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Membrane-bound IgE on B cells is increased during *Clonorchis sinensis* infection

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ABSTRACT

A high level of serum IgE is a hallmark of helminthic disease. Secretory IgE can bind FcεRI or FcεRII/CD23. The combination of IgE and FcεRI, a high-affinity interaction, has long received attention and is believed to facilitate helminth control, while the properties of CD23-bound IgE have long been unexplored. Here, we established a *Clonorchis sinensis* (*C. sinensis*) infection model with different mouse strains and investigated membrane-bound IgE on B cells during infection. We show that after infection, the increase in CD23 expression on B cells was obvious, even in relatively resistant C57BL/6 mice, as well as in susceptible BALB/c and FVB mice. Although the serum IgE amount was lower in C57BL/6 mice than in BALB/c and FVB mice, the level of IgE binding to peripheral B cells was also elevated. Additionally, the IgE on B cells was soon undetectable in vitro due to dissociable binding. The results of the present study demonstrate the dramatic increase in CD23-bound IgE on B cells after *C. sinensis* infection. The significance of CD23-bound IgE in Ag transport and presentation has gained consideration in allergy development for its potential ability to promote the Th2 response. Therefore, even though the association of IgE and CD23 is not as substantial as that of IgE and FcεRI, membrane-bound IgE on B cells may be worth further study regarding clonorchiasis and other parasitic infections.

1. Introduction

Different from other immunoglobulins that are well known for their immune protection effect, IgE is the least abundant type but is best known as a mediator of type I hypersensitivity reactions, resulting in psychological and physical discomfort and even death (Wu and Zarrin, 2014). In host defense, IgE is traditionally thought to have to evolved against metazoan parasites. However, the functional significance in fighting against this infection is undefined (Oettgen, 2016). As with all other Ig isotypes, IgE is produced as secretory or membrane isoforms. The main function of sIgE is the recognition of foreign antigens (Ags), a role shared by all Abs. Membrane-bound IgE (mIgE) exists in different forms. The IgE B cell receptor (BCR), which only exists on IgE-switched B cells, is found at very low frequencies, even in helminth infection (Wu and Zarrin, 2014; Yang et al., 2012). Another form, Fc receptor-loaded IgE, is mainly bound to the cell membrane by two different receptors, the high-affinity receptor FcεRI and the low-affinity receptor CD23 (FcεRII) (Kraft and Kinet, 2007; Vercelli et al., 1989). FcεRI, found mainly on mast cells and basophils, binds IgE with high affinity and has attracted extensive attention for its central role in allergic sensitization. CD23, expressed mainly on B cells, binds to IgE-Fc with lower affinity than FcεRI and has been noted for its regulation of IgE production (Dhaliwal et al., 2012). However, the roles of CD23-bound IgE in Ag transport and presentation that potentially promote the Th2 response in allergic reactions have been actively investigated (Getahun et al., 2005; Griffith et al., 2011; Pirron et al., 1990; Sutton and Davies, 2015).

Clonorchis sinensis, a foodborne parasite that dwells in the bile duct of the liver, is prevalent in parts of eastern Asia, including China, Korea and Vietnam. It is estimated that approximately 15 million people become infected via ingestion of raw or undercooked fish meat containing metacercariae of *C. sinensis*(Qian et al., 2016). Along with damage to bile duct epithelial cholangiocytes and hepatocytes, damage-associated molecular pattern (DAMP) molecules, such as heat shock protein, IL-25, and IL-33, and worm-derived and excretory-secretory Ag of *C. sinensis* induce a strong Th2 response, which contributes to parasite control as well as pathological damage, such as liver fibrosis (Yan et al., 2017; Yu

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et al., 2016; Zhao et al., 2018). IgE production is a key event in Th2-based responses (Oettgen, 2016). Here, in view of this background, to investigate the potential effect of CD23-bound IgE during parasite-Ag presentation and its corresponding Th2-based response, we detected IgE-bound B cells during *C. sinensis* infection and found that IgE's binding capacity for B cells increased clearly after infection, which suggested the roles of IgE as a Th2 response amplifier as well as the protective antibody in parasite infection.

2. Materials and methods

2.1. Parasite

Metacercariae of *C. sinensis*were obtained by digesting the second intermediate host, infectious *Pseudorasbora parva,* with artificial gastric juice as described elsewhere(Yu et al., 2016). In brief, the infected fish were chopped into minced fish and then digested with artificial gastric juice (0.7% pepsin A and 0.1% HCl) at 37 °C for 12 h. The undigested tissues were removed by a 100-μm filter sieve. The filtrate was left standing for 1 h. Then, the metacercariae were identified and collected from the sediment under a dissecting microscope.

2.2. Mice

Female age-matched and specific-pathogen-free C57BL/6, BALB/c and FVB mice (Beijing Vital River Laboratory Animal Technology Co., Ltd) were maintained in the SPF laboratory of the model animal research center of Xuzhou Medical University (standard rodent chow and water available ad libitum; 12 h light/dark cycle). All animal experimental protocols and procedures were approved by the Animal Care and Use Committee of Xuzhou Medical University according to the guidelines of National Laboratory Animal Ethics Committee of China. Mice were administered 45 metacercariae by intragastric intubation and were sacrificed on days 14, 28 and 56 postinfection. Serum from each mouse was collected and stored at −80 °C.

2.3. Preparations of single-cell suspensions from lymphoid organs and livers

Single-cell suspensions from lymphoid organs and livers were prepared by mechanical disruption. For lymph nodes, antibody incubations and detections were performed as soon as possible after the node was ground. For the spleen, the single-cell suspensions were treated with red blood cell lysis buffer before antibody incubations. Isolation of liver white blood cells was performed using procedures with minor modifications (Yu et al., 2016). Briefly, livers were pressed through a 200-gauge stainless steel mesh. After washing with 0.01 M sterile phosphate-buffered saline (PBS) with 2% heat-inactivated fetal bovine serum (FBS), cells were resuspended in 2 ml 40% Percoll (Solarbio, Beijing, China), overlaid on 3 ml 70% Percoll and then centrifuged at 2500 rpm for 25 min. The lymphocytes were collected from the 40/70% Percoll interface.

For the mIgE dissociation experiment, cells from the liver-draining portal lymph node were placed in 24-well tissue culture plates (2×10^6 cells/ml/well) with RPMI-1640 that contained 10% FBS at 37 °C, 5% CO_2 immediately after being treated as single cells. Cells were collected at various time points for the mIgE analysis.

2.4. Flow cytometry

After blocking the Fc receptor with anti-mouse CD16/CD32, single-cell suspensions were incubated at 4 °C for 30 min with appropriate dilutions of one or more of the following fluorescently labeled mAbs: anti-CD19-APC, anti-CD23-PE-Cy7, anti-CD80-PE, anti-CD86-PercpCy5.5, anti-IgD-Pacific-Blue, anti-IgM-PercpCy5.5, anti- IgE-FITC (Clone : 23G3), anti-IgE-PE (Clone: RMA-1) and its isotype control. All antibodies were purchased from ebioscience (San Diego, CA, USA) or BioLegend (San Diego, CA, USA). Cells were analyzed by a FACS CantoII flow cytometer (BD Bioscience).

2.5. ELISA

IgE was quantified in the serum of mice (diluted 1/50) by the mouse IgE ELISA Ready-SET-GO system (ebioscience, San Diego, CA, USA). Measurements were performed according to the manufacturer's instructions. To detect the dissociation of IgE from B cells, 1×10^8 of liver-draining portal lymph node cells isolated on ice were resuspended in 500 μl RPMI-1640 that contained 10% FBS and placed at 37 °C, 5% CO_2. The IgE in cellular supernatants was quantified by an IgE ELISA Ready-SET-GO kit at 0 h and 8 h post-resuspension.

2.6. Statistical analysis

Values are expressed as the mean ± SEM. The statistical analysis of the data was performed by Student's *t*-test and ANOVA with Bonferroni's post-test using GraphPad Prism 7 software. The difference was considered to be statistically significant if $P < 0.05$.

3. Results

3.1. Changes in serum IgE levels

Among the three strains, the serum IgE in uninfected C57BL/6 mice

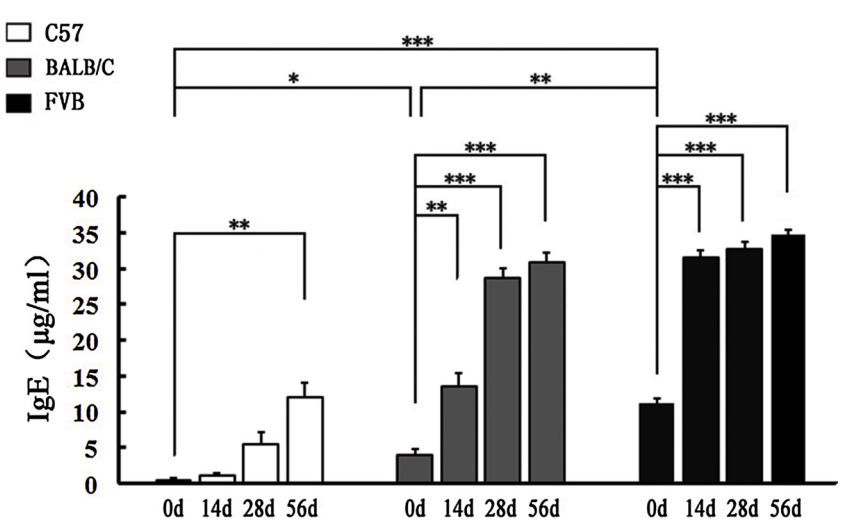

Fig. 1. Concentration of serum IgE in mice of different strains infected with *C. sinensis*.
Serum IgE was different among these strains at day 0, and the increase in serum IgE was dissimilar among these strains. For C57BL/6 mice, the IgE increased at days 28 and 56 post-infection. For BALB/c and FVB mice, the IgE increased as early as day 14 postinfection. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ANOVA with Bonferroni's post-test, the data represent means ± SEM per time point, n = 4~6).

was nearly undetectable. However, in uninfected BALB/c or FVB mice, the feeding conditions were the same as in C57BL/6 mice (Fig. 1). The differences among those strains are consistent with those in previous reports (De Vooght et al., 2010; Shimada et al., 2008). Compared to that of the control, the serum IgE in C57BL/6 mice was not obviously high at 28 d postinfection. However, the quantity difference is notable after 56 d postinfection. Serum IgE increased dramatically in BALB/c mice from 14 d to 28 d postinfection, while this dramatic increase in FVB mice occurred during the 0 d to 14 d postinfection period (Fig. 1).

3.2. Increase in CD23 expression on B cells during C. sinensis infection

The gross observations and histopathological changes of the *C. sinensis*-infected mouse liver have been described in several papers(Kim et al., 2012; Uddin et al., 2012; Zhang et al., 2017), but we also found a very unusually enlarged liver-draining portal lymph node, which is located just outside the liver where the hepatic portal vein enters) (Fig. S1). Therefore, although the liver is usually the only organ impaired by *C. sinensis*, we detected the expression level of CD23 in the liver, liver-draining portal lymph node and spleen, demonstrating the dissemination of secreted Ags and the accessory mediators of inflammation via systemic circulation.

Overall, the level of CD23 on B cells from lymph nodes, spleens and livers of *C. sinensis*-infected BALB/c mice was increased significantly compared with that of corresponding samples from noninfected mice ($P < 0.01$, Fig. 2). The result of FVB mice is similar. However, for *C. sinensis*-infected C57BL/6 mice, the increased expression of CD23 on B cells in spleens and livers was not obvious but was apparent in liver-draining portal lymph nodes (Fig. S2). The increased expression of the costimulated molecule CD86 on $CD23^+$ B cells in lymph nodes and spleens indicated the increasing ability of Ag presentation (Fig. 3). However, the expression of costimulated molecule CD80 was slightly changed on these B cells (Fig. 3).

3.3. Increase in membrane-bound IgE (mIgE) on B cells after C. sinensis infection

In addition to acting directly as professional Ag present cells, CD23-positive B cells were reported to capture IgE-complexed Ags in circulation and transport them to splenic B cell follicles that may also enhance antibody and T cell responses in *C. sinensis* infection via increased presentation of complexed Ag. Therefore, we assessed the mIgE of liver-draining portal lymph nodes and liver cells. Most of the B cells from the lymph node were mIgE positive, even in uninfected C57BL/6, whose serum IgE was nearly undetectable (Fig. S3B). After infection, the MFI of mIgE increased dramatically in the lymph nodes of all three strains of mice (Fig. 4). In FVB and BALB/c mice, after 4 w of *C. sinensis* infection, mIgE became detectable on CD19-positive B cells in the liver (Fig. 4). Fig. S3 shows the representative flow cytometry scatter plots of the rat IgG1 kappa isotype control (isotype control of anti-IgE-PE) per group of C57BL/6 mice. Additionally, mIgE could also be detected by another anti-IgE clone (Fig S3).

3.4. The ex vivo dynamics of membrane IgE on B cells in the lymph nodes of C. sinensis-infected BALB/c mice

The mIgE easily dissociated from B cells. Because the mIgE became undetectable as time passed (Fig. 5A), the MFI of CD23 only showed a slight decrease after the lymphocyte suspension was allowed to stand for 8 h (Fig. 5B). Furthermore, the IgE ELISA of the suspensions gave a positive result (Fig. 5A). These results indicated that IgE changed its form from membrane-bound to the secretory form. Unlike mIgE, other BCR (mIgM or mIgD) that were expressed by most $CD19^+$ cells did not show such a dramatic decrease, though they may have shown a slight change after 8 h of cultivation (Fig. 5C).

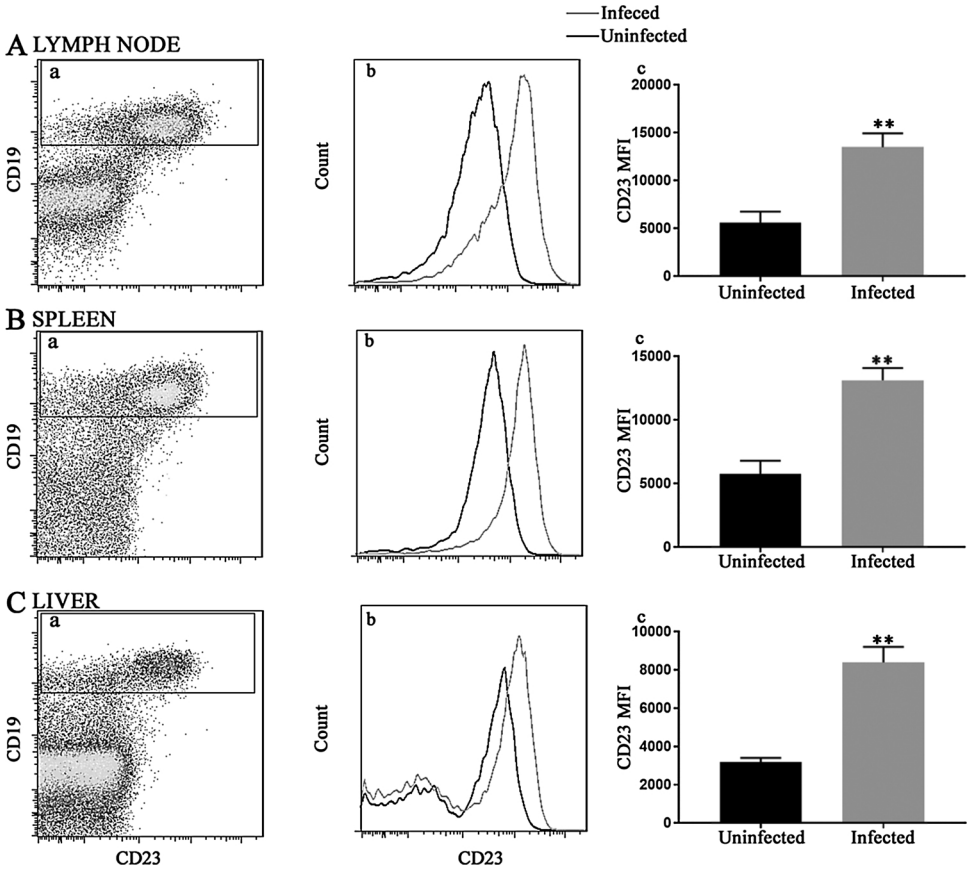

Fig. 2. Expression of CD23 on B cells after *C. sinensis* infection.
Lymphocytes from the liver-draining portal lymph nodes (A), spleens (B) and livers (C) of BALB/c mice at day 28 postinfection were harvested and stained for analysis by flow cytometry. a) CD23 was detected in $CD19^+$ cells, and this panel is a representative of uninfected mice. b) This panel is a representative of each group. The black curve represents the uninfected mice, and the gray curve represents the infected mice. c) The mean fluorescence intensities (MFI) of CD23 on $CD19^+$ cells were analyzed. (**$p < 0.01$, ***$p < 0.001$, Student's *t*-test, n = 4~6).

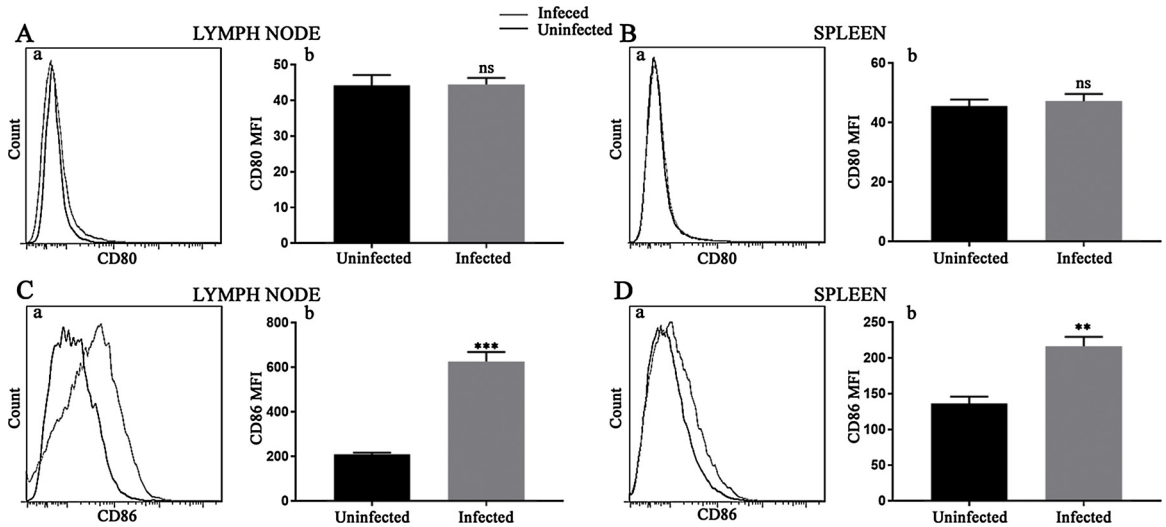

Fig. 3. Expression of CD80/86 on B cells in liver-draining portal lymph nodes and spleens from *C. sinensis*-infected BALB/c mice.
$CD19^+$ cells from the liver-draining portal lymph nodes (A, C) and spleens (B, D) from BALB/c mice at day 28 postinfection were analyzed by flow cytometry. a) This panel is a representative of each group. The black curve represents the uninfected mice, and the gray curve represents the infected mice. b The mean fluorescence intensities (MFI) of CD80/86 on $CD23^+CD19^+$ cells were analyzed. (n = 4~6, $^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t*-test).

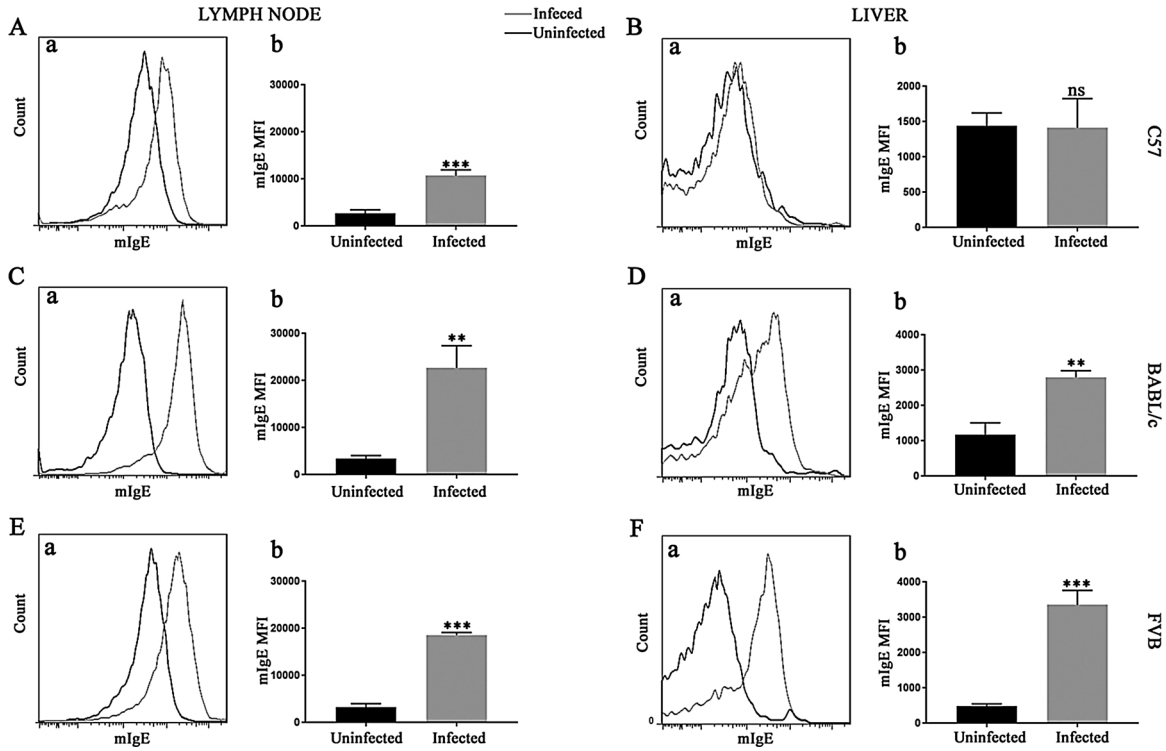

Fig. 4. Expression of membrane IgE (mIgE) on B cells from liver-draining portal lymph nodes and livers of *Clonorchis sinensis*-infected mice.
Lymphocytes from the liver-draining portal lymph nodes (A, C, E) and livers (B, D, F) of C57BL/6 (A, B), BALB/c (C, D) and FVB (E, F) mice were harvested 28 days postinfection and then stained as soon as quickly for the mIgE analysis on $CD19^+$ cells by flow cytometry. a) This panel is a representative of each group. The black curve represents the uninfected mice, and the gray curve represents the infected mice. b) The mean fluorescence intensities (MFI) of mIgE on $CD19^+$ cells were analyzed. ($^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t*-test, n = 4~6).

4. Discussion

Elevated production of IgE is a characteristic immune response during infection with helminths such as *C. sinensis* (Uddin et al., 2012). The traditional view is that IgE production is a consequence of the broader Th2 response, which is one of the key factors of parasite elimination (Oettgen, 2016). In fact, with the clarification of the dual anti-infection and pathological tissue remodeling functions of Th2type cytokines, the function of IgE during parasite infections may need to be reevaluated since in allergic disease, IgE is a key factor in amplifying a pathological Th2 response (Matucci et al., 2018).

Different mouse strains are prone to displaying different immune responses when encountering foreign invaders. C57BL/6 and BALB/c mice are regarded as prototypic Th1- and Th2-type mouse strains, respectively (Jovicic et al., 2015; Watanabe et al., 2004). Our previous studies and others have shown that different strains of mice have

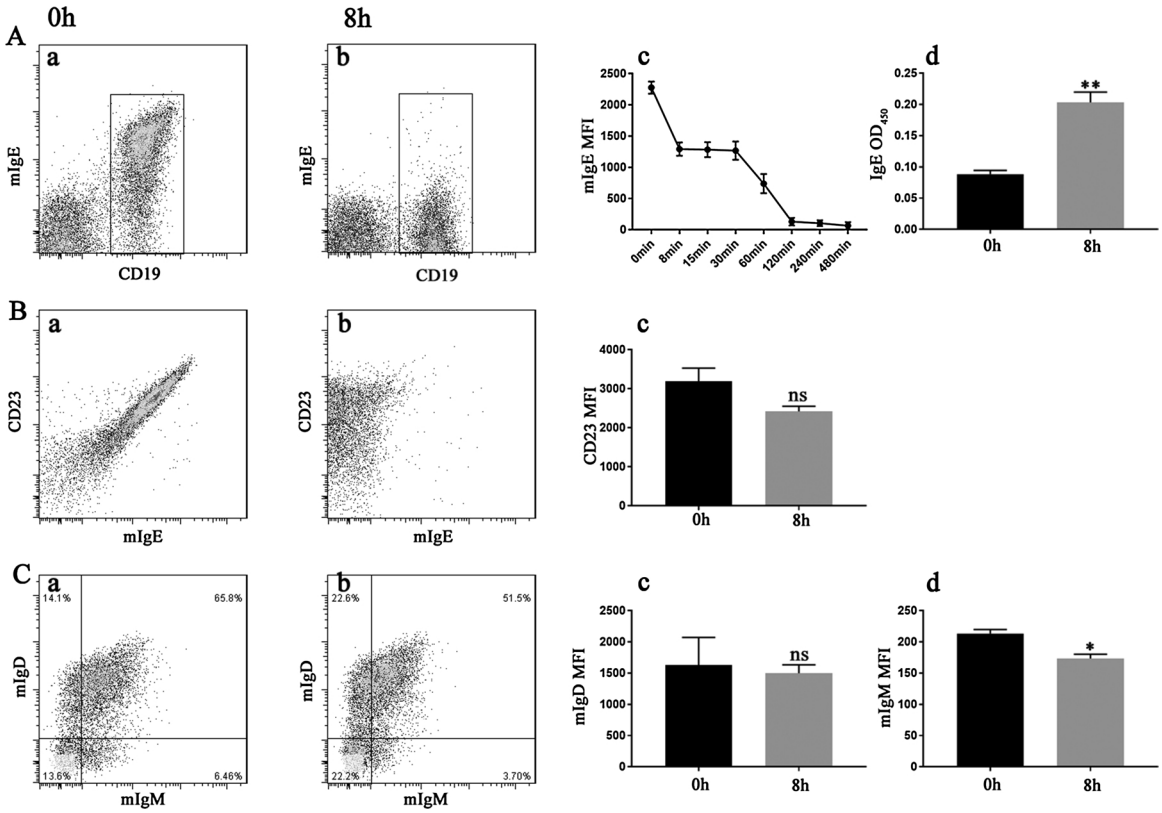

Fig. 5. *Ex vivo* dynamics of membrane IgE on B cells in the lymph node of *C. sinensis*-infected BALB/c mice.
Lymphocytes from the liver-draining portal lymph nodes of *C. sinensis*-infected BALB/c mice were harvested and then stained for analysis by flow cytometry at different time points. The expression of mIgE (A), CD23 (B), mIgD (C) and mIgM (C) on CD19$^+$ cells was analyzed at set time points up to 8 h after lymph node cell separation. a) This panel is a representative of the detection carried out directly after the node was grinded and kept on ice (0 h). b) This panel is a representative of the detection carried 8 h after lymph node cell separation. c and d) The mean fluorescence intensities (MFI) of CD23 (B), mIgD (C) or mIgM (C) on CD19$^+$ cells were analyzed. A-c) The MFI of mIgE on CD19$^+$ cells was analyzed at set time points. A-d) The level of sIgE in the supernatants of lymph node cells (2×10^8 cells/ml) detected at 0 and 8 h. (**$p < 0.01$, Student's *t*-test, n = 3).

different susceptibilities to *C. sinensis* infection. BALB/c and FVB strains are more susceptible to infection than C57BL/6 mice. The worm form in BALB/c and FVB mice is more well developed than that in C57BL/6 mice (Kim et al., 2012; Uddin et al., 2012; Zhang et al., 2017). In our present study, we found that the amount of serum IgE in FVB mice was higher than that in BALB/c, and the level in C57BL/6 was the lowest among the three strains of adult mice. In C57BL/6 mice, a somewhat resistant strain, we found that IgE was not clearly higher than that in uninfected mice, even at 28 d postinfection, but the gap at 56 d postinfection was notable. The difference in susceptibility to *C. sinensis* infection in different mouse strains may partially be attributed to an aberrant Th2 response (Choi et al., 2003), as *C. sinensis* cannot develop in severe combined immunodeficient (SCID) or athymic nude mice (Yoon et al., 2001).

CD23, the so-called low-affinity IgE receptor, is essential in the regulation of serum IgE levels (Hibbert et al., 2005). However, for the broad cellular distribution, a potentially important role in immunity has been discussed. Both in vivo and in vitro, CD23-bound IgE enhanced B cell Ag presentation to T cells and expanded T cell levels. Ag combined with IgE on CD23 that is co-crosslinked to BCR enhanced the Ag signals (Kehry and Yamashita, 1989). CD23-bound IgE facilitates Ag endocytosis of B cells as APCs (Pirron et al., 1990). In contrast, it was suggested that CD23$^+$ B cells capture IgE-complexed Ags in circulation and transport them to splenic B cell follicles where Ags were taken up by DCs or exosomal-mediated transfer from B cells to DCs (Engeroff et al., 2017; Martin et al., 2014). The above findings suggest an important role for CD23-bound IgE in the promotion of specific T cell responses. In humans, it was reported that circulating CD23$^+$ cells are preloaded with IgE (Griffith et al., 2011; Mwinzi et al., 2009). In the present study, we found that the mice fed in an SPF environment bearing IgE on CD23$^+$ B cells in lymph nodes showed almost undetectable serum IgE. Although, not as in the lymph nodes, a high proportion of IgE-bearing B cells in the livers and spleens in uninfected mice was not detected, this does not mean that the peripheral CD23$^+$ B cells were not loaded by IgE because mIgE may be dissociated from CD23 during lymphocyte preparation. For flow cytometry, liver and spleen samples must go through density gradient centrifugation or lysis of red blood cells, which may cause the dissociation of IgE from CD23 in vitro. Therefore, our results from normal mice support the view that CD23$^+$ cells circulate preloaded with IgE, even though the concentration of free serum IgE is not high. After *C. sinensis* infection, it may not be appropriate to denote CD23 as the low-affinity receptor. We found that even after long-term centrifugation and washing, IgE could also be detected on the surface of CD23$^+$ B cells infiltrating the livers of *C. sinensis*-infected mice. CD23 binds IgE at both a high and low affinity (Hibbert et al., 2005; Kilmon et al., 2001). This dual affinity is related to the oligomerization of CD23 (Gould et al., 1991). Low-affinity binding occurs when one molecule of IgE is bound to one CD23 molecule, and high-affinity binding of IgE to CD23 occurs when a molecule of IgE is bound by multiple CD23 receptors at the same time (Kilmon et al., 2001). As CD23 belongs to the C-type (calcium-dependent) lectin-like superfamily, Ca^{2+} has been reported to induce conformational changes of CD23 and increase its affinity for IgE (Yuan et al., 2013). In the present study, in lymphocytes from the livers of *C. sinensis*-infected BALB/c or FVB mice, mIgE was still detectable on B cells, despite their preparation by long-term centrifugation and washing treatment. This

indicates the possibility of affinity enhancement between CD23 and IgE after infection, which is rarely studied in parasitic infections. However, while affinity is changed between CD23 and IgE, the mIgE involved is still dissociable.

It has been long recognized that increased IgE is associated with parasitic helminth infection, and the immunological function of IgE remains controversial (Black et al., 2010). The hypotheses for the protective role of IgE in parasitic control are mainly focused on the interaction of IgE with FcεRI on myeloid cells (Oettgen). However, overall, B cells may interact with IgE more frequently than myeloid cells due to the quantitative advantages of CD23, although the level of intimacy is not as high. in vitro and in vivo studies demonstrate that IgE enhances Ag-specific Ab and T cell responses via $CD23^+$ B cells in chronic allergic disease (Pirron et al., 1990). However, the role of IgE in Ag presentation during parasite infections has not been studied. The present study suggests an important role for CD23-bound IgE in immunity to the parasite, considering that $CD23^+$ B cells were found to be more prominent and showed a gradually stable combination with IgE during infection with *C. sinensis*.

The major pathologic changes of chronic *C. sinensis* infection occur in the bile ducts, leading to eventual bile duct hyperplasia and liver fibrosis around the hepatic bile ducts (Yan et al., 2015). This pathologic change is associated with a highly polarized Th2 response (Choi et al., 2003; Zhang et al., 2017). Accumulating evidence shows that in chronic allergic disease, IgE production is not only a consequence of the Th2 response but also a cascade amplifier that promotes an immune system imbalance (to a Th2 bias) that exacerbates tissue damage (Oettgen, 2016). Regarding *C. sinensis* infection, a prominent Th2 commitment involved in infection may play a significant role in early parasite elimination, but it may also be a key factor in promoting liver fibrosis.

In summary, our study shows that during *C. sinensis* infection, CD23-bound IgE on B cells increases dramatically. The significance of CD23-bound IgE during Ag transport and presentation is gaining consideration in allergy development for its potential ability to promote the Th2 response. Therefore, even though the association of IgE and CD23 is not very substantial, membrane-bound IgE on B cells may be worth further study in clonorchiasis and other parasitic infections.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the manuscript entitled “Membrane-bound IgE on B cells is increased during *Clonorchis sinensis* infection”.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imbio.2019.03.004.

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Supplementary material

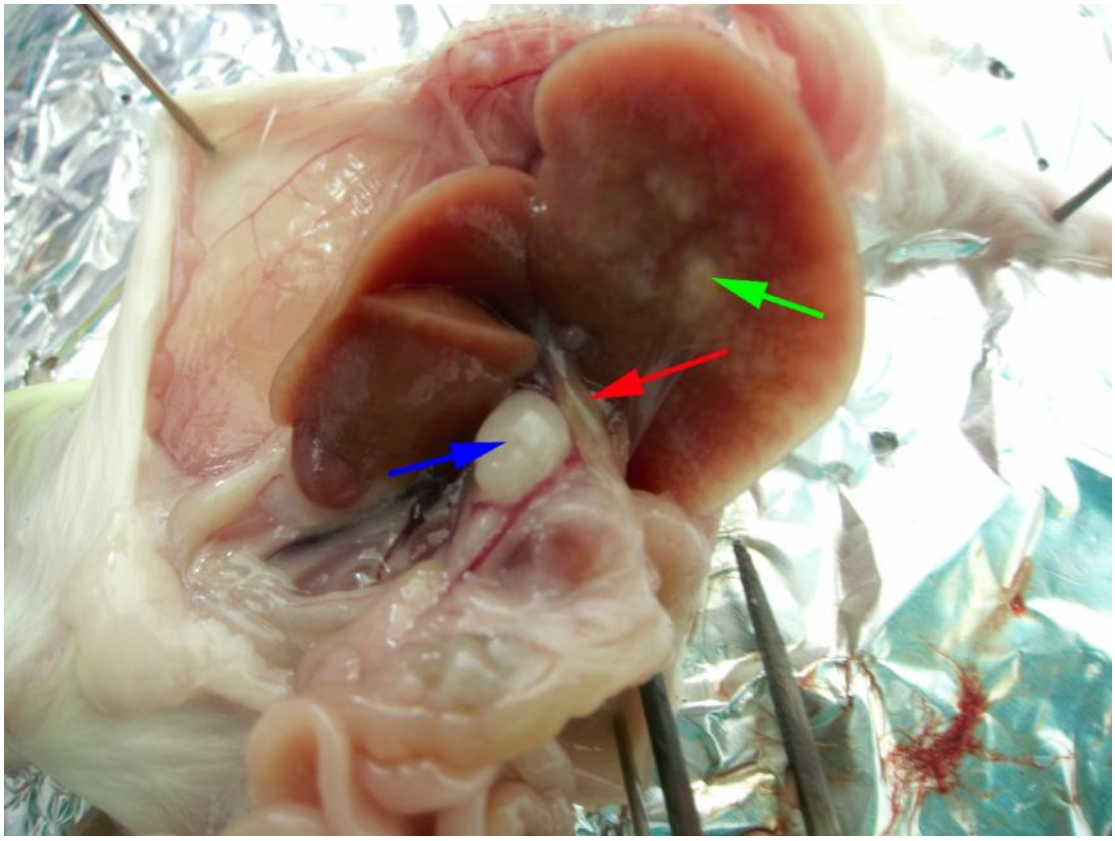

Fig. S1. The liver of a BALB/c mouse 28 days postinfection with *C. sinensis*. Note the inflammation region (green arrow), severe common bile duct dilatation (red arrow), and enlarged liver-draining portal lymph node (blue arrow).

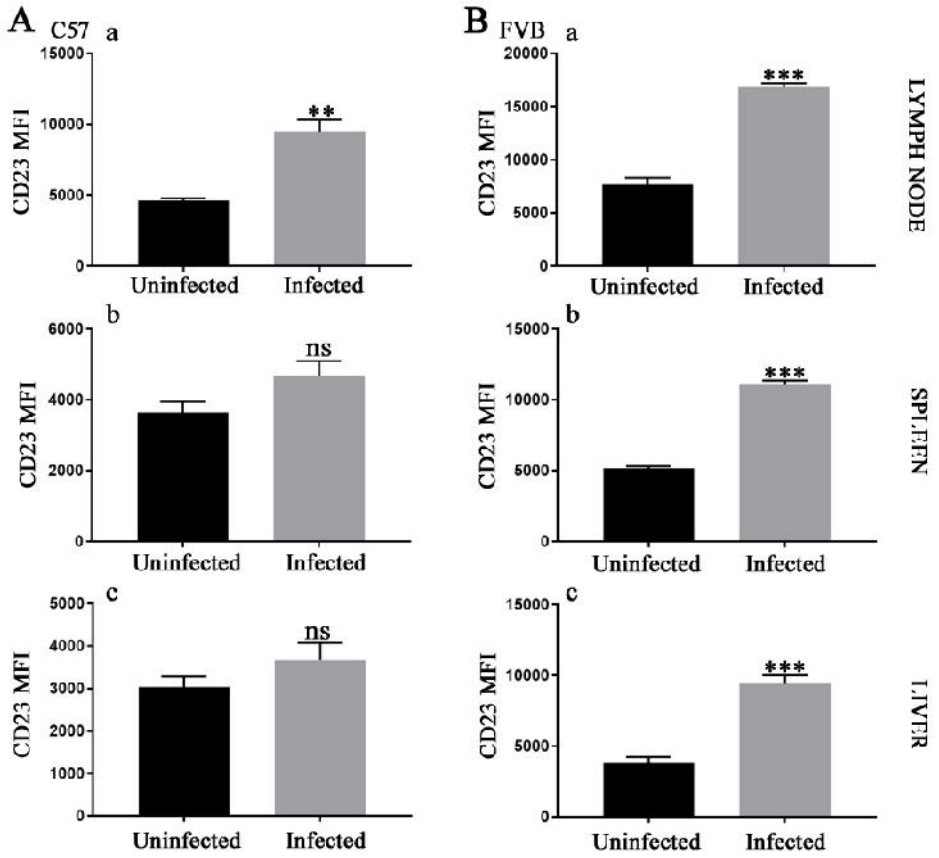

Fig. S2. Expression of CD23 on B cells of C57BL/6 and FVB mice after infection.
Mean fluorescence intensities (MFI) of CD23 on $CD19^{+}$ cells in liver-draining portal lymph nodes (a), spleens (b) and livers (c) of C57BL/6 (A) and FVB (B) mice at day 28 postinfection with *C. sinensis* were analyzed. (**p<0.01, ***p<0.001, Student's t test, n=4~6)

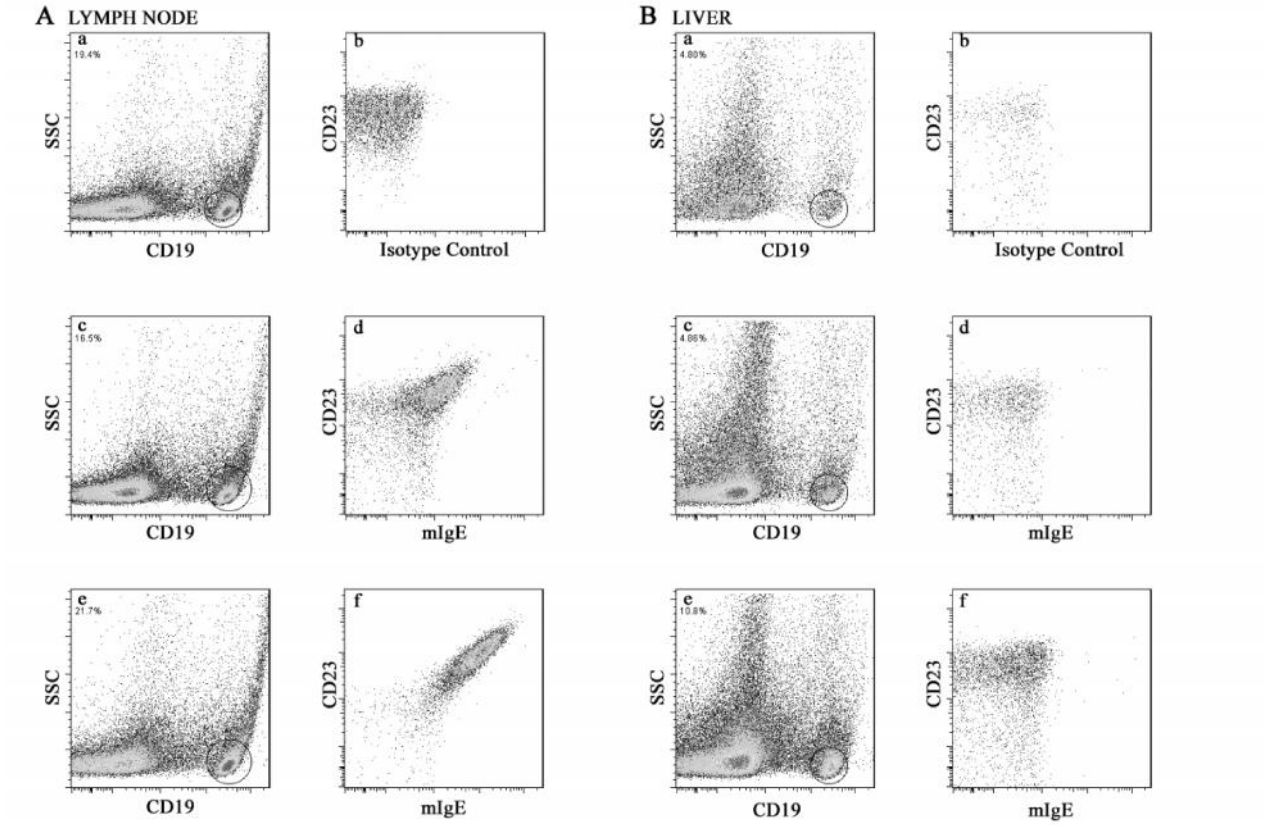

Fig. S3. Expressions of CD23 and membrane IgE (mIgE) on B cells after infection.
Expression of CD23 and mIgE on $CD19^+$ cells in liver-draining portal lymph nodes (A) and livers (B) of C57BL/6 mice 28 days postinfection with *C. sinensis*. a, c and e) CD23 and mIgE was detected in $CD19^+$ cells. b) The isotype control of ant-IgE-PE. d) Expression of CD23 and mIgE on $CD19^+$ cells of uninfected mice. f) Expression of CD23 and mIgE on $CD19^+$ cells of infected mice.

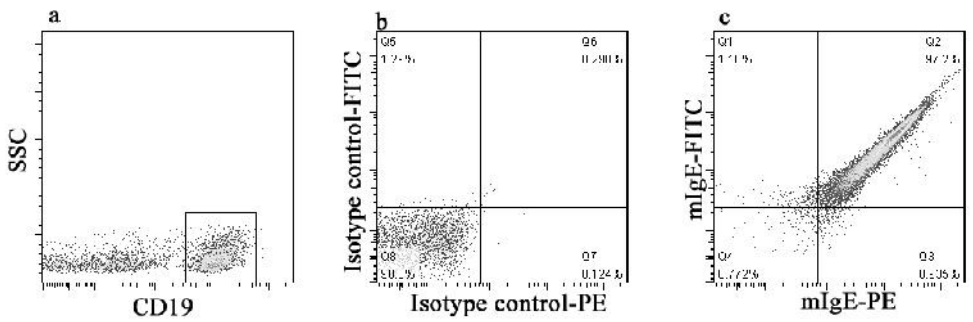

Fig. S4. Membrane IgE (mIgE) on B cells in liver-draining portal lymph nodes were tested by two different monoclonal antibodies: anti-IgE-PE (Clone: RMA-1) and anti-IgE-FITC (Clone：23G3). a) mIgE was detected in $CD19^+$ cells. b) Isotype control. c) Colabeling by the two antibody clones.