄生虫による腹部症状●

腹痛，下痢，下血などの症状は，寄生虫が消化管やその近傍に寄生して，機械的刺激や炎症反応などの刺激を起こすことが原因である．寄生数が少なければ症状はほとんどないが，虫体が大きい場合などでは少数寄生でも発症しうる．寄生虫は種によって決まった部位に寄生するので，臨床症状から寄生虫の種類を大まかに推定することは可能で，診断の出発点になる．

1. 急激に発症する腹痛

上部消化管に寄生虫が存在して急な痛みで発症する場合がある．もっとも代表的なのは胃アニサキス症である．典型的には，夕食に新鮮なサバやイカを食べたところ，夜中に突然心窩部が差し込むように激しく痛み出して寝ていられなくなったという経過をとる．届出数は少ないが，毎年2,000～3,000例くらい発生していると推定されており，夜間の当直で遭遇する可能性が高い．内視鏡による虫体の摘出が診断と治療を兼ねる．

他に上部消化管で急性の痛みを引き起こすものに回虫がある．現代日本で回虫の生活環が維持されているとは思えないが，患者は存在する．回虫は狭いところにもぐり込む性質があるので，胆管に入り込んで胆管を閉塞させ急性胆嚢炎を起こす．この場合，腹部超音波検査で回虫が胆管内の異物として認められる．

その他の例として，幼虫移行症による炎症性腸閉塞がある．ドロレス顎口虫や旋尾線虫タイプXが消化管の腹膜側から組織に侵入し，激しい好酸球性炎症を起こして機能的イレウスに陥る．一般に，寄生虫感染による顕著な好酸球増多は，寄生虫(原虫を除く)の幼虫が腸管外の組織を移行しているときか，成虫が腸管外に寄生しているときにみられる．したがって，著明な好酸球増多があって腹部症状を訴えるときは，腸管外の寄生虫が腹

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◎マラリアでも悪心，嘔吐，下痢，腹痛などの腹部症状を訴えることがある．
◎著明な好酸球増多があって腹部症状を訴えるときは，腸管外の寄生虫が腹部症状の原因になっていることを考える．

表1 病変部位別寄生虫リスト

寄生部位	寄生虫	主な症状	主な診断方法	国内感染
胃	アニサキス	急な胃痛	虫体確認	＋
肝―胆道	ランブル鞭毛虫	下痢・腹痛	便検査(シスト，栄養体)	＋
	赤痢アメーバ(肝膿瘍)	季肋部痛・発熱	抗体検査	＋
	肝蛭	季肋部痛・発熱	抗体検査	＋
	腸管住血吸虫(虫卵)	肝機能障害	抗体検査・便検査(虫卵)	－
	単包虫	季肋部痛・発熱	抗体検査	－
	多包虫	腹痛・黄疸	抗体検査	＋
	回虫	急な腹痛	虫体確認	＋
小腸	ランブル鞭毛虫	下痢・腹痛	便検査(シスト，栄養体)	＋
	クリプトスポリジウム	下痢・腹痛	便検査(オーシスト)	＋
	サイクロスポーラ	下痢・腹痛	便検査(オーシスト)	－
	横川吸虫	下痢・腹痛	便検査(虫卵)	＋
	腸管住血吸虫(成虫)	下痢・腹痛	抗体検査・便検査(虫卵)	－
	広節(日本海)裂頭条虫	下痢・腹痛	虫体確認・便検査(虫卵)	＋
	無鉤条虫		虫体確認・肛門周囲虫卵検査*	－
	有鉤条虫		虫体確認・肛門周囲虫卵検査*	－
	小型条虫	下痢・腹痛	便検査(虫卵)	＋
	回虫	下痢・腹痛	虫体確認	＋
	糞線虫	下痢・腹痛	便検査(幼虫)	＋
		体重減少		
大腸	赤痢アメーバ	粘血便・下痢・腹痛	便検査(シスト，栄養体)	＋
	腸管住血吸虫(成虫)*	下痢・腹痛	抗体検査・便検査(虫卵)	－
	鞭虫	下痢・腹痛(多数寄生時)	虫体確認・便検査(虫卵)	＋
	蟯虫	下痢・腹痛(多数寄生時)	虫体確認・肛門周囲虫卵検査*	＋

顎口虫と旋尾線虫タイプXはこの表に入っていない．
*いわゆるセロファンテープ法．

部症状の原因になっていることを考える．

2. 肝異常陰影

肝臓に寄生したり病変を引き起こす寄生虫は多い(表1)．肝臓は腫大し，慢性の季肋部痛，悪心，嘔吐，発熱などを伴う．肝臓の画像検査により何らかの異常陰影が得られるので，問題は原因の鑑別リストに寄生虫が入っているかいないかにかかっている．一般的に，寄生虫疾患の場合は，画像が派手な割に全身状態は良好である．

詳しくは「肝障害，肝脾腫，肝エコー異常」の項目で述べられるが，画像自体から寄生虫を疑う場合として「境界明瞭な肝膿瘍」がある．孤発性であればアメーバ性肝膿瘍を疑うべきだし，多発性であれば単包虫症の可能性もある．内臓幼虫移行症，肝蛭症，住血吸虫症，多包虫症などでは，肝異常陰影に加えて末梢血好酸球増多がみられる．

3. 慢性の下痢・腹痛

下痢が慢性的に続き，培養で起因菌が検出されず，または抗菌薬による治療が無効のときには，まず寄生虫感染を疑うべきである．通常の感染性胃腸炎は，免疫不全でなければ早くて2～3日，遅くとも1週間くらいから治癒に向かう．抗菌薬

- 理由のわからない慢性の下痢では，寄生虫感染を疑い便検査を行うことが必須である．
- 潰瘍性大腸炎と診断する前に，便検査，潰瘍部の生検などによりアメーバの有無を確認しなければならない．
- 赤痢アメーバ感染が確定したら他の性感染症(梅毒，HIV 感染など)も検索すべきである．

の効かない慢性の下痢はウイルス性でも細菌性でもないことを示唆しているが，非感染性の胃腸炎の検討に入る前に，ぜひとも寄生虫の検索を行う必要がある．

これまでに，アメーバ性大腸炎なのに潰瘍性大腸炎としてステロイド投与を受けていた例(多数)，横川吸虫症であるのに悪性疾患疑いで長期の入院を余儀なくされた例，重症の糞線虫症による吸収不良によって体重減少と低蛋白血症が起きていたにもかかわらず神経性食思不振症が疑われていた例などがある．理由のわからない慢性の下痢では，寄生虫感染を疑い便検査を行うことが必須である．

下痢や腹痛の原因になる寄生虫としては，原虫類が赤痢アメーバ，クリプトスポリジウム(通常慢性化はしない)，ランブル鞭毛虫，サイクロスポーラなどがあり，吸虫類では横川吸虫，有害異形吸虫，腸管住血吸虫(マンソン住血吸虫，日本住血吸虫，メコン住血吸虫，インターカラーツム住血吸虫)がある．条虫類では広節裂頭条虫，無鉤条虫，有鉤条虫，小型条虫が下痢を起こしうる．線虫類では回虫，鞭虫，蟯虫などの重症例で慢性の下痢を起こすが，現在の日本でこれら腸管線虫の多数寄生例は皆無といってよい．線虫類で問題にすべきは糞線虫である(後述)．

寄生虫と症状の関係は，小腸寄生では原虫，吸虫，条虫ともに下痢は軟便から水様性までさまざまだが，ほとんどの場合便潜血はみられない．一方，大腸寄生では粘膜破壊を伴うものが多く，粘血便やテネスムスを認める．以下に，特に赤痢アメーバ感染症と糞線虫症について述べる．

a. 赤痢アメーバ感染症

赤痢アメーバは熱帯から亜熱帯を中心に全世界に分布している．病型にはアメーバ性腸炎とアメーバ性肝膿瘍がある．ほとんどの場合粘膜病変が認められるが，肝膿瘍だけの症例もある．

アメーバ性肝膿瘍の画像所見は特徴的で，境界明瞭な孤発性の膿瘍(右葉に多い)を形成する．肝膿瘍には抗体検査が診断法として有効である．アメーバ性大腸炎では粘膜に潰瘍が形成され，増悪と寛解を繰り返す．ステロイド投与により炎症が抑制されるために症状が軽快し，一見治療がうまくいったように錯覚することがあるが，あくまで錯覚である．潰瘍性大腸炎と診断する前に，便検査，潰瘍部の生検などによりアメーバの有無を確認しなければならない．

赤痢アメーバ症の治療はメトロニダゾール(商品名：フラジール，250 mg 錠)が第一選択薬である．欧米での標準的用量は2,250 mg/日(1日9錠，分3)を10日間であるが(用法・用量保険適応外)，食欲不振，悪心，嘔吐などの消化器症状が出現しやすい．なお，わが国では赤痢アメーバ症の多くは性行為感染症である[1]．赤痢アメーバ感染が確定したら他の性感染症(梅毒，HIV 感染など)も検索すべきである．

b. 糞線虫症

少数寄生では無症状で気付かれないことも多いが，多数が寄生すると下痢や腹痛，腹部膨満感，吸収不良を起こす．もっとも重篤な病態は播種性糞線虫症と呼ばれ，大腸粘膜から侵入した幼虫が体内のいたる所に血行性にばらまかれる．粘膜侵入時に腸内細菌を引き連れて入り，敗血症，細菌性髄膜炎，細菌性肺炎などの重篤な合併症を併発する．発症のきっかけは，加齢や免疫抑制薬投与などによる宿主の免疫力低下である[2]．成人T細胞白血病(ATL)のキャリアが白血病発症前に糞線虫症を発症することはよく知られている．ただし，臨床的に免疫不全がなくても重症化する事例がある．

糞線虫の特徴は自家感染を起こすことである．

- 原因不明の慢性の下痢を起こしている患者が南西諸島出身であったら，何十年も島を離れていても糞線虫症を疑わなくてはならない．
- どんな患者でも感染症の可能性があるときには「最近海外旅行には行かれましたか」と聞く習慣をつけておいたほうがよい．

自家感染とは，小腸に寄生しているメスが産んだ虫卵が孵化し，幼虫の一部がF型と呼ばれる感染型の幼虫に発育して大腸粘膜や肛門周囲の皮膚から経粘膜または経皮的に侵入し，消化管に到達して成熟することをいう．自家感染の結果，成虫の寿命は1～2年であるにもかかわらず何十年も感染が持続する．

宿主免疫能が低下するとF型感染幼虫に発育する幼虫の割合が増え，結果として体内の成虫の数が増え，寄生虫体数が膨大になる．かくして重症糞線虫症，播種性糞線虫症に陥る．わが国では糞線虫は南西諸島に分布しているので，子供時代にこの地方に住んでいて中年を過ぎて発症することがある．原因不明の慢性の下痢を起こしている患者が南西諸島出身であったら，何十年も島を離れていても糞線虫症を疑わなくてはならない．

糞線虫症の治療はイベルメクチン(商品名：ストロメクトール，3 mg錠)が第一選択薬である．200 mg/kg/日，1日1回，朝食1時間前に服用，2週間後に再度同量服用を原則とするが，免疫不全や播種性糞線虫症があるときには1～2週間隔で，糞線虫が陰性化するまで4回以上投与する[3]．経口摂取不能の場合は錠剤を粉砕してイレウス管などから投与するが，緊急倫理審査を経て動物用イベルメクチン注射薬を静注したという報告もある[4]．

問診のすすめかた

寄生虫感染をいくらかでも疑った場合に確認すべきポイントとしては，海外渡航歴，国内の居住歴，食事歴がある．

途上国への渡航歴があれば寄生虫感染のリスクは増す．いわゆる旅行者下痢症のうち，寄生虫ではランブル鞭毛虫，赤痢アメーバ，クリプトスポリジウムが多い．国内感染がまれであるものにはサイクロスポーラがあげられる[5]．途上国からの帰国者では検査すべき病原体の種類と緊急度が大幅に変わってくることを考えると，どんな患者でも感染症の可能性があるときには「最近海外旅行には行かれましたか」と聞く習慣をつけておいたほうがよい．

表2　腹部症状を起こしうる寄生虫に感染する食品のリスト

	寄生虫	食品
線虫	アニサキス	イカ，サバなど
	旋尾線虫タイプX	ホタルイカ
	顎口虫	ドジョウ，ヘビ(マムシ)，淡水魚
吸虫	横川吸虫	アユ，ウグイ
	肝吸虫	モロコ，タナゴなど
	肝蛭	水草，ウシ(肝)
条虫	広節(日本海)裂頭条虫	サケ，マスなど
	無鉤条虫*	ウシ
	有鉤条虫*	ブタ
	旋毛虫*	ブタ，クマ，アザラシなど

*国内では感染しない．

居住歴が寄生虫感染と関係することがある．具体的には，北海道ではエキノコックス症を，南西諸島(薩南諸島と琉球諸島)では糞線虫症を考えなければならない．どちらも致命的になりうる寄生虫疾患である．

日本国内では寄生虫には食品から感染することが多い．したがって，感染性胃腸炎を診察するときの問診リストに寄生虫関連の食品も加えておくのがよい(表2)．通常の食中毒と違う点は，新鮮な食材ほど寄生虫感染のリスクは増すことである．また，野生動物(鳥類，獣，爬虫類，両生類，淡水産の魚類，甲殻類)を十分に加熱せずに食べれば寄生虫感染のリスクが高い．精をつけるためなどの理由でいろいろな人がいろいろなものを口にする．本人は決して「変なもの」を口にしたとは

◎野生動物(鳥類，獣，爬虫類，両生類，淡水産の魚類，淡水産の甲殻類)を十分に加熱せずに食べれば寄生虫感染のリスクが高い．
◎寄生虫を疑ったときの便検査では，日を変えて複数回施行する必要があることと，直接塗抹では検出できない寄生虫があることに留意する．

思っていないので，丁寧な聞きかたを心掛けるべきである．

検査のすすめかた

腸管寄生原虫やヒト腸管内で成熟する吸虫，条虫，線虫では，嚢子や栄養体，虫卵，幼虫，成虫などが糞便とともに排出される．したがって，これらの寄生虫を疑ったときには便検査を行う必要がある．便検査に際しての留意点としては，1回かぎりではなく日を変えて複数回施行することと，通常の直接塗抹では検出できない寄生虫があることを認識しておくということである(「糞便検査」の項参照)．

寄生虫によっては産卵数が少ないので，集卵法(横川吸虫や住血吸虫など)や特殊な検出法(糞線虫症における普通寒天平板培地法)が用いられることがある．しかしながら，下痢の原因が寄生虫であれば寄生数は多いと考えるべきで，直接塗抹法で調べる価値は十分にある．

内視鏡検査は，内視鏡が届く範囲に肉眼レベルの寄生虫がいる場合きわめて有効である．上部消化管では回虫，アニサキス，大腸内視鏡では鞭虫や広節(日本海)裂頭条虫が発見される．広節裂頭条虫では，内視鏡で追いかけたが回盲部から奥の方に逃げられたという例が多い．カプセル内視鏡は，自然状態の寄生虫を観察できるという点で寄生虫学者としては興味深い．

抗体検査が有効な寄生虫は組織侵入性があるものに限られるので，幼虫移行症や腸管以外の臓器に寄生する虫など，便検査が無効な寄生虫疾患に対して有用である．寄生虫の抗体検査は，(株)エスアールエル，宮崎大学医学部寄生虫学，国立感染症研究所寄生動物部などで実施している．

おわりに

寄生虫感染症に関する相談があるときは，最寄りの大学の寄生虫学・医動物学関係の研究室，あるいは国立感染症研究所寄生動物部に連絡するのがよい．筆者が所属する宮崎大学医学部寄生虫学分野でも検査依頼や相談に応じている．詳しくは研究室ホームページを参照いただきたい(http://www.miyazaki-med.ac.jp/parasitology/detail.htm，相談専用メールアドレスは kessei@med.miyazaki-u.ac.jp)．

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好酸球性髄膜炎を呈したドロレス顎口虫症の1例

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Key Words: ドロレス顎口虫，好酸球性髄膜炎，カニ

はじめに

寄生虫感染症による好酸球性髄膜炎の原因としては広東住血線虫症がよく知られているが，他の寄生虫感染は原因として稀である．淡水産カニ摂食後に発熱，頭痛を呈した好酸球性髄膜炎で，ドロレス顎口虫感染が原因と考えられる稀な1例を経験したので報告する．

症例

患者：71歳，女性，無職

主訴：びりびりする頭痛

既往歴：めまい症，卵巣嚢種，子宮筋腫にて子宮全摘、下肢静脈瘤術後

生活歴：趣味で無農薬野菜を栽培

現病歴：20XX年10月24日，ゆでた淡水産カニ（モクズガニと思われる）を食べた．12月10日頃から朝は37度台，夜は38度台の発熱を繰り返すようになった．12月14日，びりびりするような神経痛様の激しい頭痛が出現した．12月15日，脳神経外科専門病院を受診し，頭部MRIを撮影されたが，異常は認められなかった．

12月16日，当院内科一般外来受診．項部硬直を認め，髄膜炎の疑いで当科に緊急入院となった．

入院時一般身体所見：体温37.5度と微熱を認めるほかに特記すべき異常を認めなかった．

入院時神経学的所見：意識清明だが苦悶様表情で項部硬直を認めた．眼球運動は正常であり，構音障害はなかった．左三叉神経第Ⅰ枝，第Ⅱ枝領域，左後頭神経領域にびりびりする神経痛様の痛みを訴えていた．四肢・体幹に感覚・運動障害は認めないが，頭痛のため歩行不能であり，光過敏，

A case of Eosinophilic Meningitis probably due to *Gnathostoma doloresi* Infection

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聴覚過敏を認めた.

入院時, 入院後検査所見 : 血算 (分画異常なし, 好酸球増加なし), 一般生化学検査に異常なし. 甲状腺機能, 免疫グロブリン分画, 各種自己抗体, 各種真菌マーカーは陰性であった.

髄液検査 : 初圧 20cmH_2O 以上, 細胞数 229/mm^3 (リンパ球 146, 好中球 60), 蛋白定量 54mg/dl, 糖定量 61mg/dl, Cl 114mEq/l, IgG 7.7mg/dl, アルブミン 39.9mg/dl, ADA 6.9IU/l. 抗酸菌, EBV, HSV, HHV6 PCR はすべて陰性, 細菌培養陰性. グロコット染色で真菌を認めなかった. 細胞診にて Class Ⅰであり, 好酸球 1+, リンパ球 ±であった.

頭部・頸部造影 MRI;異常所見を認めなかった.

入院後経過;ウイルス性髄膜炎の診断にて対症療法を開始したが, 神経痛様の頭痛は改善しなかった. 12 月 28 日に血液中好酸球が 25.5%と増加し, 同日再検した髄液細胞診で, 髄液中に多量の好酸球の出現 (36.9%) を認め, 好酸球性髄膜炎と診断した. 症状が髄膜炎のみであることと, 淡水産カニの摂食歴があることから広東住血線虫症, 肺吸虫症を鑑別に挙げ, プラジカンテル 2,400mg/日, プレドニゾロン 40mg/日で治療を開始した.

原因検索として血清抗寄生虫スクリーニングを実施したところ, ドロレス顎口虫抗原に対し反応がみられたため, 顎口虫と広東住血線虫抗原について血清と髄液の寄生虫に対する抗体を測定した. その結果, 両者に対する抗体が有意に増加しており, いずれかの寄生虫の感染を疑わせた (図 1). さらに原因虫種確定のために交差吸収試験を施行したところ, 髄液中抗体はドロレス顎口虫抗原に対して特異的に反応することが判明した (図 2). 以上より, ドロレス顎口虫の髄腔内感染が濃厚に疑われた. そのためアルベンダゾール 600mg/日の投与を 7 日間行った. 髄液所見は改善

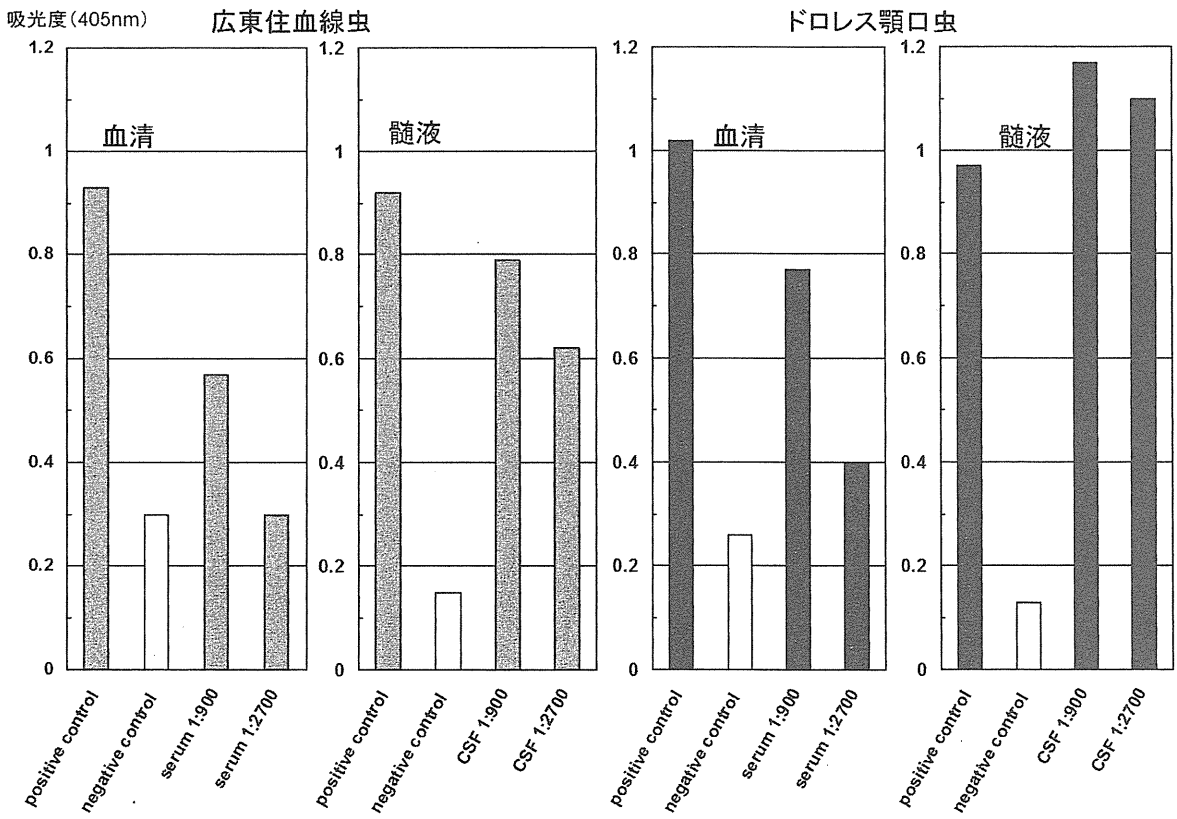

図 1　広東住血線虫とドロレス顎口虫の抗原抗体反応試験 (microtiter plate ELISA)

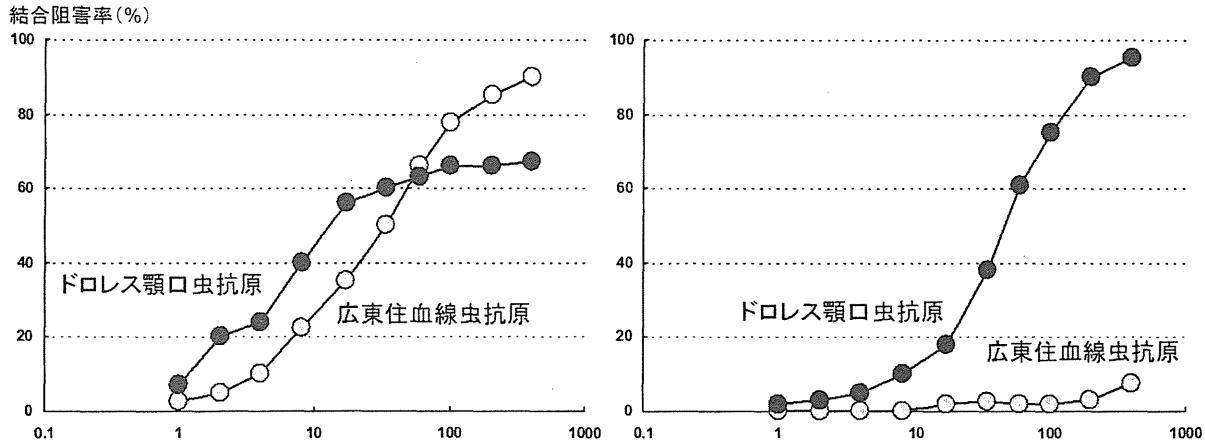

図2　患者血清の広東住血線虫またはドロレス顎口虫抗原への結合を，それぞれの抗原により阻害した．広東住血線虫抗原への結合は広東住血線虫抗原とドロレス顎口虫抗原の療法で阻害されたが（左），ドロレス顎口虫抗原への結合は広東住血線虫抗原では阻害できなかった（右）．これは，患者血清の広東住血線虫抗原への結合は交差反応であることを意味する．

し，神経痛様頭痛も残存していたものの改善傾向であり，2月24日退院した．経過を通じて3回施行した頭部・頸髄造影MRIで異常所見は認めず，また眼症や幼虫皮膚爬行症の所見もみられなかった（図3）．

考察

ドロレス顎口虫は水棲魚類，水棲節足動物などの摂取により感染し，眼症や幼虫皮膚爬行症、イレウス[1)]などをきたした報告がある．中枢神経への感染は根神経炎，髄膜脳炎などの形をとり，海

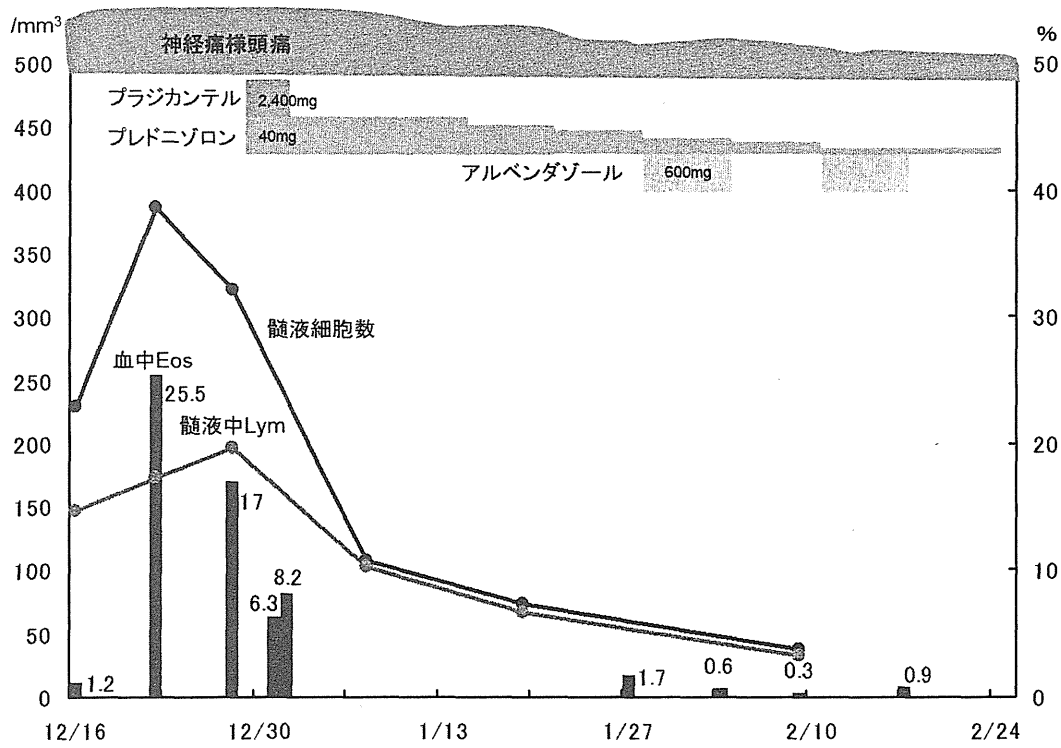

図3　入院中経過

外では四肢麻痺，くも膜下出血などを伴い，MRIで脳実質に造影病変などを認める例が報告されている[2)3)]．本症例では症状は好酸球性髄膜炎のみであり，我々が検索した限りでは報告はなかった．日本での顎口虫感染は現在ほとんどがドロレス顎口虫によるものとされるが，顎口虫の種特定は困難であり，日本顎口虫の感染も可能性として考えられる．

本例では神経痛様頭痛は発症6か月経過後も持続しており，顎口虫感染では初期治療を誤った場合の再発の報告もあるため[4)]，今後も慎重な経過観察が必要である．

好酸球性髄膜炎の原因として，広東住血線虫症の他に，ドロレス顎口虫も鑑別の一つとして重要と考えられる．

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