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Polymeric Immunoglobulin Receptor in Intestinal Immune Defense against the Lumen-Dwelling Protozoan Parasite Giardia

Barbara J. Davids,* J. E. Daniel Palm, † Michael P. Housley, ‡ Jennifer R. Smith, § Yolanda S. Andersen, ‡ Martin G. Martin, § Barbara A. Hendrickson, ‡ Finn-Eirik Johansen, ¶ Staffan G. Sva¨rd, ‡ Frances D. Gillin,* and Lars Eckmann 2‡

The polymeric Ig receptor (pIgR) is conserved in mammals and has an avian homologue, suggesting evolutionarily important functions in vertebrates. It transports multimeric IgA and IgM across polarized epithelia and is highly expressed in the intestine, yet little direct evidence exists for its importance in defense against common enteric pathogens. In this study, we demonstrate that pIgR can play a critical role in intestinal defense against the lumen-dwelling protozoan parasite Giardia, a leading cause of diarrheal disease. The receptor was essential for the eradication of Giardia when high luminal IgA levels were required. Clearance of Giardia muris, in which IgA plays a dominant role, was severely compromised in pIgR-deficient mice despite significant fecal IgA output at 10% of normal levels. In contrast, eradication of the human strain Giardia lambia GS/M, for which adaptive immunity is less IgA dependent in mice, was unaffected by pIgR deficiency, indicating that pIgR had no physiologic role when lower luminal IgA levels were sufficient for parasite elimination. Immune IgA was greatly increased in the serum of pIgR-deficient mice, conferred passive protection against Giardia, and recognized several conserved giardial Ags, including ornithine carbamoyltransferase, arginine deiminase, α-enolase, and α- and β-giardins, that are also detected in human giardiasis. Corroborative observations were made in mice lacking the J chain, which is required for pIgR-dependent transepithelial IgA transport. These results, together with prior data on pIgR-mediated immune neutralization of luminal cholera toxin, suggest that pIgR is essential in intestinal defense against pathogenic microbes with high-level and persistent luminal presence. The Journal of Immunology, 2006, 177: 6281–6290.

The intestinal tract faces a physiological challenge in that it must provide ready access to ingested nutrients and also protection against food-borne microbes. This challenge is met by multiple specific and nonspecific host defenses that establish and maintain a selective intestinal barrier. Prominent among these defenses is secretory IgA (SIgA), the daily intestinal production of which in humans and mice exceeds that of any other isotype (1, 2). IgA is synthesized by mucosal B cells and plasma cells as a dimeric Ab with a molecular mass of ~350 kDa and is composed of two Ig α H chains, two Ig L chains, and a joining (J) chain. The latter is required for stabilization of polymeric IgA, because mice lacking the J chain have a diminished capacity to produce stable polymeric IgA (3). Upon secretion by IgA-producing cells in the lamina propria, the vast majority of polymeric IgA is transported into the intestinal lumen where it binds to Ags, thus promoting clearance through the luminal bulk flow and preventing access to the mucosa.

Transport of large quantities of IgA across the intestinal epithelium requires active mechanisms, because the intact epithelial barrier shows only low permeability for large proteins. This task is accomplished by a specialized transport protein, the polymeric Ig receptor (pIgR), which is coded for by the highly conserved gene Pigr in mammals and belongs to the superfamily of class I transmembrane receptor proteins (4). It is expressed constitutively by epithelial cells in the intestinal tract and lungs, as well as in hepatocytes in rodents and lagomorphs (2). Epithelial expression is further induced by proinflammatory stimuli such as TNF-α or IFN-γ or by microbial infection (5–7). Transfection of non-pIgR-expressing polarized epithelial cells with Pigr in culture confers the ability to transport polymeric IgA, as well as polymeric IgM, in a basolateral-to-apical direction (8). Release of luminal SIgA is achieved by cleavage of pIgR, whose extracellular domain, known as secretory component, remains covalently bound to IgA.

Mice deficient for Pigr cannot transport polymeric IgA into the intestinal lumen (9, 10). These mice are generally healthy, although they show increased levels of circulating IgA, as well as IgG, and increased immune reactivity against Ags from commensal bacteria (9), suggesting that pIgR-dependent IgA excretion into the intestinal lumen is important for preventing immune activation by enteric commensals and food Ags. The mice also exhibit modest increases in intestinal IgA-secreting cells and intraepithelial lymphocytes (11, 12), although the functional significance of these observations is unclear. Moreover, deficiency in pIgR interfered

1 Abbreviations used in this paper: pIgR, polymeric Ig receptor; ADI, arginine deiminase; OCT, ornithine carbamoyltransferase; SlgA, secretory IgA.

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3 Abbreviations used in this paper: pIgR, polymeric Ig receptor; ADI, arginine deiminase; OCT, ornithine carbamoyltransferase; SlgA, secretory IgA.
with normal immune protection against infection with the respiratory pathogens Streptococcus pneumoniae, Mycobacterium tuberculosis, and the influenza virus in the lungs and nasal cavity in mice (13-15), indicating a role of the receptor in immune defense against respiratory pathogens. However, pIgR was not required for B cell-dependent eradication of the murine enteric bacterial pathogen Citrobacter rodentium, which resides predominantly in the lumen and in close proximity to the epithelium (16, 17), nor was pIgR essential in adaptive immune defense against normal oral inocula of the invasive bacteria Salmonella typhimurium (17, 18).

Therefore, despite the evolutionary conservation of pIgR and the presumed physiologic importance of the intestinal SIGA system, little actual evidence exists in support of a critical role of pIgR in host defense against microbial pathogens in the intestinal tract, which is the major entry portal for food and waterborne pathogens in mammals.

The protozoan flagellate Giardia infects an estimated 280 million people each year worldwide (19) and is the most common cause of waterborne diarrheal disease in the United States (20). Infection can be asymptomatic, but frequently causes varying degrees of diarrhea, nausea, abdominal cramps, and malabsorption. Infection is initiated by the consumption of contaminated water and occasionally by person-to-person contact or contaminated food. Trophozoites, the vegetative and disease-causing form of the parasite, colonize the small intestine where they reside with the epithelium. Because of this tropism, host defenses against Giardia must act luminaly (21). Studies in mice have shown that IgA is critical for eradication of the murine parasite Giardia muris, which establishes a robust infection in its natural host (22). By comparison, IgA is less important in murine immune defense against the human parasite Giardia lamblia (22), possibly because it is less well adapted to mice. In addition, CD4 T cells (23, 24), mast cells (25, 26), and IL-6 (27, 28) are known to influence immune defense against Giardia, although they are likely to act by indirect mechanisms.

The goal of the present studies was to define the physiologic importance of pIgR and transepithelial IgA transport in host defense against Giardia as a prototypical protozoan parasite of the mammalian intestinal tract. Using two different murine models of giardiasis, we found that pIgR is critical in intestinal immune defense against the luminal parasite when high levels of luminal IgA are required but not under conditions of lower IgA requirements. These results suggest that pIgR has an important function in intestinal host defense against lumen-dwelling enteric parasites.

Materials and Methods

Mice

Mice deficient for pIgR, the J chain, or the Ig α H chain were described previously (3, 9, 29). pIgR- and IgA-deficient mice were on a mixed B6 × 129 genetic background so that wild-type littermates from heterozygous breeding pairs were used as controls. J chain-deficient mice were backcrossed for >12 generations to a BALB/c background, and wild-type BALB/c mice (Taconic Farms) were used as controls. Genotypes were confirmed by PCR analysis of tail DNA. All animal studies were reviewed and approved by the University of California San Diego Institutional Animal Care and Use Committee.

Giardia strains and infection protocol

The following Giardia strains were used in these studies: G. muris (obtained from M. Belosevic, University of Alberta, Edmonton, Canada) (30), G. lamblia strain GS/M-83-H7 (American Type Culture Collection (ATCC) 50581) (31), and G. lamblia strain WB, clone C6 (ATCC 50803) (32). G. muris was maintained by regular in vivo passage through mice. G. lamblia GS/M and WB/C6 were maintained by in vitro culture of trophozoites in TYI-S-33 medium (33). For mouse infections with G. muris, cysts were enriched by sucrose flotation, counted in a hemacytometer under a phase-contrast microscope, and given by oral gavage in 0.2 ml of water at 10⁵ cysts/mouse (22). For G. lamblia GS/M infections, trophozoites were administered by oral gavage at 10⁷/mouse in 0.2 ml of TYI-S-33 medium (22). At different times after infection, trophozoites in the small intestine were enumerated using a hemacytometer as previously described (22). Briefly, the small intestine was opened longitudinally, placed in 5-10 ml of ice-cold PBS for 10 min, and shaken vigorously to detach trophozoites, which were counted in a hemacytometer under a phase-contrast microscope. The detection limit of the assay was ~ 2 × 10³ trophozoites per small intestine. For cyst detection, fecal pellets were collected over a 2-3 h-period and homogenized in PBS. Cysts were enriched by sucrose flotation and counted in a hemacytometer using a phase-contrast microscope. The detection limit of the cyst assay was ~ 2 × 10³ cysts per gram of feces.

RNA isolation and RNase protection assay

Total RNA was isolated from the small intestine by a modified phenol/ guanidine isothiocyanate method (TRizol reagent; Invitrogen Life Technologies). RNase protection assays were done as described previously (34). Briefly, radioactively labeled cRNA probes for pIgR, sodium glucose co-transporter-1, and the ribosomal protein S2 were generated by in vitro transcription of cDNA in the presence of [32P]UTP. The radiolabeled probes were mixed with 2.5 μg of sample RNA and hybridized overnight at 42°C, after which remaining single-stranded RNA was digested with a combination of RNase A and T1 (Ambion). The reaction mixture was precipitated, denatured, and resuspended on a 5% polyacrylamide gel for 2 h, which was then placed on a filter paper and dried. The autoradiographic signal intensities of protected fragment bands were quantified by using a PhosphorImager (Molecular Dynamics) and analyzed with ImageQuant software (Molecular Dynamics). Bands were normalized by using the ribosomal protein S2 RNA signal in each lane (34).

Fecal and serum Ig assays

Serum was collected by tail vein bleeding, diluted 1/10 into PBS containing 2 mM EDTA, and stored at ~80°C until use. For fecal Ig assays, feces (100–200 mg) were collected from individual mice over a 2-h period, weighed, homogenized in 2 ml PBS, centrifuged at 18,000 × g to remove insoluble material, and stored at ~80°C until use. Levels of IgA, IgM, and IgG were determined by ELISA using mouse isotype-specific Abs from Southern Biotech as described previously (35).

Determination of anti- giardial Ig titers

Antigiardial Ab titers were assayed by whole-cell ELISA. Microtiter plates (Immulon 4 HBX; Thermo Electron) were coated with trophozoites by adding a 50 μl/well suspension of 10⁹/ml in PBS followed by drying overnight at room temperature under constant air flow. Parasites were fixed with 0.15% glutaraldehyde in 0.15 M sodium phosphate for 5 min at room temperature, after which the plates were quenched with 0.15 M glycine (pH 7.0) for 1 min and blocked in 5% dried nonfat milk and 1% goat serum in PBS for 1 h at room temperature. Plates were incubated with serial dilutions of sera or fecal extracts (diluted in PBS containing 1% BSA and 1% goat serum) for 1 h at room temperature, washed with PBS, and further incubated with HRP-labeled goat anti-mouse IgA, IgM, or IgG (Southern Biotech; 1/1,000 diluted in 1% goat serum in PBS) for 1 h at room temperature. After washing, bound HRP was detected with tetramethylbenzidine/H₂O₂ in 0.1 M sodium acetate buffer (pH 6.0). Reactions were stopped with 1.2 M sulfuric acid, and absorbances were read at 450 nm and, for background subtraction, at 750 nm.

Assays of anti- giardial activity

Antigiardial activity of sera was determined by agglutination assays and passive immune protection tests in mice. For agglutination assays, G. lamblia GS/M trophozoites (2 × 10⁷/ml) were mixed with 10% mouse serum in TYI-S-33 medium and incubated on ice for 15 min, after which they were transferred to a cell culture plate and further incubated at 37°C for 30 min. Photographs of the plates were taken with the aid of a phase-contrast microscope. For passive immunizations, adult C57BL/6J mice were infected i.p. with 50 μl of immune serum, preimmune serum, or PBS 2 h before oral infection with 5 × 10⁷ G. lamblia GS/M trophozoites and then thereafter for 4 days, at which point trophozoites were enumerated in the small intestine.

One-dimensional immunobl ot analysis of giardial extracts

Total protein extracts of G. lamblia GS/M or WB/C6 were prepared from lysates of in vitro culture using trichloroacetic acid in the presence of a protease inhibitor mixture (Complete Mini; Roche) as described previously (36). Proteins (20 μg) were separated under reducing conditions on a 4–20% SDS-polyacrylamide gel and electrotransferred to a nitrocellulose
membrane (Hybond ECL; Amersham Biosciences), which was blocked overnight at 4 °C in PBS containing 5% dried nonfat milk, 1% goat serum, and 0.1% Tween 20. Membranes were stained with individual preimmune or immune sera diluted 1:200 in staining buffer (PBS containing 1% dried milk, 1% goat serum, and 0.1% Tween 20) for 1 h at room temperature, washed three times over 30 min in PBS with 0.1% Tween 20, incubated with HRP-labeled goat anti-mouse IgA (1/700 dilution in staining buffer; Southern Biotech), washed, and developed with ECL reagents (Amersham Biosciences).

Molecular Ag identification

Giardial Ags were identified by two-dimensional immunoblot analysis combined with mass spectrometry as described previously (37). Briefly, total cell extracts of G. lamblia WB/C6 trophozoites were prepared by sonication in PBS containing a protease inhibitor mixture (Complete Mini; Roche) followed by the addition of 4 vol of two-dimensional sample buffer (9.9 M urea, 4% Igepal CA630, and 2.2% Phenyldiamine (pH 3–10); Amersham Biosciences). In the first dimension, isoelectric focusing was performed on a Multiphor II horizontal electrophoresis system (Amersham Biosciences) according to the manufacturer’s instructions using ReadyStrip immobilized pH gradient strips (Bio-Rad) with a linear pH gradient of 5–8. The second dimension was run on a 12% SDS-polyacrylamide gel. Proteins were visualized using a modified silver stain (PlusOne silver staining kit; Amersham Biosciences) or transferred to a polyvinylidene difluoride membrane (Hybond ECL; Amersham Biosciences) or blocked with 5% milk, 1% goat serum, and 0.05 Tween 20) and incubated for one hour with the indicated primary and secondary antibodies. 

Results

plgR is required for clearance of G. muris

To define the importance of plgR in intestinal host defense against a prototypical lumen-dwelling enteric pathogen, we conducted infection studies with the murine parasite G. muris. Mice genetically deficient for plgR and their wild-type littermate controls were inoculated with cysts by the natural oral route, and infectious burden was determined over time by enumerating the numbers of the vegetative trophozoite form in the small intestine. Wild-type mice had maximal trophozoite numbers one week after infection and cleared infection thereafter, with a >5000-fold reduction in infectious load by 7 wk (Fig. 1A) and complete eradication in >40% of mice at that time. plgR-deficient mice had similar trophozoite numbers as wild-type mice early after infection but failed to control infection subsequently. At 7 wk they had >1000-fold higher trophozoite numbers in the small intestine compared with controls (Fig. 1A). In parallel, fecal cyst shedding was significantly higher in plgR-deficient mice compared with controls (Fig. 1, B and C). These data show that plgR is critical for clearance of G. muris from the murine host. To place these results into perspective, we compared the findings in plgR-deficient mice with those in mice lacking IgA, because we found previously that IgA is required for G. muris eradication (22). No significant difference was observed in trophozoite numbers between the two knockout mouse strains at 1 or 7 wk (Fig. 1A), indicating that plgR- and IgA-deficient mice exhibited similar defects in regard to immune defense against G. muris.

The level of epithelial plgR expression determines the rate of active transport of polymeric IgA and IgM across polarized epithelia (8). Mice heterozygous for plgR (plgR+/−) displayed a ∼50% decrease in small intestinal plgR mRNA expression relative to wild-type (plgR+/+) mice (Fig. 2A). Concurrently, fecal IgA levels were reduced by ∼50% in plgR heterozygous mice (Fig.
importance of J chain in immune defense against G. muris

To confirm the importance of transepithelial Ig transport into the intestinal lumen for anti-Giardia immunity, we used J chain-deficient mice as a different genetic model of secretory Ab deficiency. The J chain is needed for stabilization of polymeric IgA and its transport across polarized epithelia (3, 40). J chain-deficient mice were colonized by G. muris as well as wild-type controls after 1 wk but failed to control and eradicate infection thereafter (Fig. 3). Given that the J chain-deficient mice were on a different genetic background (BALB/c) than the pIgR-deficient mice (B6 × 129) yet exhibited a similar immunological defect as pIgR-deficient mice, the results underline the fact that transport of polymeric IgA across the epithelium is critical for immune defense against G. muris.

Role of pIgR and J chain in immune defense against G. lamblia GS/M

G. muris is a natural parasite of mice but is not known to infect humans. To explore the role of pIgR in murine immune defense against a clinically relevant strain of Giardia, we conducted infection studies with the GS/M strain of G. lamblia, which, unlike other G. lamblia strains, can colonize normal adult mice (41). PlgR-deficient and control mice had similar trophozoite numbers 4 and 7 days postinfection, and both groups of mice eradicated the infection within 3 wk (Fig. 4). Furthermore, J chain-deficient mice exhibited no impairment or delay in eliminating G. lamblia GS/M infection (data not shown). Thus, neither deficiency in the pIgR nor the J chain had an impact on murine immune defense against G. lamblia GS/M. In contrast, in parallel studies we found that IgA-deficient mice showed a significant defect in clearing G. lamblia GS/M after 5 wk (Fig. 4), which confirmed our prior findings (22).

pIgR-deficient mice have reduced but significant levels of fecal IgA

The observation that IgA contributed to immune defense against G. lamblia GS/M and yet pIgR was not required for clearance suggested that IgA, in amounts sufficient for effective defense, can reach the intestinal lumen without active transport by pIgR. This notion appeared to be inconsistent with previous reports that pIgR-deficient mice have little or no luminal IgA (9, 10) and, therefore, prompted us to re-examine the levels of luminal IgA as reflected

**FIGURE 2.** Heterozygosity for pIgR compromises mucosal IgA output but not G. muris clearance. A. Uninfected mice lacking pIgR (pIgR<sup>−/−</sup>), heterozygous for pIgR (pIgR<sup>+/−</sup>), or wild-type littermate controls (pIgR<sup>+/+</sup>) were analyzed by RNase protection assay for small intestinal mRNA expression of pIgR or, as controls, the nutrient transporter sodium glucose cotransporter 1 (SGLT1), the brush border enzyme lactase, and the ribosomal protein S2. A representative autoradiograph is shown on the left. Signal intensities of specific bands were quantified with a PhosphorImager system and are expressed as the percentage relative to the mean value of wild-type controls normalized for S2 mRNA levels. Data are mean ± SEM of 3–4 individual mice. *, p < 0.05 relative to wild-type mice. B. Fecal homogenates of uninfected wild-type (pIgR<sup>+/+</sup>), heterozygous (pIgR<sup>+/−</sup>), or wild-type littermate control mice were assayed for IgA by ELISA. Results are mean ± SEM (n = 6 mice). *, p < 0.05 relative to wild-type mice. For comparison, IgA output in pIgR-deficient (pIgR<sup>−/−</sup>) mice is shown in Fig. 5A. C. Heterozygous mice (○) and wild-type littermate controls (●) were infected with G. muris cysts, and trophozoite numbers were determined at the indicated times after infection. Data are mean ± SEM of 7–8 mice per time point. *, p < 0.05 compared with wild-type control at the same time point. The gray line depicts the detection limit of the assay. For comparison, trophozoite numbers in pIgR<sup>−/−</sup> mice are shown in Fig. 1A.

**FIGURE 3.** J chain-deficient mice fail to clear G. muris. Mice lacking the J chain (○) and their wild-type controls (●) were infected orally with G. muris cysts, and trophozoite numbers in the small intestine were determined at the indicated times. Data are mean ± SEM (n = 5 mice/time point). The gray line depicts the detection limit of the assay. *, Significant increase (p < 0.05) compared with wild-type controls.

*pIgR expression is not regulated during G. muris infection*

Given the importance of pIgR in mediating immunity to G. muris, we asked whether pIgR expression might be regulated during the course of infection as was observed for other conditions of mucosal immune activation (4). However, pIgR mRNA levels, which correlate with pIgR-dependent epithelial IgA transport capacity in vitro (8) and in vivo (39), were not significantly different in the small intestine between G. muris-infected and -uninfected mice after 1 or 3 wk (data not shown). These results suggest that G. muris infection does not alter plgR-dependent transepithelial transport of polymeric IgA or IgM. In support of this conclusion, levels of total fecal IgA did not differ significantly between Giardia-infected mice and uninfected controls (see Fig. 5).
Serum IgA from pIgR-deficient mice has antiGiardial activity
The finding that significant levels of fecal IgA were present in pIgR-deficient mice suggested that it could exert antiGiardial activity in the intestinal lumen of the mice. To determine whether this might be the case, we examined IgA-dependent antiGiardial activity of mucosally produced IgA, which accumulates in the serum because it is not transported into the lumen in pIgR-deficient mice (9, 10). Whole-cell ELISA analysis revealed a strong increase in antiGiardial IgA, but not IgM or IgG, in the serum of pIgR-deficient mice by 4–6 wk after G. lamblia GS/M infection (Fig. 6A). By comparison, wild-type mice exhibited only a modest increase in antiGiardial serum IgA but strong increases in IgG titers after infection (Fig. 6A). Next, we examined the serum for IgA-dependent antiGiardial activity. Incubation of in vitro grown G. lamblia GS/M trophozoites with serum immune serum from pIgR-deficient mice caused marked agglutination, whereas preimmune serum of the same mice or immune serum from wild-type mice did not (Fig. 6B, and data not shown). Furthermore, immune serum from pIgR-deficient mice conferred passive protection against G. lamblia GS/M when administered systemically (Fig. 6C). In contrast, administration of immune serum from wild-type mice did not protect against Giardia (Fig. 6C), indicating that antiGiardial IgG in these mice was insufficient to exert protective functions in vivo. Taken together, these data and the results above (Figs. 4 and 5) suggest that pIgR-deficient mice produce high levels of antiGiardial IgA upon infection, of which the fraction that reaches the intestinal lumen is small but sufficient to contribute to G. lamblia clearance.

Unique and conserved Ags recognized by antiGiardial IgA from pIgR-deficient mice
The development of a strong and protective antiGiardial IgA response in pIgR-deficient mice provided an opportunity to use immune serum from the mice to identify relevant giardial Ags recognized by mucosally produced IgA not found at significant levels in the serum of normal mice. This opportunity was particularly

by fecal IgA output in the different groups of our mice. pIgR-deficient mice had readily detectable fecal IgA by ELISA, although the levels were ∼10-fold lower than those in wild-type mice (Fig. 5A). As expected, IgA-deficient mice had no detectable fecal IgA (Fig. 5A). Infection with G. lamblia GS/M did not alter fecal IgA output significantly in either of the mice (Fig. 5A). Detection of fecal IgA in pIgR-deficient mice was not an ELISA artifact, because immunoblot analysis of fecal extracts revealed the expected size of Ig α H chains (Fig. 5B). By comparison, fecal IgG levels were modestly (∼2-fold) increased in pIgR-deficient mice, whereas fecal albumin output was comparable between the different groups of mice (Fig. 5, A and B). Serum analysis revealed ∼50-fold elevated IgA levels in pIgR-deficient mice (Fig. 5A), which is consistent with prior reports (9, 10) and suggests that pIgR deficiency led to an increase in the gradient for IgA across the intestinal epithelium. Further analysis of the relationship between serum and fecal IgA levels in pIgR-deficient mice revealed ratios similar to those found for IgG in both pIgR-deficient and wild-type mice but very different from the ones observed for IgA in wild-type mice (Fig. 5C). Thus, IgA can reach the intestinal lumen in the absence of pIgR, albeit with a reduced efficiency.

FIGURE 5. pIgR-deficient mice have significant fecal IgA output. A, Fecal homogenates were prepared and serum was collected from wild-type (WT), pIgR-deficient (pIgR−/−), or IgA-deficient (IgA−/−) mice either uninfected (○) or 5 days after infection with G. lamblia GS/M trophozoites (■) and assayed by ELISA for levels of IgA (top panels) and IgG (bottom panels). Data are mean ± SEM (n = 6 mice/group). The gray lines depict the detection limit of the assay. *Significant increase (p < 0.05) compared with wild-type controls.

FIGURE 4. pIgR-deficient mice can eradicate G. lamblia. Mice deficient for pIgR ( ), IgA (△), or their wild-type littermate controls ( ■) were infected orally with 10⁷ G. lamblia GS/M trophozoites, and trophozoite numbers in the small intestine were determined at the indicated times. Data are mean ± SEM (n = 6 mice/time point). The gray line depicts the detection limit of the assay.

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Abs of the indicated isotypes were determined by whole-cell ELISA. Data from uninfected (F) or left uninfected (FE) C57BL/6 mice were injected i.p. daily with PBS or with serum from GS/M – 6 wk before collection (Immune) were mixed with in vitro grown GS/M trophozoites. After 4 days, trophozoite numbers in the small intestine were determined. Data are mean ± SEM (n ≥ 8 mice/group). B, Sera from uninfected pIgR-deficient mice (Pre-immune) or from pIgR-deficient mice infected with G. lamblia GS/M 4–6 wk before collection (Immune) were mixed with in vitro grown G. lamblia GS/M trophozoites and incubated for 45 min, after which photographs were taken with a phase-contrast microscope. C, Normal C57BL/6 mice were injected i.p. daily with PBS or with serum from pIgR −/− or pIgR −/+ mice either before infection (Pre) or 4–6 wk after infection with G. lamblia GS/M (Imm) and infected orally with 5 × 10⁶ G. lamblia GS/M trophozoites. After 4 days, trophozoite numbers in the small intestine were determined. Data are mean ± SEM (n = 4 mice/group). *, Significant decrease (p < 0.05) compared with PBS-injected mice.

FIGURE 7. Serum IgA from pIgR-deficient mice detects conserved giardial Ags. Total cell lysates of in vitro grown trophozoites of G. lamblia GS/M (G) and G. lamblia WB/C6 (C) were prepared, size fractionated by reducing, denaturing gel electrophoresis, and blotted onto a membrane. Blots were stained with serum from uninfected pIgR-deficient mice (Pre) and from pIgR-deficient mice infected with G. lamblia GS/M 4–6 wk previously (Immune) as primary Abs and HRP-labeled anti-mouse IgA as secondary Abs. Arrowheads indicate common Ags recognized by anti-giardial IgA in both Giardia strains. Each blot shows staining results using serum from an individual animal.

To define the molecular identity of conserved giardial Ags, we separated total trophozoite extracts of G. lamblia WB/C6 using two-dimensional gel electrophoresis and identified proteins recognized by immune IgA through immunoblotting and subsequent mass spectrometry (37). Immune IgA detected several Ags with molecular masses ranging from 30 to 65 kDa and pI values of 5.5–7.8 (Fig. 8). Mass spectrometric analysis of the proteins corresponding to the Ag spots identified 12 giardial proteins, including OCT, ADI, α-enolase, and different members of the giardin family (Table I). One Ag, OCT, was recognized by all of the seven individual immune sera tested, whereas the other Ags were recognized by only some of the sera. Furthermore, several immune sera detected most of the 12 Ags, whereas others recognized only one or a few of them (Table I). Thus, individual mice varied considerably in the spectrum of conserved giardial Ags recognized by their immune IgA. Interestingly, most of the Ags identified in the murine studies were previously shown to be recognized by Abs in immune sera or breast milk from humans with giardiasis (37, 38) (Table I), suggesting similar immune responses to Giardia in humans and mice.

To confirm the identity of the Ags identified by mass spectrometry, we synthesized three of the Ags, OCT, ADI, and α-enolase, as recombinant proteins in E. coli and purified and used them in immunoblots. Immune IgA from pIgR-deficient mice recognized all three recombinant Ags, although recognition was variable because OCT was strongly recognized by some but not at all by other sera, whereas ADI was only weakly recognized by most of the sera (Fig. 9). Nonetheless, these results confirm the mass spectrometric analysis but also suggest that some of the native Ags may be recognized in a modified form not produced in bacteria or may be partially destroyed upon purification.

**Discussion**

PlgR is highly conserved in mammals and a functional homologue has been identified in birds (43), suggesting that the receptor has
evolutionarily important functions in vertebrate host defense. It is highly expressed in the intestine, yet prior studies have provided little direct evidence for the importance of pIgR in defense against common enteric microbial pathogens (16–18). The work reported here demonstrates that pIgR can play a critical role in intestinal defense against the protozoan enteric parasite \textit{Giardia} as revealed by the combined use of two different \textit{Giardia} species, \textit{G. muris} and \textit{G. lamblia} GS/M, and gene-targeted mice lacking IgA, the J chain, or one or both alleles of pIgR. The receptor was required for eradication of \textit{Giardia} under conditions of high luminal IgA requirements, because IgA-dependent eradication of \textit{G. muris} was compromised in pIgR\textsuperscript{−/−} mice, which were found to have only 10\% of normal fecal IgA output. In contrast, IgA-dependent clearance of \textit{G. lamblia} GS/M was not affected by the absence of pIgR, indicating that pIgR had no apparent physiologic role when lower

<table>
<thead>
<tr>
<th>Spot ID</th>
<th>Ag OCT</th>
<th>GenBank Accession No.</th>
<th>Predicted Molecular Mass (kDa)</th>
<th>Mouse</th>
<th>Human</th>
<th>Ref. 37</th>
<th>Ref. 38</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ornithine carbamoyltransferase</td>
<td>AF069576</td>
<td>36.4</td>
<td>●</td>
<td></td>
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<td>●</td>
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<tr>
<td>2</td>
<td>Arginine deiminase</td>
<td>U49236</td>
<td>64.1</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>3</td>
<td>α-Enolase</td>
<td>AF465774</td>
<td>48.2</td>
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<td>●</td>
<td>●</td>
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<tr>
<td>4</td>
<td>α1-Giardin</td>
<td>X52485</td>
<td>33.9</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>5</td>
<td>α2-Giardin</td>
<td>M34550</td>
<td>33.9</td>
<td>●</td>
<td>●</td>
<td>●</td>
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</tr>
<tr>
<td>6</td>
<td>α7.1-Giardin</td>
<td>AF514358</td>
<td>32.8</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>*</td>
<td>α11-Giardin</td>
<td>YT81330</td>
<td>35.0</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>7</td>
<td>β-Giardin</td>
<td>M36728</td>
<td>29.4</td>
<td>●</td>
<td>●</td>
<td>●</td>
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</tr>
<tr>
<td>8</td>
<td>α2-Tubulin</td>
<td>AF176672</td>
<td>50.6</td>
<td>●</td>
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<td>9</td>
<td>\textit{Giardia} trophozoite antigen-2</td>
<td>AF514362</td>
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<td>*</td>
<td>Fructose-1,6-bisphosphate aldolase</td>
<td>XM_774313</td>
<td>35.2</td>
<td>●</td>
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</tbody>
</table>

\textsuperscript{a}Ags were identified by mass spectrometry of proteins extracted from spots specifically recognized by antigiardial IgA from pIgR-deficient mice previously infected with \textit{G. lamblia} GS/M trophozoites. A representative silver-stained gel and immunoblot, along with the corresponding spot identification (ID) numbers, is shown in Fig. 8. Ags labeled with an asterisk are not represented in Fig. 8. Data from seven individual mice are shown. ●, Respective antigen was detected by immune IgA of a given mouse. For comparison, published data from human giardiasis patients are shown in the last two columns.
IgA in human immune defense against particular strains of Giardia, a notion supported by the observation that the ratios of circulating Ig to fecal Ig output were comparable for IgA and IgG in these mice, as adult mice express only low levels of high-capacity intestinal IgG transporters (62). Thus, the concentration gradients and, hence, the driving forces and permeability across the intestinal and hepatic epithelium appear to be similar for IgA and IgG in the absence of plgR.

Mice devoid of the J chain showed the same phenotype in regard to Giardia clearance as those lacking plgR. Similarly, effective intestinal immune protection against cholera toxin depended on the J chain (44). Mice lacking the J chain exhibit ~10-fold reduced IgA levels in intestinal lavage fluid (3), which resembles our findings in plgR−/− mice. Together, these data suggest that deficiency in plgR and the J chain have similar physiologic consequences for IgA-dependent immune protection in the intestinal tract. An important biochemical difference between plgR and J chain deficiency is that serum IgA from plgR−/− but not J chain−/− mice can be transported across polarized epithelial cells, although it is markedly elevated in both mutant mouse strains (3). Dimeric IgA can form in the absence of the J chain (3), but plgR-dependent epithelial transport is severely curtailed (40). As a practical consequence, we could demonstrate that plgR-deficient mice provide a convenient model to examine the role of IgA in passive intestinal immune protection. The utility of this model is further supported by our observation that Ag-specific serum IgG levels were negligible in plgR-deficient but not wild-type mice upon Giardia infection. High mucosal IgA levels in plgR−/− mice (9) might prevent Ag-dependent activation of IgG isotype switching or IgG production in the intestine or systemic sites, although it is also possible that Ag-specific IgA may mask Ags for in vitro recognition by IgG.

Using immune IgA from plgR-deficient mice as a novel probe, we identified at least 12 giardial Ags at the molecular level. Our identification strategy focused on conserved Ags present even in only distantly related strains of G. lamblia. We did not characterize Ags unique to the infecting strain of G. lamblia GS/M, although our one-dimensional immunoblot analysis showed that such Ags exist. In particular, members of the variant surface protein family, which are diverse among giardial strains and major targets of the murine and human adaptive immune response (63–65), were not among the conserved Ags revealed in our work. Instead, the identified Ags belonged to different protein categories not associated with surface antigenic variation. For example, ADI and OCT are enzymes involved in giardial metabolism of arginine, an important energy source for trophozoites (66–68). They are normally present in the cytoplasm and thus would be expected to be inaccessible to host immune recognition, yet we found that they can be released upon contact with epithelial cells (S. Svärd, unpublished data). Other Ags of interest are the α-giardins, which constitute a gene family with more than 20 members in G. lamblia and display homology to vertebrate annexins, proteins characterized by their calcium- and phospholipid-binding properties (69). Several α-giardins, particularly α1 and α2, are localized at the trophozoite plasma membrane and may play a role in parasite attachment to the intestinal epithelium (69, 70). Importantly, most of the Ags in our murine studies were also recognized by Abs in immune sera or breast milk from humans infected with G. lamblia (37, 38). This overlap suggests that the Ag specificity of the murine and human
immune responses to \textit{G. lamblia} are remarkably similar and provide important validation for the use of themurine giardiasis models for understanding and preventing the human disease.

**Disclosures**

The authors have no financial conflict of interest.

**References**


