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P2X<sub>7</sub> Receptor-Dependent Intestinal Afferent Hypersensitivity in a Mouse Model of Postinfectious Irritable Bowel Syndrome

Christopher Keating,*<sup>1</sup> Pablo Pelegrin,†<sup>1</sup> Carlos M. Martínez,† and David Grundy*<sup>1</sup>

The ATP-gated P2X<sub>7</sub> receptor (P2X<sub>7</sub>R) was shown to be an important mediator of inflammation and inflammatory pain through its regulation of IL-1β processing and release. Trichinella spiralis-infected mice develop a postinflammatory visceral hypersensitivity that is reminiscent of the clinical features associated with postinfectious irritable bowel syndrome. In this study, we used P2X<sub>7</sub>-R knockout mice (P2X<sub>7</sub>-R<sup>−/−</sup>) to investigate the role of P2X<sub>7</sub>-R activation in the in vivo production of IL-1β and the development of postinfectious visceral hypersensitivity in the T. spiralis-infected mouse. During acute nematode infection, IL-1β–containing cells and P2X<sub>7</sub>-R expression were increased in the jejunum of wild-type (WT) mice. Peritoneal and serum IL-1β levels were also increased, which was indicative of elevated IL-1β release. However, in the P2X<sub>7</sub>-R<sup>−/−</sup> animals, we found that infection had no effect upon intracellular, plasma, or peritoneal IL-1β levels. Conversely, infection augmented peritoneal TNF-α levels in both WT and P2X<sub>7</sub>-R<sup>−/−</sup> animals. Infection was also associated with a P2X<sub>7</sub>-R-dependent increase in extracellular peritoneal lactate dehydrogenase, and it triggered immunological changes in both strains. Jejunal afferent fiber mechanosensitivity was assessed in uninfected and postinfected WT and P2X<sub>7</sub>-R<sup>−/−</sup> animals. Postinfected WT animals developed an augmented afferent fiber response to mechanical stimuli; however, this did not develop in postinfected P2X<sub>7</sub>-R<sup>−/−</sup> animals. Therefore, our results demonstrated that P2X<sub>7</sub>-Rs play a pivotal role in intestinal inflammation and are a trigger for the development of visceral hypersensitivity. The Journal of Immunology, 2011, 187: 1467–1474.

Inflammation can induce long-term alterations in sensory-signaling processes within the gastrointestinal tract, giving rise to chronic abdominal symptoms, including pain (1). This visceral hypersensitivity is an important clinical feature associated with irritable bowel syndrome (IBS) (2). Although the development of IBS is linked to prior infection in one well-defined patient cluster, the so-called ‘postinfectious’ IBS (PI-IBS) patients, the pathophysiological mechanisms underlying the generation and maintenance of visceral hypersensitivity remain poorly understood.

Visceral nociceptive information from the gut is relayed to the CNS via the actions of many ion channel and receptor pathways (3), including ATP-mediated P2X receptor pathways (4). For example, P2X<sub>2/3</sub> receptors, ion channels present on sensory neurons, are activated by low (<1 µM) levels of ATP and can be sensitized after inflammation or nerve damage (5–7). However, P2X<sub>7</sub> receptors (P2X<sub>7</sub>-Rs) are located predominantly on immune cells, such as macrophages and microglia, and their activation in response to cellular danger signals, such as high (>&gt;100 µM) concentrations of ATP, triggers a series of physiological events that culminates with the processing and release of the proinflammatory cytokine IL-1β (8–10).

IL-1β is pronociceptive; treatment of mice with IL-1β mediated thermal and mechanical hyperalgesia (11, 12), whereas intrathecal injections of IL-1β increased the release of excitatory neurotransmitters (13). Furthermore, pharmacological blockade of IL-1β attenuated the hyperalgesia associated with microglial activation (14). IL-1β was also implicated in the delayed rectal allodynic response to colorectal distension following i.p. LPS treatment in rats (15). Increased IL-1β mRNA levels were demonstrated in IBS patients (16), and in an animal model of PI-IBS, enhanced expression of both IL-1β mRNA and protein was detected (17).

The knowledge that P2X<sub>7</sub>-Rs are involved in regulating IL-1β processing and release, and that their activation is in response to the presence of cellular danger signals, supports an upstream signaling role for these receptors in regulating inflammation-induced nociceptive behavior. For example, in models of chronic inflammatory airway disease, P2X<sub>7</sub>-R expression is increased on human and mouse macrophages/dendritic cells (18, 19). P2X<sub>7</sub>-R antagonists were shown to decrease the inflammatory response in an animal model of arthritis (20), and P2X<sub>7</sub>-R knockout mice possess reduced inflammatory thermal hyperalgesia and nerve injury-induced mechanical allodynia (21). Furthermore, P2X<sub>7</sub>-R-dependent release of IL-1β was implicated in mediating LPS-induced hypersensitivity in the spinal cord associated with microglial activation (8). The recent development of highly selective P2X<sub>7</sub>-R antagonists demonstrated a specific role for this receptor in mediating nociceptive signaling during chronic pain.
states, thereby opening new therapeutic research avenues for the treatment of chronic pain.

We hypothesized that P2X7R activation would be implicated in the development of postinfectious visceral hypersensitivity and investigated this using an animal model of postinfectious gastrointestinal dysfunction, the *Trichinella spiralis*-infected mouse (22). Mice infected with *T. spiralis* develop a transient inflammatory response that resolves to leave a dysfunctional gut characterized by altered motor and sensory nerve function reminiscent of IBS (17, 22, 23). In the current study, we used P2X7R−/− mice (21) to investigate the role of P2X7Rs and IL-1β release in establishing an inflammatory response to *T. spiralis* infection. Following this, the contribution of P2X7-Rs toward the generation of visceral hypersensitivity was assessed.

**Materials and Methods**

**Animals and experimental treatment**

The generation of this P2X7R−/− strain was described previously (21). Adult male P2X7R−/− (C57BL/6 background) and C57BL/6 (wild-type [WT]) mice were used in accordance with the United Kingdom Animals Scientific Procedures Act (1986). The *T. spiralis* colony was maintained through serial infection of adult male NIH Swiss or C57BL/6 mice, as previously described (24). P2X7R−/− and WT mice were infected with *T. spiralis* larvae (in a mouse/mouse ratio of one larva/gavage) using oral gavage (0.2 ml in 0.9% saline), as previously described (23). Control mice included naive and sham-infected animals (0.9% saline). Experiments were performed at designated time points postinfection (PI).

**Skeletal muscle worm burden**

Skeletal muscle worm burdens of postinfected animals were determined by homogenizing the skeletal muscle of skinned, eviscerated mice in a mini food processor. The homogenate was then incubated for 1.5–2 h at 37°C in a solution (500 ml) containing 0.9% saline, 0.5% hydrochloric acid, and 0.5% pepsin, as previously described (24). Following this, the digestate was strained through several layers of gauze and washed with 0.9% saline, and the worms were allowed to settle at unit gravity. The supernatant was removed, and following three additional washes, larvae were collected, resuspended in 0.9% saline, and counted under a dissection microscope.

**Analysis of cytokine release**

Animals were killed by cervical dislocation, and the peritoneal cavity was lavaged with 3 ml sterile PBS (Invitrogen). Recovered PBS was centrifuged for 5 min at 800 × g, and a 50-μl aliquot was taken for analysis using a commercially available ELISA (see below). Serum was also collected unprocessed IL-1

**Histochemistry and immunocytochemistry**

Jejunal segments were obtained from uninfected or infected mice (day 8 PI) and were fixed for 24 h at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Samples were embedded in paraffin, and 4-μm-thick sections were obtained. Sections were deparaffinized, rehydrated, and treated for 5 min in citrate buffer (pH 6) at 121°C. Endogenous peroxidase activity was blocked using 0.3% H2O2 solution (Dako Diagnostics) for 5 min. Sections were blocked for 20 min with 3% BSA (Dako Diagnostics) supplemented with 1% swine serum (Dako Diagnostics) or rabbit serum (Vector Labs) for T-CD3 or IL-1β immunostaining, respectively. Three well-validated primary Abs were used for immunostaining: a polyclonal rabbit anti-CD3 Ab (Dako Diagnostics) was used for both CD3+ T cells and P2X7, whereas a polyclonal rabbit anti-rat IgG Ab (Dako Diagnostics) was used for IL-1β. After washing with PBS, sections were incubated with the avidin–HRP complex (Vector Labs) and used in accordance with the manufacturer’s instructions. Positive staining was revealed with 3-3'-diaminobencidine (Dako Diagnostics). Finally, sections were counterstained with Harris’s hematoxylin. Positive reactions were identified by a pericellular (CD3 immunostaining) or cytoplasmic (IL-1β immunostaining) brown-dark precipitation. To quantify group differences in the cellular inflammatory infiltrate composition, average numbers of polymorphonuclear neutrophils (PMNs), plasma cells, and CD3+ T cells were calculated by counting in 10 high-power fields (×40). H&E-staining protocols were used for histopathological analysis.

**Peritoneal macrophage isolation**

Peritoneal macrophages were collected after washing the peritoneal cavity with 3 ml sterile PBS, and macrophages were isolated by adherence to plastic wells and centrifuged with F4/80 immunostaining. Ten fields of view were counted for each animal to estimate the number of peritoneal macrophages.

**Lactate dehydrogenase assay**

A cytotoxicity detection kit (Roche) was used in accordance with the manufacturer’s instructions.

**Tissue preparation for afferent nerve recordings**

Physiological recordings of jejunal mesenteric afferent nerve activity were made from uninfected and postinfected P2X7R−/− (≥28 d PI) and WT mice. Animals were killed by cervical dislocation, and their abdominal cavity was opened and bathed in cold Krebs solution (in mM: NaCl 120; KCl 5.9; NaH2PO4 1.2; MgSO4 1.2; NaHCO3 15.4; CaCl2 2.5; glucose 11.5) that was gassed with carbogen (95% O2, 5% CO2). Three-centimeter segments of jejunum with an attached mesenteric bundle were harvested 10–20 cm proximal to the ileocecal junction, placed into an organ chamber (8 ml) and superfused with gassed Krebs solution (8 ml/min) maintained at 33–34°C. Segments were securely attached at either end to an input and outlet port. The input port was connected to a syringe pump that allowed continuous intraluminal perfusion of Krebs solution through the segments (0.2 ml/min) when the outlet port was open but allowed periodic distension when closed. Intraluminal pressure was recorded via a pressure amplifier (NL108; Digitimer, Letchworth, U.K.) connected in series with the input port. A mesenteric nerve was dissected out from the mesenteric bundle and drawn into a suction electrode.

The preparation was stabilized for 60 min before any experimental procedures were started. Jejunal afferent mechanosensitivity was tested by distending the preparation to an intraluminal pressure of 60 mm Hg using a ramp-distension protocol. This protocol allowed for the activation of low-threshold (LT) and high-threshold (HT) mechanosensitive fibers and was repeated at 1000-s intervals to test the reproducibility of the nerve response to ramp distensions and the response during test conditions. Nerve activity was recorded using a Neurolog headstage (NL100, Digitimer), and electrical signals were amplified (NL104, filtered) (NL125, band pass 200–3000 Hz), and acquired (20-KHz sampling rate) on a personal computer through a Micro 1401 M-Pro interface running Spike2 software (Cambridge Electronic Design, Cambridge, U.K.).
from “n” assays. For the physiological experiments, data are presented as mean firing frequencies (imp/s) and plotted using a 10-s time constant. Stimulus response curves (mean afferent discharge plotted against increases in intraluminal pressure) for group activities were plotted using a customized script program (CED, Cambridge, U.K.). In the physiological experiments, naive and sham-infected animals showed no difference and were pooled for subsequent analysis. Data are expressed as mean ± SEM. Significant differences among all data sets were determined using either the Student t test (paired or unpaired) or 1- or 2-way ANOVA with additional post hoc tests where necessary; p < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 4.00 (San Diego, CA).

Results
Trichinella infection in WT and P2X7R-/- animals
Total worm burdens were determined using enzymatic digestion of skeletal muscle of animals infected with T. spiralis larvae at 56 d PI. Worm burdens were significantly increased in post-infected P2X7R-/- mice (34,400 ± 8,500; n = 4) compared with WT mice (18,400 ± 1,400; n = 11; p < 0.01).

Immunohistological characterization of T. spiralis infection in WT and P2X7R-/- mice
Histological analysis of uninfected animals revealed a normal ultrastructure in both strains, in which villus height, crypt depth, and number of Paneth cells were similar (Fig. 1A, 1C, 1E, Table I). Occasionally, lymphoid follicles were observed in the lamina propria of these animals. A small increase in the stromal cellularity was observed in the villous regions of P2X7R-/- mice (Fig. 1C), which coincided with increased numbers of CD3+ T cells and plasma cells (Table I).

During the acute phase of T. spiralis infection, we observed similar ultrastructural changes in WT and P2X7R-/- animals, which were characterized by atrophy of jejunal villi, intestinal crypt proliferation, a marked decrease in the villi/crypt ratio (Fig. 1B, 1D, Table I), proliferation of lymphoid follicles located within intestinal crypts, and Paneth cell hyperplasia (Fig. 1E, Table I).

P2X7-R expression is upregulated in WT mice following T. spiralis infection
We found a significant (3-fold increase) in P2X7-R expression in T. spiralis-infected WT mice at 8 d PI, mainly on lamina propria macrophages (Fig. 2). In uninfected WT mice, P2X7-R expression was similar to basal levels, with 37 ± 2 positive cells compared with 134 ± 0.8 positive cells PI (n = 10 fields each; p < 0.001).

P2X7-R-mediated IL-1β release is an essential signaling step during the inflammatory response to T. spiralis
We studied whether P2X7-R-mediated IL-1β release was occurring during T. spiralis infection. Low levels of IL-1β expression were observed in uninfected WT and P2X7R-/- mice (Fig. 3i, iii, Table I). In the infected WT animals, intracellular IL-1β expression was increased in the lamina propria-associated inflammatory cells compared with controls (Fig. 3i, ii, Table I). However, intracellular IL-1β expression in the lamina propria inflammatory cells of P2X7R-/- animals remained at basal levels following T. spiralis infection (Fig. 3ii, iv, Table I).

Released IL-1β levels were significantly increased in the serum at 2 d PI (p < 0.0001, versus control) and were also higher in the spleen (although this did not reach significance) (Fig. 4A). However, IL-1β levels were unchanged in the peritoneum (Fig. 4A). At 8 d PI, serum IL-1β levels had normalized to control values, but IL-1β levels were significantly augmented in the peritoneum (p < 0.001, versus control; Fig. 4A). However, in the P2X7R-/- animals, peritoneal, serum, or splenic levels of IL-1β had not increased at either 2 or 8 d PI compared with their respective controls (Fig. 4A).

TNF-α levels were significantly increased in the peritoneal lavage at 2 d PI in both WT (p < 0.01, versus control) and P2X7R-/- animals (p < 0.01, versus control; Fig. 4B). However, TNF-α levels had normalized to control levels in both WT and P2X7R-/- animals at 8 d PI (data not shown).

A hallmark of P2X7-R activation in immune cells is the induction of apoptosis and cell death (28). To assess this, we measured free...
peritoneal lactate dehydrogenase (LDH) levels after *T. spiralis* infection. Peritoneal LDH levels were significantly increased in WT mice during *T. spiralis* infection at 2 and 8 d PI compared with uninfected controls (*p < 0.01 and *p < 0.05, respectively; Fig. 4C). However, peritoneal LDH levels were unaltered in the peritoneum of infected P2X7R^−/−^ animals (Fig. 4C), demonstrating a crucial in vivo role for P2X7R activation in immune-mediated cell death that normally occurs following parasite challenge.

**P2X7R deficiency alters immune cell populations toward adaptive immunity**

We examined the effect of *T. spiralis* infection on the main immune cell populations in both strains of mice. In uninfected animals there was a clear imbalance between the resident immune cell populations in both strains of mice. In uninfected animals only. Scale bars, 20 μm.

**FIGURE 2.** P2X7R expression is upregulated in WT mice following *T. spiralis* infection. Photomicrographs demonstrating anti-P2X7R immunostaining of jejunal segments from uninfected and infected (8 d PI) WT mice. Following infection, P2X7R expression was increased in WT animals. Positive stained cells (arrowheads); nonspecific background (*). Scale bars, 20 μm.

**FIGURE 3.** IL-1β expression is differentially altered in the jejunum of *T. spiralis*-infected WT and P2X7R^−/−^ mice. Photomicrographs demonstrating anti–IL-1β immunostaining of jejunal segments from uninfected and *T. spiralis*-infected (8 d PI) WT and P2X7R^−/−^ mice. Following *T. spiralis* infection, intracellular IL-1β levels are increased in the WT animals only. Scale bars, 20 μm.

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### Table I. Jejunum histological score and inflammatory infiltrate after *T. spiralis* infection in WT and P2X7R^−/−^ mice

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th><em>T. spiralis</em> Infected</th>
<th><strong>WT</strong></th>
<th><strong>P2X7R^−/−^</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height (μm)</td>
<td>355.70 ± 8.18</td>
<td>442.08 ± 11.74</td>
<td>253.09 ± 11.22***</td>
<td>248.00 ± 6.69***</td>
</tr>
<tr>
<td>Villus crypt height (μm)</td>
<td>55.67 ± 2.03</td>
<td>63.50 ± 1.97</td>
<td>114.22 ± 4.36***</td>
<td>122.61 ± 3.29***</td>
</tr>
<tr>
<td>Villus/crypt ratio</td>
<td>6.39</td>
<td>6.96</td>
<td>2.22</td>
<td>2.02</td>
</tr>
<tr>
<td>No. of Paneth cells</td>
<td>234 ± 0.99</td>
<td>289 ± 1.22</td>
<td>400 ± 2.85***</td>
<td>515 ± 2.45***</td>
</tr>
<tr>
<td>No. of PMNs</td>
<td>31 ± 0.28</td>
<td>16 ± 0.16*</td>
<td>203 ± 1.69***</td>
<td>93 ± 0.67***</td>
</tr>
<tr>
<td>No. of CD3+ T cells</td>
<td>851 ± 3.90</td>
<td>1679 ± 5.21*</td>
<td>1078 ± 5.75**</td>
<td>1957 ± 4.70***</td>
</tr>
<tr>
<td>No. of plasma cells</td>
<td>74 ± 0.42</td>
<td>138 ± 0.90*</td>
<td>163 ± 0.91***</td>
<td>275 ± 2.72***</td>
</tr>
<tr>
<td>No. of eosinophils</td>
<td>15 ± 1.67</td>
<td>21 ± 3.14</td>
<td>48 ± 2.49***</td>
<td>33 ± 2.13***</td>
</tr>
<tr>
<td>No. of IL-1β cells</td>
<td>104 ± 0.81</td>
<td>106 ± 1.21</td>
<td>246 ± 1.42***</td>
<td>109 ± 0.67†</td>
</tr>
<tr>
<td>Inflammatory score</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM; n = 5–6 animals/group and n = 10–18 quantified fields of view.

***p < 0.001 versus uninfected group, **p < 0.01 versus uninfected group, *p < 0.05 versus uninfected group, †p < 0.001 versus WT infected group.

−, minimum inflammatory score; +, ++, ++++, increasing inflammatory score.
Macrophage size increased in WT animals, during acute infection, from 11.5 ± 0.3 μm in control animals (n = 23) to 13.5 ± 0.24 μm in infected animals (n = 29; p < 0.001), whereas in P2X7R−/− animals, it remained unchanged: 11.8 ± 0.3 μm (n = 26) and 12.1 ± 0.23 μm (n = 39), respectively (p > 0.1).

The early infectious phase in P2X7R−/− animals was associated with a cellular infiltrate that was mainly composed of CD3+ T cells and plasma cells (Fig. 5A, Biv, Table I). PMN and eosinophils numbers were also increased in P2X7R−/− animals during acute infection, but these increases were significantly less than those observed in WT animals (Table I).

P2X7R is important for the development of mesenteric afferent nerve mechano-hypersensitivity following T. spiralis infection

The effect of transient inflammation upon jejunal afferent nerve mechanosensitiveness was investigated in WT and P2X7R−/− animals using a repeated ramp-distension protocol. In uninfected WT preparations, ramp distensions induced a biphasic increase in afferent nerve activity, corresponding to the activation of LT and HT mechanosensitive afferent neurons (Fig. 6A, Biv). In T. spiralis-postinfected WT animals (>28 d PI), jejunal ramp distensions evoked an augmented afferent discharge compared with controls (Fig. 6B). Afferent discharge in response to jejunal ramp distension in the uninfected P2X7R−/− animals also followed a biphasic profile (Fig. 6C). However, in the postinfected P2X7R−/− animals, afferent discharge in response to ramp distension was no different from that observed in the uninfected P2X7R−/− animals (Fig. 6C). It was also noted that the uninfected WT and P2X7R−/− animals had similar response profiles (p > 0.05; two-way ANOVA), suggesting that P2X7R deficiency does not affect basal mechanosensitivity in jejunal afferent fibers.

We summarized these results in a series of bar graphs to illustrate the change in afferent discharge at LT and HT distension pressures; the LT response was taken as the change in afferent discharge occurring between 0 and 20 mm Hg change in intraluminal pressure, and the HT response was taken between 20 and 60 mm Hg. In postinfected WTs, both LT and HT threshold responses were significantly augmented compared with controls (Fig. 6D, 6E). However, in postinfected P2X7R−/− animals, both LT and HT afferent discharge were unchanged from controls (Fig. 6D, 6E).

Discussion

The development of persistent visceral pain following transient enteric inflammation is a characteristic feature of PI-IBS, although the mechanisms contributing to its initiation and maintenance are poorly defined. A number of studies implicated P2X7R-signaling pathways as important mediators in the development of inflammatory and somatic inflammatory pain. In this study, we provide conclusive evidence that P2X7R is involved in mediating the development of visceral hypersensitivity in an animal model of PI-IBS. We demonstrated that P2X7R expression was increased in the jejunum of mice infected with T. spiralis and that development of postinfectious afferent nerve hypersensitivity, which is a hallmark of this model (23), was dependent upon P2X7R activation. Infection was also associated with a P2X7R-dependent increase in IL-1β expression and release, which seemed to be an important feature in establishing an innate immune response against T. spiralis infection. Consequently, this study presents P2X7R as a potential target for the development of novel therapeutics to treat postinflammatory visceral pain.

Our data showed that T. spiralis infection in P2X7R−/− animals resulted in a clear attenuation of the innate inflammatory response, coupled with a decreased expression and release of proinflammatory IL-1β. These factors are known to be involved in the sensitization of sensory nerves and contribute to the development of postinflammatory pain (8, 11–13, 15). The results presented in this article also complement studies in which P2X7R deletion or pharmacological blockade reduced the development of hyperalgesia in models of inflammatory and neuropathic pain in response to P2X7R-mediated IL-1β release (21, 29, 30). In addition, our observation that infection increased TNF-α levels in both WT and P2X7R−/− animals implies that this cytokine is not essential for the development of afferent nerve mechano-hypersensitivity.
It is well established that activation of P2X7Rs is a key component in the pathway controlling the release of mature IL-1β from macrophages (10). Released IL-1β is a crucial mediator in the establishment of the inflammatory reaction in response to cellular danger signals and infections (10, 31). In this study, we clearly demonstrated that in vivo release of IL-1β in WT animals is present in peritoneal, serum, and spleen supernatants during the early enteric phase of *T. spiralis* infection. However, in P2X7R<sup>−/−</sup> mice, there was no increase in IL-1β release during the early phase of *T. spiralis* infection, whereas IL-1β expression was also unchanged from basal levels. This demonstrates that, although P2X7Rs do not seem to contribute to basal IL-1β levels, the immune cell environment in the knockout animals (or the lack of P2X7R itself) contributes to downregulating both the release of mature IL-1β and the expression of pro–IL-1β in response to infection. This could be well explained by the fact that P2X7R stimulation induces MAPK signaling (32–34), and the expression of pro–IL-1β is strongly dependent on this signaling cascade (8).

Although *T. spiralis* infection resulted in similar morphological changes within the jejunum of WT and P2X7R<sup>−/−</sup> animals, we...
found that the composition of their inflammatory infiltrate differed. Compared with WT animals, uninfected P2X7R−/− animals possessed altered basal leukocyte populations characterized by increased numbers of CD3+ T cells in their lamina propria, elevated peritoneal macrophage numbers, and decreased PMN numbers. In WT animals, T. spiralis infection resulted in an early strong innate inflammatory response that was induced by a P2X7R-dependent increase in IL-1β and associated with increased numbers of peritoneal macrophages, eosinophils, and intestinal-associated PMNs but with little apparent increase in CD3+ T cells. T. spiralis infection of P2X7R−/− animals resulted in an attenuated release of IL-1β and a weak recruitment of innate immune cells, such as PMNs and eosinophils, to the lamina propria. Therefore, the innate response to T. spiralis infection seems to have been replaced in P2X7R−/− mice by the induction of an adaptive immune response involving CD3+ T cells and plasma cells. The mechanisms behind this are unknown, but we propose that this may reflect a compensatory response to infection in the knockout animals.

The P2X7R gene in rodents was recently shown to possess a novel splice variant that encodes an alternative receptor with a different intracellular N terminus and first transmembrane domain. This receptor is still functional in the knockout mice used in this study (35), and expression patterns show this isoform is enriched in cells in the thymus and spleen of WT and P2X7R−/− mice, suggesting that it is involved in a specific function in these tissues (36). Therefore, this may account for the increased numbers of CD3+ T cells that we observed in the P2X7R−/− animals. We suggest that functional P2X7-Rs in the T cells of knockout animals may be attempting to compensate for the deficiency of P2X-Rs in their innate macrophages and dendritic cells (36) and so establish an effective, but attenuated, immune response against T. spiralis infection, based upon an adaptive, and not innate, immune response.

Peritoneal macrophage numbers increased throughout the course of infection in the P2X7R−/− animals, reaching higher levels than those observed in WT infected mice at 8 d PI. No IL-1β release was found at this time point, demonstrating that the innate immune response in P2X7R−/− mice was absent rather than delayed. The decrease in peritoneal macrophage numbers at 8 d PI in the WT animals may reflect the beginning of resolution of the inflammatory response, whereas in the P2X7R−/− animals, peritoneal macrophage migration continues in an attempt to offset the defective inflammatory response occurring in these animals. We also noticed that a greater skeletal muscle larval worm burden was seen in the postinfected P2X7R−/− animals compared with their WT counterparts. IL-1β stimulates secretion of other cytokines, such as endothelial GM-CSF and G-CSF (37). These cytokines stimulate granulocyte proliferation, which is a key feature in the host response to aid parasite clearance (38). These data suggested that the P2X7R-dependent IL-1β-mediated inflammatory response is involved in aiding parasite expulsion and, consequently, limiting the secondary parasite infection.

We hypothesized a role for P2X7-R in the development of postinfectious visceral hypersensitivity in T. spiralis-infected mice. P2X7-R involvement in augmented nociceptive signaling was demonstrated previously (8, 21, 39). Therefore, we assessed the effect of T. spiralis infection upon the response of jejunal afferent fibers to mechanosensitive stimuli in postinfected WT and P2X7R−/− animals. As expected, the postinfectious phase of T. spiralis infection was associated with the development of a profound hypersensitivity to mechanical stimulation in the jejunal afferent fibers of WT mice. However, development of postinfectious afferent mechano-hypersensitivity was absent in the P2X7R−/− animals. Uninfected WT and P2X7R−/− animals possessed similar mechanosensitive response profiles, suggesting that normal visceral afferent mechanosensitivity is preserved in the knockout animals. Previous studies also showed that basal nociceptive responses are unaltered in either P2X7R−/− mice or in WT animals treated with P2X7-R antagonists (8, 21). Therefore, the development of postinfectious afferent hypersensitivity in WT animals and its absence in the P2X7R−/− mice suggest that P2X7-R plays an important role in initiating the pathological changes resulting from an inflammatory insult. We also ruled out the possibility that the development of afferent hypersensitivity was delayed in the P2X7R−/− animals, because we saw no differences in afferent mechanosensitivity recorded from preparations taken at 28 or 56 d PI in either the WT or the knockout animals.

This study demonstrated that P2X7-R activation is involved in the generation of visceral sensory nerve dysfunction in an in vivo model of inflammation. We showed that T. spiralis infection invokes a P2X7-R-dependent and -independent inflammatory response in which the P2X7-R-dependent inflammatory response seems to be essential in generating postinfectious afferent hypersensitivity. This has direct clinical relevance in the study of novel analgesic therapy for visceral pain, because it presents P2X7-R as a potential target to decrease postinfectious afferent nerve hypersensitivity.

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Disclosures
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