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# Differences in the Importance of Mast Cells, Basophils, IgE, and IgG versus That of CD4+ T Cells and ILC2 Cells in Primary and Secondary Immunity to *Strongyloides venezuelensis*

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**ABSTRACT** There is evidence that mast cells, basophils, and IgE can contribute to immune responses to parasites; however, the relative levels of importance of these effector elements in parasite immunity are not fully understood. Previous work in *Il3*-deficient and c-*kit* mutant *Kit^{W/W-v}* mice indicated that interleukin-3 and c-Kit contribute to expulsion of the intestinal nematode *Strongyloides venezuelensis* during primary infection. Our findings in mast cell-deficient *Kit^{W-sh/W-sh}* mice and two types of mast cell-deficient mice that have normal c-*kit* ("Hello *Kit*ty" and MasTRECK mice) confirmed prior work in *Kit^{W/W-v}* mice that suggested that mast cells play an important role in *S. venezuelensis* egg clearance in primary infections. We also assessed a possible contribution of basophils in immune responses to *S. venezuelensis*. By immunohistochemistry, we found that numbers of basophils and mast cells were markedly increased in the jejunal mucosa during primary infections with *S. venezuelensis*. Studies in basophil-deficient *Mcpt8^{DTR}* mice revealed a small but significant contribution of basophils to *S. venezuelensis* egg clearance in primary infections. Studies in mice deficient in various components of immune responses showed that CD4+ T cells and ILC2 cells, IgG, FcRγ, and, to a lesser extent, IgE and FcεRI contribute to effective immunity in primary *S. venezuelensis* infections. These findings support the conclusion that the hierarchy of importance of immune effector mechanisms in primary *S. venezuelensis* infection is as follows: CD4+ T cells/ILC2 cells, IgG, and FcRγ > mast cells > IgE and FcεRI > basophils. In contrast, in secondary *S. venezuelensis* infection, our evidence indicates that the presence of CD4+ T cells is of critical importance but mast cells, antibodies, and basophils have few or no nonredundant roles.

**KEYWORDS** Immunoglobulin E, basophils, mast cells, parasites

**S**trongyloides stercoralis is endemic mainly in tropical and subtropical areas and is estimated to infect 30 million to 100 million people worldwide (1, 2). *Strongyloides venezuelensis* is a rodent parasite with a similar life cycle that is used as an experimental model of *S. stercoralis* (3). Prior work has implicated mast cells (MCs) (4–15) and interleukin-3 (IL-3) (5, 7, 12) in immune resistance to primary infections with *S. venezuelensis*. IL-3 is essential for the increases in levels of jejunal MCs and blood basophils

observed after *S. venezuelensis* infection (7, 12), and *Kit^{W/W-v}* mice (markedly deficient in MCs) (16, 17) that were also deficient in *Il3* exhibited a more pronounced defect in *S. venezuelensis* expulsion during primary infections than did either *Kit^{W/W-v}* or *Il3*-deficient mice, suggesting that basophils also might contribute to *S. venezuelensis* clearance (7).

However, the importance of MCs and basophils in *S. venezuelensis* immunity was not proven by such work. For example, the MC-deficient mice used in many of the studies (4, 6, 7, 9, 11, 14), *Kit^{W/W-v}* mice, have several c-Kit-related defects in addition to their MC deficiency (17–26) and, until recently, no basophil-deficient mice were available to assess specifically the roles of basophils in this setting. In the present study, we used basophil-deficient mice and three types of MC-deficient mice to examine the roles of basophils and MCs in primary and secondary infections with *S. venezuelensis*. Because it has been reported that IgG1 and IgE antibodies (Ab) can function in concert to enhance expulsion of *S. venezuelensis* during primary infections but that neither type of antibody had a nonredundant function in such clearance (14), we also tested mice deficient in antibodies, IgE, the FcR γ chain, and FcεRI alpha, as well as mice with deficiencies in T cells and B cells. These experiments revealed that MCs, basophils, IgG, and IgE have distinct roles in primary versus secondary infections with *S. venezuelensis*.

## RESULTS

**Mast cells and, to a lesser extent, basophils contribute to expulsion of *S. venezuelensis* in primary infections.** Both IL-3 and c-Kit can enhance clearance of a primary infection with *S. venezuelensis* (7). The role of c-Kit is consistent with other lines of evidence indicating possible contributions of MCs to host resistance to this nematode (4, 6, 11, 27). While it is well known that MC numbers increase greatly in the jejunum of wild-type mice during primary infections with *S. venezuelensis* (6–9, 11), it was not known to what extent basophils might also infiltrate such tissues. Using an anti-mouse mast cell protease 8 (mMCP-8) Ab (28) recognizing the basophil-specific marker mMCP-8, we found that basophils were undetectable by immunohistochemistry in the jejunum of uninfected wild-type mice (Fig. 1A, left panel) but that many basophils were present 7 days after the first *S. venezuelensis* inoculation (Fig. 1A, middle and right panels).

It has been suggested that MCs and basophils might have complementary and/or partially overlapping roles in certain immune responses to parasites (7, 27, 29–31). We used genetic approaches to investigate the relative contributions of MCs and basophils in primary and secondary infections with *S. venezuelensis*. MC-deficient WBB6F$_1$-*Kit^{W/W-v}* mice exhibited delayed expulsion of *S. venezuelensis* in primary infections (6, 7, 11), but such mice have other abnormalities related to their *c-kit* mutations that may influence the response to this parasite by mechanisms unrelated to their MC deficiency (17, 32–35).

Therefore, we tested genetically engineered MC-deficient *Cpa3-Cre;Mcl-1^{fl/fl}* "Hello *Kit*ty" mice, in which a marked MC deficiency, and also a substantial reduction in basophils, occurs because of a mechanism independent of mutations affecting *c-kit* (36). Hello *Kit*ty mice exhibited a 13-day delay in achieving cessation of *S. venezuelensis* egg excretion compared to the corresponding control *Cpa3-Cre;Mcl-1^{+/+}* MC- and basophil-sufficient mice (Fig. 1B). Another genetically engineered MC-deficient mouse (with normal c-*kit*), the MasTRECK (Tg/+) mouse (32), exhibited a 7-day delay in cessation of egg excretion compared to diphtheria toxin (DT)-treated littermate wild-type control (+/+) or phosphate-buffered saline (PBS)-treated MasTRECK mice (Fig. 1C; also see Fig. S1A in the supplemental material).

Beyond a profound MC deficiency, both Hello *Kit*ty mice (36) and, at early times after DT treatment, DT-treated MasTRECK mice (32) have significantly reduced numbers of basophils. We therefore also tested MC-deficient C57BL/6J-*Kit^{W-sh/W-sh}* mice (37), which have Kit-related MC deficiency but have slightly increased numbers of basophils (37). Like the Hello *Kit*ty mice, *Kit^{W-sh/W-sh}* mice exhibited a 12-day delay in cessation of *S. venezuelensis* egg excretion compared to the corresponding wild-type mice (Fig. 1D).
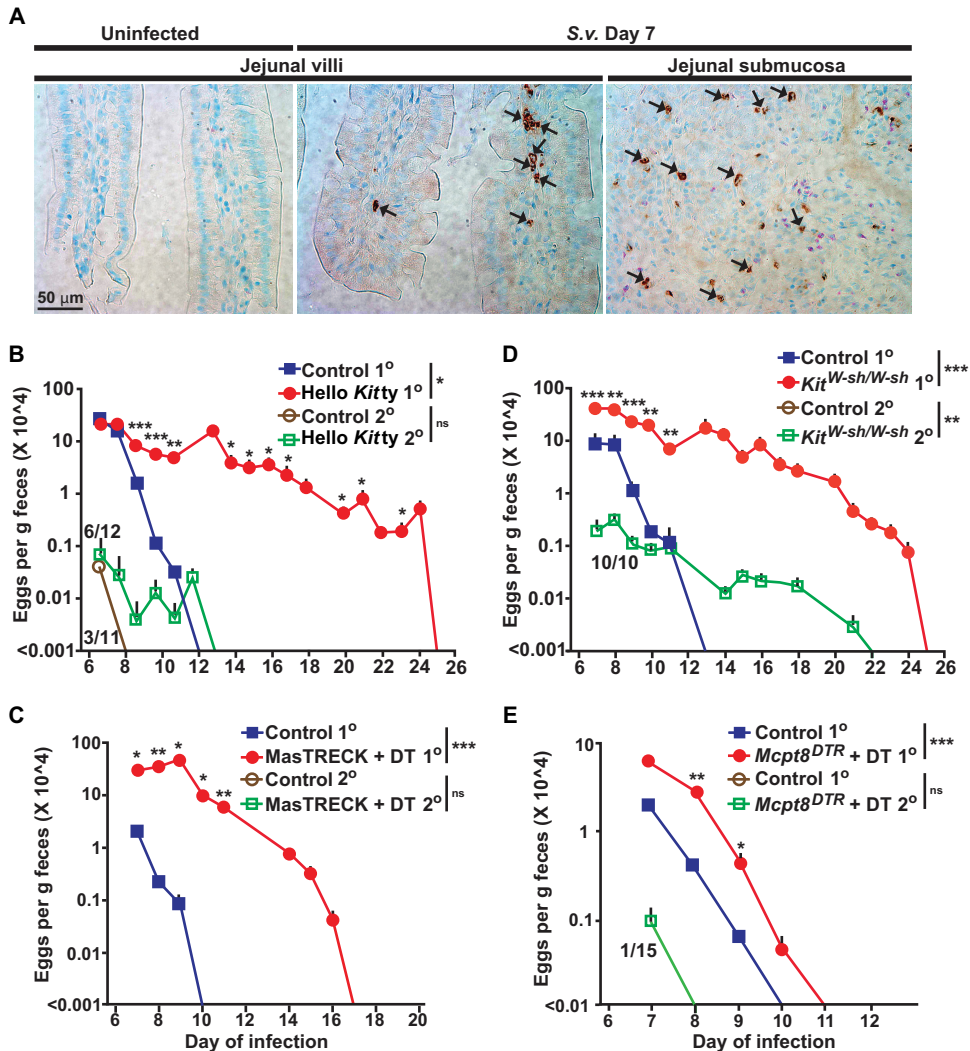
**FIG 1** Roles of mast cells and basophils in primary and secondary *S. venezuelensis* infections. (A) Immunohisto-chemical staining with an anti-mMCP8 Ab (DAB [3,3′-diaminobenzidine] substrate) to visualize basophils (some are indicated with small black arrows) and Giemsa counterstaining (4-μm-thick, paraffin-embedded sections) in sections of jejunum from wild-type C57BL/6 mice 7 days after inoculation with 10,000 *S. venezuelensis* L3. The left and middle panels are representative images from jejunal villi, and the right panel is a representative image from jejunal submucosa. *S. v.*, *S. venezuelensis*. (B to E) Kinetics of *S. venezuelensis* egg excretion after inoculation of 10,000 *S. venezuelensis* L3 in primary infections (1°) or secondary infections (2° [in mice inoculated with 10,000 *S. venezuelensis* L3 28 days after the first inoculation]) in C57BL/6-Hello *Kit*ty and control mice ($n = 22$ each) for 1° infections (7 experiments) and C57BL/6-Hello *Kit*ty ($n = 12$) and control ($n = 11$) mice for 2° infections (4 experiments) (B), BALB/c-MasTRECK mice treated with DT ($n = 5$) and control mice ($n = 4$) for 1° infections (2 experiments) and BALB/c-MasTRECK mice treated with DT and control mice ($n = 5$ for each) for 2° infections (2 experiments) (C), C57BL/6-$Kit^{W-sh/W-sh}$ and control mice ($n = 9$ each) for 1° infections (6 experiments) and C57BL/6-$Kit^{W-sh/W-sh}$ ($n = 10$) and control ($n = 3$) mice for 2° infections (3 experiments) (D), and $Mcpt8^{DTR/+}$ mice treated with DT ($n = 26$) and control mice ($n = 27$) for 1° infections (9 experiments) and $Mcpt8^{DTR/+}$ mice treated with DT ($n = 15$) and control mice ($n = 7$) for 2° infections (2 experiments) (E). The numbers of eggs are shown as means + standard errors of the means (SEM) determined by pooling the data from all the experiments that were performed with each strain. Numerators in the fraction numbers for 2° infections indicate the number of mice for which eggs were detected, and denominators indicate the number of mice examined. Absence of fraction numbers or lines for 2° infections means that none of the mice produced detectable eggs. ***, $P < 0.0001$; **, $P < 0.001$; *, $P < 0.05$; no asterisks, $P > 0.05$ (for comparisons between each mutant mouse and the corresponding controls at each time point performed with the unpaired, two-tailed Student's *t* test and, as indicated next to the legend for groups of mice, with 2-way ANOVA to compare the time courses of the responses).

Finally, we tested $Mcpt8^{DTR/+}$ mice (38), which can be rendered deficient specifically in basophils. DT-treated $Mcpt8^{DTR/+}$ mice exhibited significantly more eggs in their feces on days 8 and 9 (and a 1-day delay in cessation of egg excretion) than the corresponding control mice (i.e., the DT-treated basophil-sufficient littermate wild-type $Mcpt8^{+/+}$

mice and PBS-treated *Mcpt8*<sup>*DTR/+*</sup> mice; see Fig. S1B in the supplemental material) (Fig. 1E). It has been shown that, during primary infection with *S. venezuelensis*, *Kit*<sup>*W/W-v*</sup> mice (4–7, 11) and IL-3-deficient *Kit*<sup>*W/W-v*</sup> mice (7) harbored more adult worms in the intestines than the corresponding control mice, as well as exhibiting a more extensively delayed cessation of egg excretion. We found that basophil-depleted DT-treated *Mcpt8*<sup>*DTR/+*</sup> mice also exhibited increased numbers of worms in the intestines at day 8 of primary *S. venezuelensis* infection compared to the corresponding wild-type mice (Fig. S2A in the supplemental material), in addition to exhibiting increased numbers of eggs in the feces on that day and also a subsequent delay in cessation of egg excretion (Fig. 1E).

In addition to monitoring primary responses to *S. venezuelensis* by counting egg excretion, we measured changes in numbers of basophils and MCs. The various control MC- and basophil-sufficient mice exhibited substantial increases in blood basophil levels during primary infections (Fig. 2A; also see Fig. S3 in the supplemental material) which peaked around days 10 to 12. Hello *Kit*ty mice, DT-treated MasTRECK mice, and DT-treated *Mcpt8*<sup>*DTR/+*</sup> mice each exhibited slight increases in blood basophil levels that were detectable ~7 to ~11 days after initiation of *S. venezuelensis* infection; however, all but the DT-treated MasTRECK mice maintained substantial basophil depletion (Fig. 2A; also see Fig. S3 in the supplemental material). The temporary basophil depletion that we saw in DT-treated MasTRECK mice is consistent with previous observations (32).
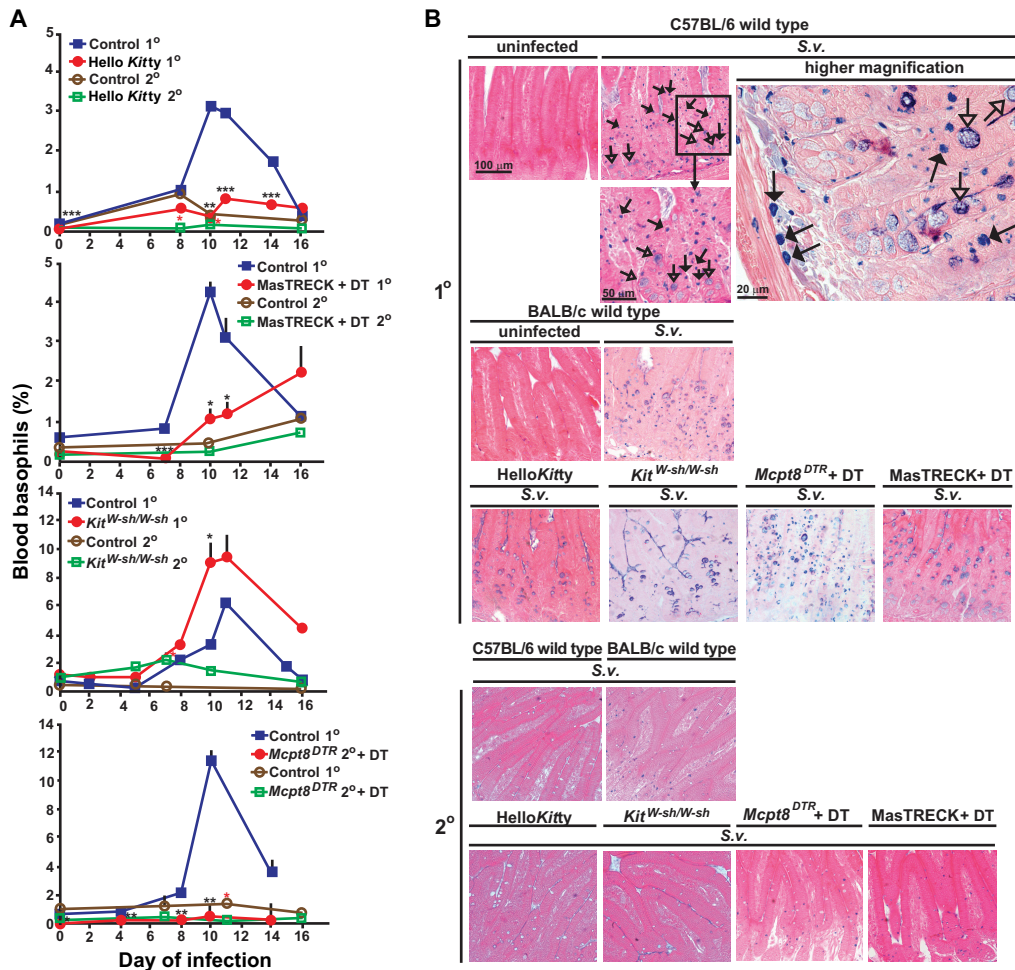
In *S. venezuelensis*-infected MC- and basophil-sufficient mice, MC levels were increased in the jejunal mucosa during the primary infection, and numbers of goblet cells also increased (Fig. 2B and C; see also Fig. 4D). In contrast, while all 3 types of MC-deficient mice exhibited increased levels of goblet cells in the jejunum (reaching levels similar to those in the corresponding MC-sufficient mice), numbers of mucosal MCs and peritoneal MCs were drastically reduced (Fig. 2B and C; see also Fig. 4D and Fig. S3 in the supplemental material). In *S. venezuelensis*-infected basophil-deficient mice, levels of both mucosal and peritoneal MCs were similar to those in the corresponding control mice (Fig. 2B and C; also see Fig. S3 in the supplemental material).

These results establish that MC deficiencies were sustained during primary *S. venezuelensis* infections in the three kinds of MC-deficient mice tested and that the basophil deficiencies are maintained during such infections in both DT-treated *Mcpt8*<sup>*DTR/+*</sup> mice and Hello *Kit*ty mice. These findings also indicate that the expansion of MC numbers observed in this setting appears not to require an important nonredundant contribution of basophils and that the jejunal goblet cell hyperplasia associated with primary infections with *S. venezuelensis* can occur largely independently of contributions of MCs or basophils.

Compared to the consistent basophil depletion observed before day 7 in DT-treated *Mcpt8*<sup>*DTR/+*</sup> and MasTRECK mice, Hello *Kit*ty mice had a larger variation in the percentage of blood basophil deficiency and also exhibited more variation in the delay in cessation of *S. venezuelensis* egg production than DT-treated MasTRECK and *Mcpt8*<sup>*DTR/+*</sup> mice. Notably, in individual Hello *Kit*ty mice, there was a strong correlation between the delay in cessation of *S. venezuelensis* egg excretion and the magnitude of their blood basophil deficiency (Fig. 2D, left panel). No such correlation was observed between the delay in *S. venezuelensis* egg clearance and the reduction in peritoneal MCs (Fig. 2D, middle panel) or between the reductions in the levels of blood basophils and peritoneal MCs (Fig. 2D, right panel). Also, all individual Hello *Kit*ty mice remained substantially MC deficient during *S. venezuelensis* infection. The strong correlation in individual Hello *Kit*ty mice between the extent of their reduction in blood basophils and the length of delay in cessation of parasite egg excretion supports the conclusion that basophils, as well as MCs, contribute to protective immunity during a primary infection with *S. venezuelensis*.

**Minor or no roles of mast cells and basophils in *S. venezuelensis* expulsion during secondary infections.** We also examined whether MCs and/or basophils contribute to cessation of *S. venezuelensis* egg excretion during secondary infections.

**FIG 2** Changes in numbers of basophils, mast cells, and epithelial goblet cells in wild-type and mast cell- and/or basophil-deficient mice during primary and secondary *S. venezuelensis* infections. (A) Blood was collected from each mouse at the indicated points after *S. venezuelensis* infection and stained with anti-IgE and anti-CD49b. Percentages of basophils in live cells were plotted. C57BL/6-Hello *Kit*ty ($n = 21$) and control ($n = 22$) mice were analyzed in both the 1° and 2° infections (separate groups of 21 and 22 mice, for a total of 86 mice). Data for 1° infections include those from 16/21 Hello *Kit*ty mice and all 22 control mice in the same 7 experiments performed as described for Fig. 1, as well as 5 additional Hello *Kit*ty mice with 1° infections which were not used as described for Fig. 1. For basophil counts in 2° infections, data are shown from all of the Hello *Kit*ty mice ($n = 11$) and control mice ($n = 12$) reported as described for Fig. 1 plus an additional 10 Hello *Kit*ty and 10 control mice with 2° infections. Data represent results for BALB/c-MasTRECK mice treated with DT and control mice (a mixture of DT-treated wild-type mice [$n = 5$] and PBS-treated BALB/c-MasTRECK mice [$n = 5$]) ($n = 10$ each for both 1° and 2° infections, for a total of 40 mice), including 5 BALB/c-MasTRECK mice treated with DT and 4 control mice for the 1° infections and 5 BALB/c-MasTRECK mice treated with DT and 5 control mice for the 2° infections. Data represent results for C57BL/6-*Kit*$^{W-sh/W-sh}$ ($n = 14$) and control ($n = 18$) mice (separate groups of 14 and 18 mice for the 1° and 2° infections, for a total of 64 mice), including 9 C57BL/6-*Kit*$^{W-sh/W-sh}$ and 9 control mice from the 6 experiments performed as described for Fig. 1 for the 1° infections and 3 C57BL/6-*Kit*$^{W-sh/W-sh}$ and 10 control mice from the 3 experiments performed as described for Fig. 1 for the 2° infections. Data for *Mcpt8*$^{DTR}$ mice treated with DT and control mice (a mixture of DT-treated wild-type mice [$n = 3$] and PBS-treated *Mcpt8*$^{DTR}$ mice [$n = 3$]) ($n = 6$ each) were from the 2 experiments performed as described for Fig. 1. ***, $P < 0.0005$; **, $P < 0.005$; *, $P < 0.05$; no asterisks, $P > 0.05$. Black asterisks (for 1° infections) or red asterisks (for 2° infections) indicate the statistical significance of results of comparisons between each type of mutant mouse and the corresponding control mice for that time point. (B and C) Jejunums were collected 14 days after 1° or 2° infections. (B) Mast cells (some indicated with filled arrows) were stained with alcian blue after fixation with Carnoy's solution (open arrows indicate goblet cells). Representative tissue images are shown in panel B, and numbers of mast cells per villus crypt unit (vcu) are shown as bar graphs in panel C (upper panel, means + SEM, $n = 9$ to 10 each; data pooled from 3 experiments). Peritoneal fluid was collected 14 days after the start of the 1° or 2° infections, and mast cells in live cells were assessed using anti-IgE and anti-c-Kit antibodies. Percentages of peritoneal mast cells in live cells are shown as bar graphs in panel C (lower panel, means + SEM, $n = 3$ to 10 each; data pooled from 2 or 3 experiments). Wild-type values for comparisons to those from mutant mice were combined from corresponding controls of the same strain (C57BL/6 or BALB/c). Black asterisks, statistical significance of results of comparisons of values for uninfected mice versus 1° or 2° infected mice in the same group (as indicated by bars). Red asterisks, statistical significance of results of comparisons of values for each type of mutant mouse and the corresponding wild-type mice for that condition (i.e., uninfected or 1° or 2° infected). Actual values are noted (means ± SEM) for low numbers; absence of

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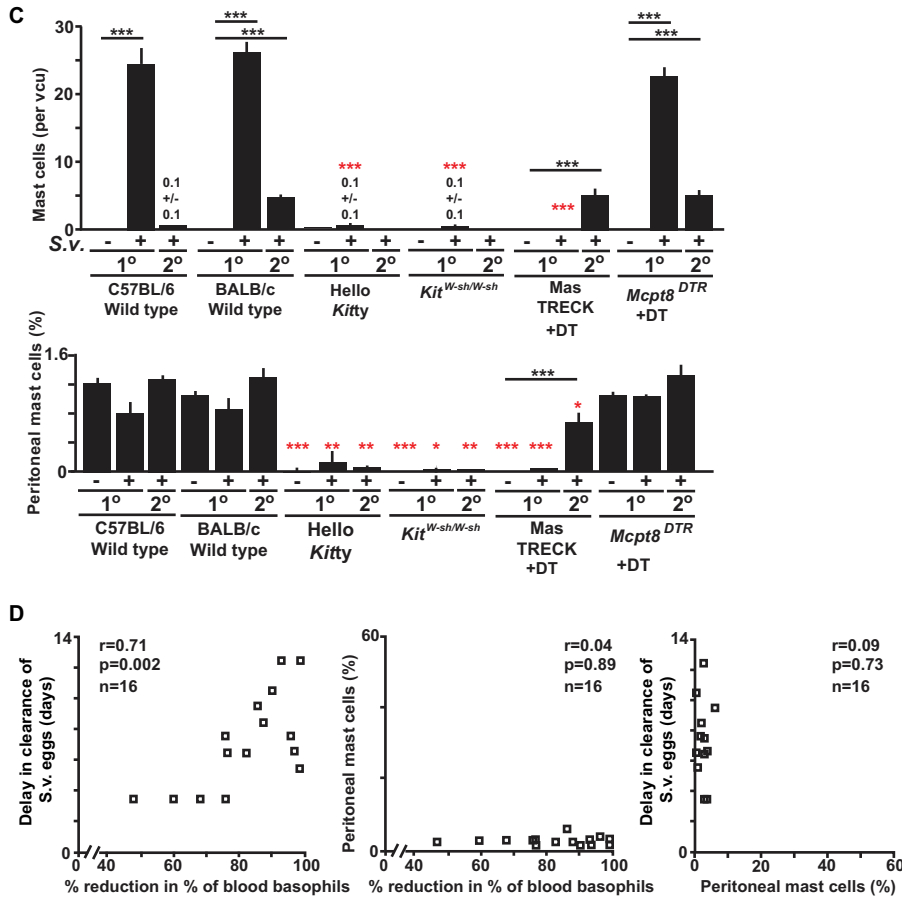
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a bar or of numbers means that none were detected. (D) (Left panel) Correlation between the delay in achieving cessation of egg excretion (defined as 0 egg count) in each Hello *Kit*ty mouse (compared to the average number of days for cessation of egg excretion in the corresponding wild-type mice in each experiment) (*y* axis) and percent reduction in the percentage of blood basophils of individual Hello *Kit*ty mice (calculated based on the average percentage of blood basophils in the corresponding control wild-type mice in each experiment) 10 to 12 days after initiation of 1° infection with *S. venezuelensis* (e.g., an 80% reduction means that that Hello *Kit*ty mouse had 20% the average percentage of basophils in the corresponding wild-type mice in that experiment]) (*x* axis) (*n* = 16). (Middle panel) Correlation between the percentage of peritoneal mast cells in that Hello *Kit*ty mouse 18 to 20 days postinitiation of 1° infection with *S. venezuelensis* (*y* axis) and the percentage of reduction in the percentage of blood basophils of individual Hello *Kit*ty mice (defined as for left panel) (*x* axis) (*n* = 16). (Right panel) Correlation between the delay in achieving cessation of egg excretion in each Hello *Kit*ty mouse (defined as for left panel) (*y* axis) and the percentage of peritoneal mast cells in that Hello *Kit*ty mouse 18 to 20 days after initiation of 1° infection with *S. venezuelensis* (*x* axis) (*n* = 16). *r*, correlation coefficient. The data in each panel are from the same 16 Hello *Kit*ty mice, all of them pooled from the data for the 16 mice in the 7 experiments performed as described for Fig. 1 for which data on basophils and peritoneal mast cells were obtained.

Several studies indicated that, in secondary nematode infections of wild-type mice, the larvae can be trapped in the skin and lungs before reaching the intestines, as reported for *S. venezuelensis* (39–41), *Strongyloides ratti* (41), and *Nippostrongylus brasiliensis* (42–44). In this study, we assessed fecal egg numbers as an indicator of whether larvae could reach the intestines and then produce eggs. Only 3 of 11 *Cpa3-Cre;Mcl-1*^{+/+} mice (the MC- and basophil-sufficient controls for Hello *Kit*ty mice) and none of the C57BL/6J-*Kit*^{+/+} mice (the MC-sufficient controls for the MC-deficient *Kit*[W-sh/W-sh] mice) excreted any eggs during secondary infections (Fig. 1B and D). Even when eggs were detected, the amounts were ~1,000-fold lower than during primary infections and eggs were detected only on day 7 (Fig. 1B). No eggs at all were detected during secondary infections in control (MC- and basophil-sufficient) mice on the BALB/cJ background (Fig. 1C and E).

Compared to the corresponding control mice, half of the Hello *Kit*ty mice and all of the $Kit^{W-sh/W-sh}$ mice exhibited delayed cessation of *S. venezuelensis* egg excretion during secondary infections, although the amounts of eggs in the feces were ~1,000-fold lower than during primary infections (Fig. 1B and D). However, the differences between the MC-deficient and corresponding control mice were not statistically significant. DT-treated MasTRECK mice and the corresponding control mice did not differ in their response to secondary infection with *S. venezuelensis*, in that none of the mice in either group exhibited any eggs in the feces (Fig. 1C). Only 1 of 15 DT-treated basophil-deficient $Mcpt8^{DTR/+}$ mice excreted any eggs during the secondary infection, and that excretion was seen only on day 7 (Fig. 1E).

Blood basophil levels were not increased in the various basophil-sufficient control mice in secondary infections to the same extent as in primary infections (Fig. 2A; also see Fig. S3 in the supplemental material). Moreover, such mice exhibited substantially fewer MCs in the jejunal mucosa than in the primary infections, especially in mice on the C57BL/6J background (Fig. 2B and C). Peritoneal MCs were not detectable after secondary infections in Hello *Kit*ty mice or $Kit^{W-sh/W-sh}$ mice (Fig. 2B and C; also see Fig. S3 in the supplemental material). DT-treated $Mcpt8^{DTR/+}$ mice and MasTRECK mice (each on the BALB/cJ background) had numbers of mucosal MCs in the jejunums after secondary infections that were similar to those in the corresponding control mice (Fig. 2B and C; also see Fig. S3 in the supplemental material). Importantly, we found that DT-treated MasTRECK mice had not only mucosal MCs but also peritoneal MCs after secondary infections, at levels similar to those in the corresponding control mice (Fig. 2C). The fact that DT-based MC depletion was no longer effective in this setting (Fig. 2C) might, in part, explain why no eggs were detected during secondary infections in DT-treated MasTRECK mice.

Taken together, these results suggest that MCs played only a minor (if any) role in resistance to *S. venezuelensis* during secondary infections in the mice analyzed and that basophils did not provide a critical nonredundant function in orchestrating cessation of *S. venezuelensis* egg excretion in these mice during the secondary infection.

**Roles of CD4$^+$ T cells, ILC2 cells, and IgG and IgE antibodies in immune responses to *S. venezuelensis*.** The more rapidly effective expulsion of *S. venezuelensis* observed in wild-type mice in the secondary infection is consistent with prior work indicating that adaptive immunity enhances host resistance to this nematode (39, 40, 45). To investigate the importance of various potential effector mechanisms in orchestrating immune responses to *S. venezuelensis*, we examined primary and secondary infections in mice deficient in different components of innate or adaptive immunity. We found that T and B cell-deficient $Rag2^{-/-}$ mice exhibited a 20-day delay (i.e., requiring 32 days in total) in cessation of *S. venezuelensis* egg excretion during primary infections and a 25-day delay (i.e., requiring 37 days in total) in the secondary infection (versus no eggs detected during secondary infections in any of the wild-type mice) (Fig. 3A), underscoring the importance of an adaptive immune response for effective secondary immune responses to this nematode (8, 11, 13, 14, 41, 46–48). In addition, we confirmed that the number of adult worms in the intestines on day 8 of the primary infection was significantly higher in the $Rag2^{-/-}$ mice than in the corresponding wild-type mice (Fig. S2B in the supplemental material), a finding consistent with the differences in the levels of egg excretion observed in these groups (Fig. 3A).

To assess contributions of CD4$^+$ T cells, we injected anti-CD4 or isotype control antibodies into wild-type C57BL/6J mice. Compared to the control group, during the primary infection mice injected with the anti-CD4 antibody exhibited a 27-day delay in cessation of *S. venezuelensis* egg excretion (Fig. 3B). During secondary infections, no eggs were detected in any of the wild-type mice injected with the isotype control antibody but eggs persisted until day 29 in the anti-CD4 antibody-treated mice (Fig. 3B). While both *Rag2*-deficient mice and anti-CD4 antibody-treated wild-type mice exhibited an elevated percentage of blood basophils at baseline compared to the corresponding control mice (reflecting the depletion of lymphocytes in the blood of such mice), neither the primary nor the secondary *S. venezuelensis* infection induced a
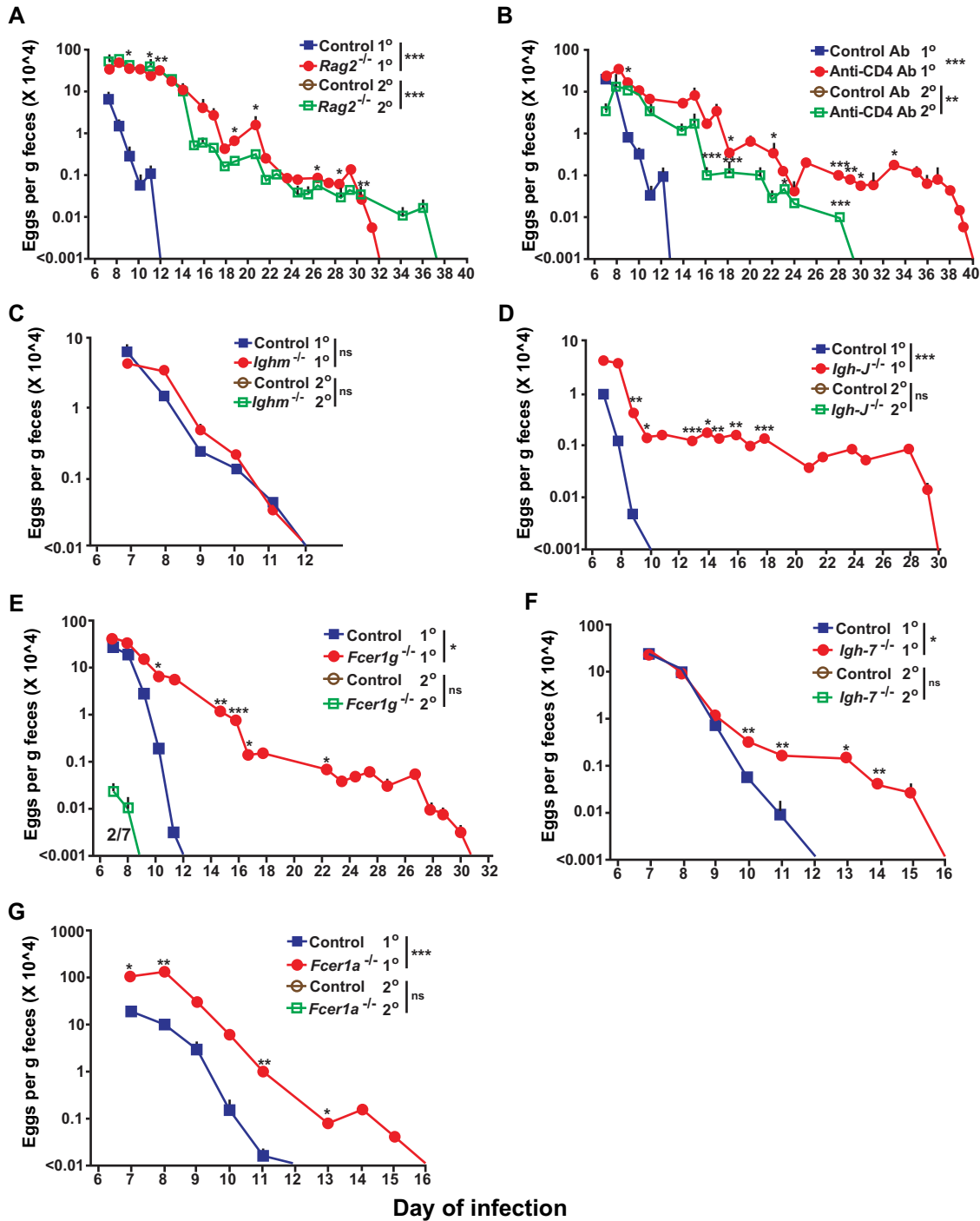
**FIG 3** Roles of T cells, ILC2 cells, antibodies, and antibody receptors in primary and secondary *S. venezuelensis* infections. Analyses of kinetics of *S. venezuelensis* egg excretion during 1° or 2° infections after inoculation with 10,000 *S. venezuelensis* L3 (second inoculations with *S. venezuelensis* were performed 42 days [panels A, B, D, E, and I] or 28 days [C, F, G, and J] after the first inoculation) in C57BL/6-*Rag2*-deficient ($n = 13$) and control C57BL/6 ($n = 4$) mice for 1° infection (3 experiments) and C57BL/6-*Rag2*-deficient ($n = 6$) and control C57BL/6 ($n = 4$) mice for 2° infection (2 experiments) (A), C57BL/6 wild-type mice treated with an anti-CD4 antibody or an isotype-matched control antibody ($n = 3$ each) (2 experiments) (B), C57BL/6-*Ighm*-deficient ($n = 9$) and control C57BL/6 ($n = 7$) mice for 1° infection (4 experiments) and C57BL/6-*Ighm*-deficient and control mice for 2° infection ($n = 5$ each) (2 experiments) (C), BALB/c-*Igh-J*-deficient ($n = 8$) and control BALB/c ($n = 5$) mice for 1° infection (3 experiments) and BALB/c-*Igh-J*-deficient and control BALB/c mice ($n = 5$ each) for 2° infection (2 experiments) (D), C57BL/6-*Fcer1g*-deficient ($n = 7$) and control C57BL/6 ($n = 6$) mice for 1° infection (3 experiments) and C57BL/6-*Fcer1g*-deficient ($n = 3$) and control C57BL/6 ($n = 7$) mice for 2° infection (3 experiments) (E), BALB/c-*Igh-7*-deficient ($n = 17$) and BALB/c-*Igh-7*-sufficient ($n = 15$) mice for 1° infection (6 experiments) and BALB/c-*Igh-7*-deficient ($n = 11$) and control BALB/c ($n = 3$) mice for 2° infection (3 experiments) (F), C57BL/6-*Fcer1a*-deficient ($n = 10$) and control C57BL/6 ($n = 8$) mice for 1° infection (3 experiments) and C57BL/6-*Fcer1a*-deficient and control C57BL/6 mice ($n = 5$ each) for 2°
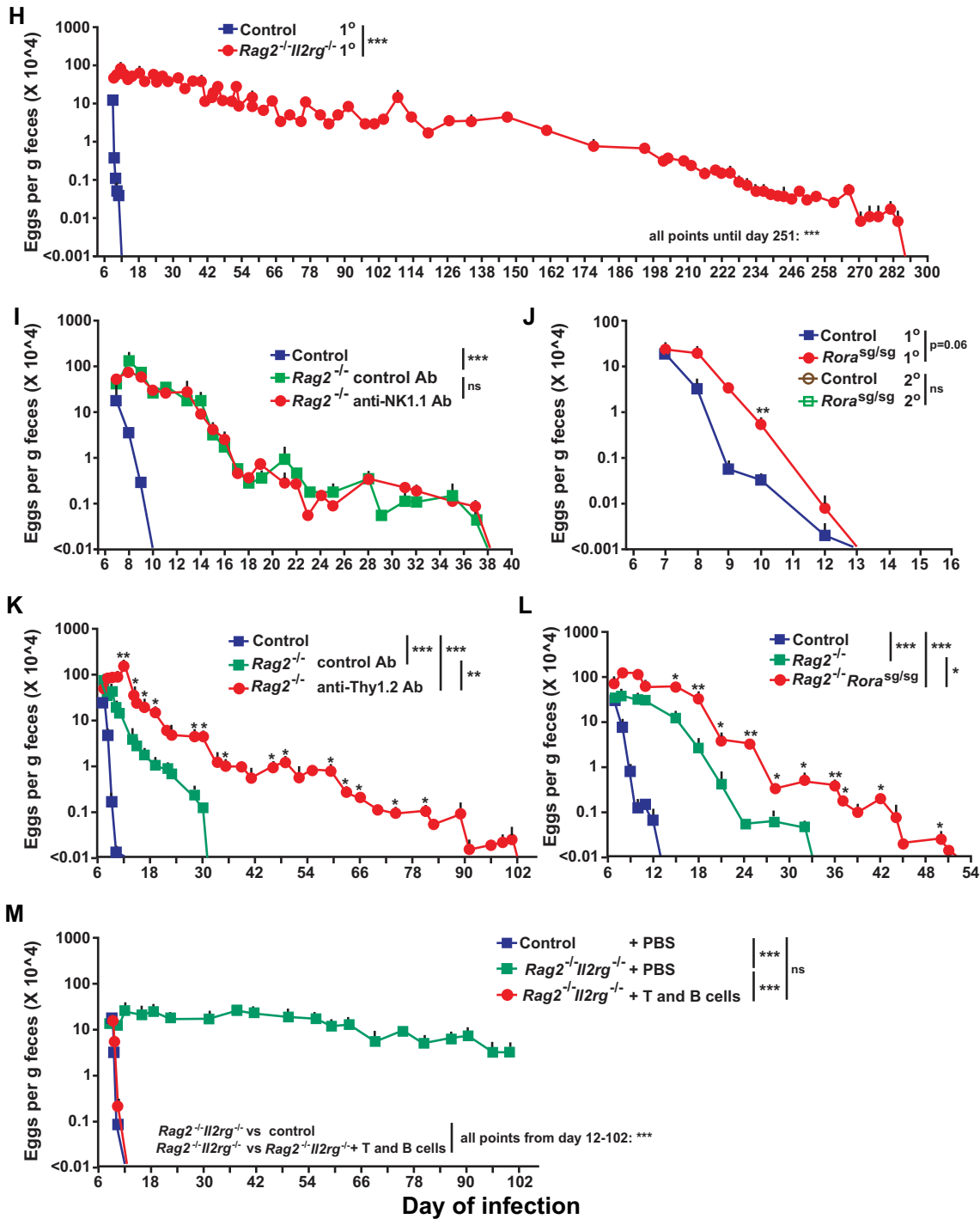
**FIG 3** Legend (Continued)

infection (2 experiments) (G), BALB/c-*Rag2Il2g* doubly deficient (n = 12) and control BALB/c (n = 10) mice for 1° infection (2 experiments) (H), C57BL/6-*Rag2*-deficient mice injected with an anti-NK1.1 antibody or an isotype-matched control antibody (n = 3 each) for 1° infection (2 experiments) (I), C57BL/6-*Rora*^sg/sg^ (n = 3) and control C57BL/6 (n = 4) mice for 1° infection (2 experiments) and C57BL/6-*Rora*^sg/sg^ (n = 4) and control C57BL/6 (n = 3) mice for 2° infection (2 experiments) (J), C57BL/6-*Rag2*-deficient mice injected with an anti-Thy1.2 antibody or an isotype-matched control antibody (n = 3 each) for 1° infection (2 experiments) (K), C57BL/6-*Rag2*-deficient mice (n = 5) and C57BL/6-*Rag2*-deficient *Rora*^sg/sg^ mice (n = 3) and the corresponding wild-type C57BL/6 (n = 3) mice for 1° infection (2 experiments) (L), and BALB/c-*Rag2Il2g* doubly deficient mice treated with PBS (n = 3) and BALB/c-*Rag2Il2g* doubly deficient mice which received splenic T and B cells from wild-type BALB/c mice (n = 3) and the corresponding control BALB/c mice treated with PBS (n = 3) for 1° infection (2 experiments) (M). The numbers of eggs are shown as means + SEM, with data derived by pooling results from all experiments performed (as indicated for each strain). The fraction number in panel E is displayed as described for Fig. 1. ***, $P < 0.0001$; **, $P < 0.001$; *, $P < 0.05$; no asterisks, $P > 0.05$ (for comparisons between each type of mutant or antibody-treated mouse or mutant mouse that received splenic T and B cells and the corresponding control mice at each time point performed with the unpaired, two-tailed Student's $t$ test and, as indicated next to the legend for groups of mice, with 2-way ANOVA to compare the time courses of the responses).

significant increase in the percentage of blood basophils (Fig. 4A; also see Fig. S4 in the supplemental material). As previously reported (11), jejunal mucosal MCs were essentially absent in $Rag2^{-/-}$ mice at baseline and did not appear in such mice after a primary infection (Fig. 4B and C). The low levels of expression of IgE on the surface of blood basophils in anti-CD4 antibody-treated wild-type mice during primary infections with *S. venezuelensis* (see Fig. S4 in the supplemental material) are consistent with an important role of CD4$^+$ T cells in the induction of an IgE response in this setting (49, 50), with resulting upregulation of surface expression of Fc$\varepsilon$RI on blood basophils (51–53). However, these *Rag2*-deficient mice and anti-CD4 antibody-treated wild-type mice exhibited levels of jejunal goblet cell hyperplasia similar to those in the corresponding control mice (Fig. 4B to D; also see Fig. S5 in the supplemental material). These results confirm that, during *S. venezuelensis* infection, CD4$^+$ T cells are essential for the expansion of jejunal mucosal MCs (11), elevation of IgE levels, and effective clearance of *S. venezuelensis* eggs (14) but are dispensable for jejunal goblet cell hyperplasia (Fig. 4B to D; also see Fig. S4 and S5 in the supplemental material).

To investigate the role of B cells in immune responses to *S. venezuelensis*, we assessed the infections in *Ighm*-deficient mice (known as $\mu$MT mice), which lack conventional B cells due to a deficiency in membrane $\mu$ heavy chain and exhibit markedly reduced levels of immunoglobulins (54). Surprisingly, *Ighm*-deficient mice did not exhibit a delay in cessation of *S. venezuelensis* egg excretion in the primary infection and, like the corresponding control mice, exhibited no detectable egg production during secondary infections. However, while *Ighm*-deficient mice had no detectable IgE on the surface of basophils before *S. venezuelensis* infection, IgE was detected on basophils on day 10 of *S. venezuelensis* infection (see Fig. S4 in the supplemental material), a finding in accord with the previously reported observation of IgE production in these mice infected with *Heligmosomoides polygyrus*, *Trichuris muris*, and *Schistosoma mansoni* (55). IgE was detected by flow cytometry on the surface of basophils after primary infections in all *Ighm*-deficient mice. On the other hand, with enzyme-linked immunosorbent assay (ELISA), which is not as sensitive as flow cytometry, total IgE was detected in 2 of 6 *Ighm*-deficient mice after secondary infections (see Fig. S6A in the supplemental material). Total IgG1 levels were elevated after primary infections in all *Ighm*-deficient mice (see Fig. S6B in the supplemental material). *S. venezuelensis*-specific IgG1 was detected in 1 of 6 *Ighm*-deficient mice after secondary infections, but much higher titers were detected in all wild-type mice after primary infections (see Fig. S6C in the supplemental material). Moreover, during primary infection with *S. venezuelensis*, *Ighm*-deficient mice and the corresponding wild-type mice developed similarly expanded populations of blood basophils, jejunal mucosal MCs, and jejunal goblet cells (Fig. 4; also see Fig. S4 in the supplemental material).

We also examined primary and secondary *S. venezuelensis* infections in *Igh-J*-deficient mice (also known as *Jh*-deficient mice), which lack all detectable antibodies from either conventional or nonconventional B cells (56). *Igh-J*-deficient mice exhibited a 20-day delay in cessation of *S. venezuelensis* egg excretion during the primary infection, suggesting an indispensable role of antibodies in the response to *S. venezuelensis*; however, like the corresponding control mice, *Igh-J*-deficient mice exhibited no detectable egg excretion during the secondary infection (Fig. 4D). Antibodies remained undetectable by ELISA (or by fluorescence-activated cell sorter [FACS] analysis of basophils) after either primary or secondary *S. venezuelensis* infection in *Igh-J*-deficient mice (data not shown), but *Igh-J*-deficient mice and the corresponding wild-type mice developed similarly expanded populations of blood basophils, jejunal mucosal MCs, and jejunal goblet cells during the primary infection (Fig. 4; also see Fig. S4 in the supplemental material).

The important role of antibodies in the primary infection with *S. venezuelensis* appears to depend on the binding of antibodies to FcRs that signal via the FcR common $\gamma$-chain (8, 10, 14), and in our model, we found that *Fcer1g*-deficient mice exhibited a 19-day delay in cessation of egg excretion (Fig. 3E), a delay very similar to that seen in *Igh-J*-deficient mice (Fig. 3D). Like *Igh-J*-deficient mice, *Fcer1g*-deficient mice exhibited
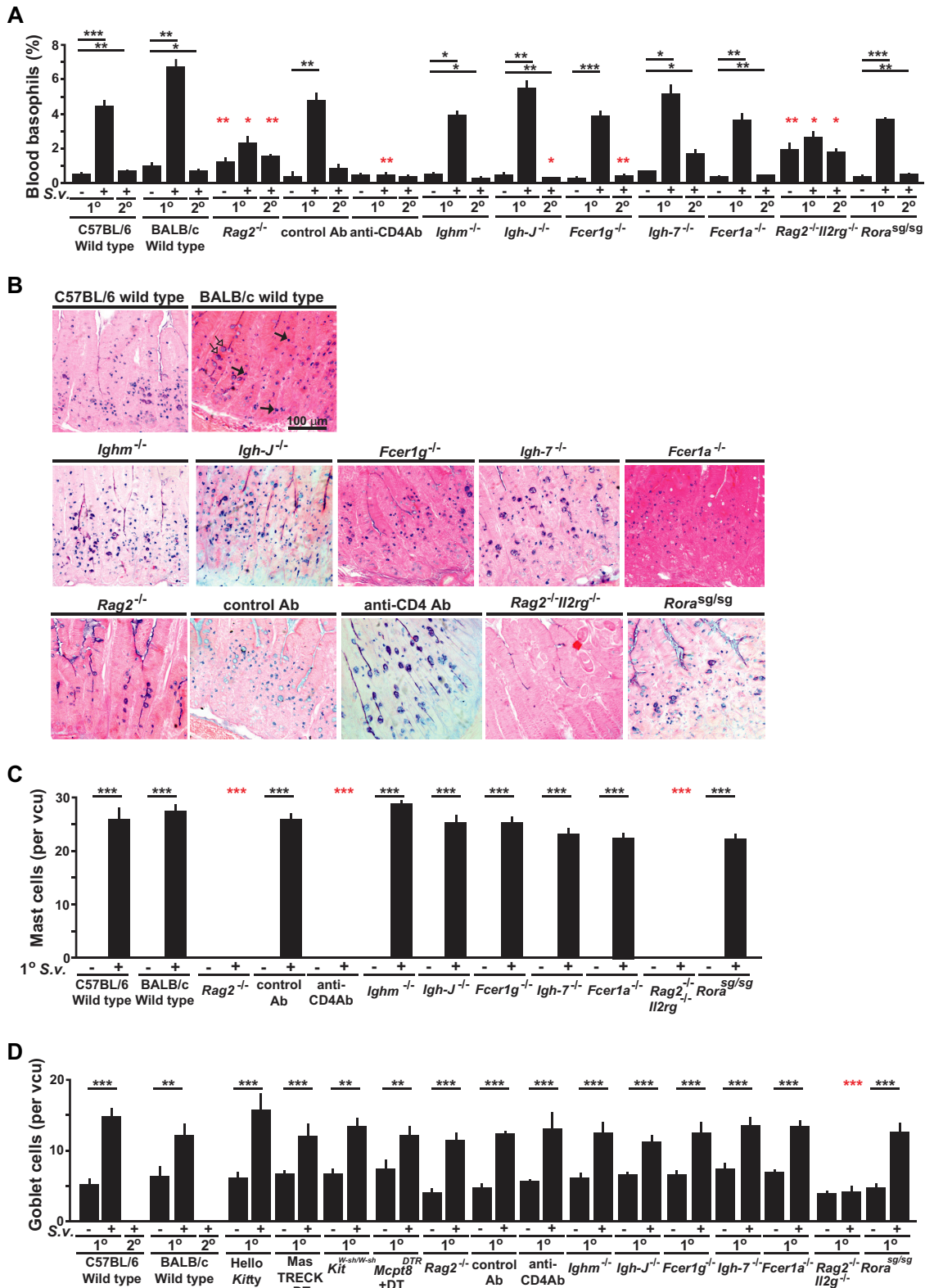
FIG 4 Changes in numbers of basophils, mast cells, and epithelial goblet cells in mice deficient in various components of immune responses during primary and secondary *S. venezuelensis* infections. (A) Blood was collected from each mouse during all of the experiments performed as described for Fig. 3A to J before infection or 10 to 12 days after 1° or 2° *S. venezuelensis* infections and stained with anti-IgE and anti-CD49b. Percentages of basophils in live cells are shown as bar graphs with means + SEM. ***, $P <$ 0.0005; **, $P <$ 0.005; *, $P >$ 0.05; no asterisks, $P >$ 0.05. Black asterisks indicate the statistical significance of results of comparisons between values for uninfected mice and 1° or 2° infected mice in the same group (as indicated by bars). Red asterisks indicate the statistical significance of results of comparisons between each type of mutant or anti-CD4 Ab-treated mouse and the corresponding

(Continued on next page)

levels of expanded populations of blood basophils, jejunal mucosal MCs, and jejunal goblet cells similar to those seen with the corresponding wild-type mice during primary *S. venezuelensis* infections (Fig. 4; also see Fig. S4 in the supplemental material). In secondary *S. venezuelensis* infections, 2 of 7 *Fcer1g*-deficient but none of the corresponding wild-type mice exhibited some egg excretion, but not after day 8 (Fig. 3E), but these differences were not statistically significant. Other groups showed that the numbers of adult worms in the intestines, as well as levels of *S. venezuelensis* egg excretion, were increased in antibody-deficient *Aicda*$^{-/-}$ mice and *Fcer1g*-deficient mice compared to the corresponding wild-type mice (8, 14).

Because FcRγ is a component of receptors for IgG and IgE, we searched for any nonredundant contribution of IgE by testing mice deficient in IgE (i.e., *Igh-7*-deficient mice) or the FcεRI α chain (i.e., *Fcer1a*$^{-/-}$ mice). IgE-deficient and FcεRI-deficient mice each had a 4-day delay to egg clearance in primary infections (Fig. 3F and G) and exhibited expanded populations of blood basophils, jejunal mucosal MCs, and jejunal goblet cells similar to those seen with the corresponding wild-type mice (Fig. 4; also see Fig. S4 in the supplemental material). Neither of these mutant mouse strains exhibited any egg production during secondary infections. These findings confirm prior evidence (14) indicating that both IgG and IgE antibodies can contribute to resistance to *S. venezuelensis* during primary infections and identify for the first time a small but nonredundant role for IgE, functioning via FcεRI, in the cessation of egg excretion in this setting. In contrast, there was no evidence of a nonredundant function of IgE or FcεRIα in egg clearance in secondary infections with *S. venezuelensis*.

We also examined *Rag2* and *Il2g* doubly deficient mice, which lack T cells, B cells, NK cells, and ILC2 cells (57), and found that they had a delay of about 270 days in egg clearance during primary infection with *S. venezuelensis* (Fig. 3H), as well as significantly increased numbers of adult worms in the intestines on day 8 of the infection (see Fig. S2C in the supplemental material). As in the *Rag2*$^{-/-}$ mice, *Rag2* and *Il2g* doubly deficient mice exhibited no significant increase in the percentage of blood basophils and virtually lacked jejunal mucosal MCs (Fig. 4A to C; also see Fig. S4 in the supplemental material). However, unlike *Rag2*$^{-/-}$ mice, *Rag2* and *Il2g* doubly deficient mice exhibited no increase in the levels of jejunal goblet cells during the primary infection (Fig. 4B and D).

Because *Rag2* and *Il2g* doubly deficient mice lack NK cells and ILC2 cells (57), in addition to analyses of T and B cells, we examined *Rag2*-deficient mice depleted of NK cells using an anti-NK1.1 antibody (see Fig. S5 in the supplemental material). Such treatment did not influence the time to egg clearance in the primary infection (Fig. 3I). We then tested ILC2-deficient *Rora*$^{sg/sg}$ mice (58). *Rora*$^{sg/sg}$ mice excreted significantly more eggs on day10 of the primary infection than the corresponding wild-type mice, but the two strains of mice did not differ in time to cessation of egg excretion (Fig. 3J). Nor did such *S. venezuelensis*-infected *Rora*$^{sg/sg}$ mice differ from the corresponding wild-type mice in *S. venezuelensis*-induced expansion of blood basophil or jejunal mucosal MC populations or in exhibiting increases in levels of jejunal goblet cells (Fig. 4; also see Fig. S3 in the supplemental material).

Finally, we used 3 approaches to assess the effect of combined deficiencies in T cells, B cells, and ILC2 cells on primary infection with *S. venezuelensis*. First, we injected anti-Thy1.2 antibodies into *Rag2*-deficient mice to deplete ILC2 cells. Compared to the wild-type control group, the isotype control antibody-injected *Rag2*-deficient mice had a 20-day delay in cessation of egg excretion (to a total of 31 days) but the Thy1.2 antibody-injected *Rag2*-deficient mice exhibited an additional 71-day delay (to a total

**FIG 4** Legend (Continued)
control mice for that condition. (B to D) Jejunums were collected 14 days after the start of 1º infections, and sections of jejunum were stained as described for Fig. 2B (in the panel showing the histology in BALB/c wild-type mice, some of the mast cells are indicated with filled arrows and some of the goblet cells are indicated with open arrows) (B), and numbers of mast cells (C) or goblet cells (D) per villus crypt unit (vcu) are shown as bar graphs (n = 4 to 12 each; data were combined from 2 or 3 experiments). Wild-type values for comparisons to those from mutant mice were combined from corresponding controls of the same strain (C57BL/6 or BALB/c).

of 102 days) (Fig. 3K). Second, we found that, compared to the 20-day delay seen in *Rag2*-deficient mice (to a total of 33 days), *Rora^{sg/sg} Rag2*-deficient mice exhibited an additional 19-day delay (to a total of 52 days) (Fig. 3L). Third, we found that the adoptive transfer of purified (>98%) splenic T cells and B cells from wild-type mice into *Rag2;Il2g* doubly deficient mice fully normalized the time to cessation of egg clearance (to a total of 13 days) (Fig. 3M). Taken together, the results shown in Fig. 3H to M suggest that NK cells made little detectable difference in the time to cessation of egg excretion in primary *S. venezuelensis* infection (Fig. 3H) but support the conclusion that both T cells and ILC2 cells can contribute to egg clearance (Fig. 3J to M). However, compared to the extraordinarily long delay in egg clearance seen in *Rag2;Il2g* doubly deficient mice (Fig. 3H), the shorter delays seen in mice deficient in T cells and/or ILC2 cells suggest that there might be an additional mechanism(s) contributing to the very long delay in egg clearance in *Rag2;Il2g* doubly deficient mice.

## DISCUSSION

*S. venezuelensis* has been used for many years as an animal model for investigating the roles of various immune cells and antibodies in the control of infections with *Strongyloides* species. Previous work suggested that MCs can contribute to control of the primary infection, but most of those studies were done with *Kit^{W/W-v}* mice, which have a profound MC deficiency but also express other Kit-related abnormalities that might influence their response to *S. venezuelensis* (17–26). To our knowledge, there have been no prior reports of studies investigating the role of basophils in this infection.

By studying 3 types of MC-deficient mice and *Mcpt8^{DTR}* mice, which can be selectively depleted of basophils, we provide additional evidence that MCs can contribute substantially to *S. venezuelensis* egg clearance during primary infections but make minor (if any) contributions during secondary infections, at least in the model of infection analyzed, whereas basophils can make a minor albeit statistically significant contribution during the primary infection but have no detectable role in the secondary infection (see Table S1 and Fig. S7 in the supplemental material).

In addition to confirming a prior report (14) indicating that IgG and IgE antibodies (in that report, acting in concert) can contribute to cessation of *S. venezuelensis* egg excretion during primary infections and prior reports that FcR$\gamma$ also has an important role in that setting (8, 10, 14), we identified a small but statistically significant contribution of IgE and Fc$\varepsilon$RI as well (see Table S1 and Fig. S7 in the supplemental material), perhaps because we inoculated our mice with a larger number of cells of third-stage infective *S. venezuelensis* larvae (L3) than did Matsumoto et al. (14). While it seems likely that parasite-specific IgE and IgG antibodies bind to Fc receptors on effector cells and that their cross-linking by parasite antigens (especially for IgE) or the activation of effector cells by antigen-IgG immune complexes then results in expulsion of the parasites (14, 59, 60), the actual roles of parasite-specific and nonspecific antibodies in this setting are still controversial (61, 62). *Ighm*-deficient mice that lack antibodies at baseline were able to produce IgG1 and IgE during primary *S. venezuelensis* infections, and *S. venezuelensis* eggs were cleared on the same day as in wild-type mice (Fig. 3C; also see Fig. S4 and S6 in the supplemental material). Total IgE (detected by flow cytometry) and total IgG1 (measured by ELISA) levels were elevated in all *Ighm*-deficient mice (see Fig. S4 and S6 in the supplemental material). *S. venezuelensis*-specific IgG1 levels were elevated in all wild-type mice; however, only 1 of 6 *Ighm*-deficient mice had detectable *S. venezuelensis*-specific IgG1 (see Fig. S6 in the supplemental material). While the latter result does not strongly support the conclusion that parasite-specific antibodies have an essential role in cessation of egg excretion during primary *S. venezuelensis* infections, it is possible that some important *S. venezuelensis* antigens (e.g., those associated with adults worms but not larvae) were not included in our extract from L3. Similarly, it would be helpful to have more-sensitive assays to detect *S. venezuelensis*-specific IgE. Clearly, more research needs to be done to understand better the roles of parasite-specific antibodies in this setting.

In confirmation of findings reported by others (11), we found that CD4$^+$ T cells contributed importantly to cessation of *S. venezuelensis* egg excretion in both primary and secondary infections. They contributed to the expansion of mucosal MCs and blood basophils in the primary infection and also to the development of an IgE response, as inferred based on increased levels of IgE on the surface of basophils (see Fig. S4 in the supplemental material and data not shown). Moreover, our evidence indicates that CD4$^+$ T cells are largely if not entirely responsible for orchestrating effective immunity during secondary infections (see Table S1 in the supplemental material).

Notably, *Rag2;Il2g* doubly deficient mice exhibited a much longer delay in cessation of *S. venezuelensis* egg production than did *Rag2*-deficient mice. In addition to lacking cytokine responses requiring *Il2g* (which encodes a component of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), *Rag2;Il2g* doubly deficient mice lack NK cells, ILC2 cells, and T cells. Since NK cell depletion in Rag2-deficient mice did not have a detectable effect on *S. venezuelensis* expulsion, NK cells are unlikely to contribute substantially to *S. venezuelensis* expulsion. However, ILC2 cell-depleted *Rag2*-deficient mice took 102 days to achieve egg clearance (71 days longer than ILC2-sufficient *Rag2*-deficient mice), and *Rag2*-deficient *Rora$^{sg/sg}$* mice took 51 days to achieve egg clearance (19 days longer than ILC2-sufficient *Rag2*-deficient mice), suggesting that there is cooperation between T cells and ILC2 cells in achieving cessation of *S. venezuelensis* egg production. This result also suggests that additional factors can contribute to the 270-day delay in egg clearance exhibited by *Rag2;Il2g* doubly deficient mice, such as effects related to the lack of cytokine signaling dependent on *Il2g*. Our work with *Rora$^{sg/sg}$* mice, which lack ILC2 cells, also suggests that ILC2 can have a supportive role in cessation of *S. venezuelensis* egg production, which is in contrast to what appears to be a major role for these cells in immune responses to *Nippostrongylus brasiliensis* (63). However, jejunal goblet cell hyperplasia was observed during primary infections with *S. venezuelensis* in *Rag2*-deficient mice but not in *Rag2;Il2g* doubly deficient mice, raising the possibility that mucus production by goblet cells can contribute to cessation of *S. venezuelensis* egg excretion, as it does during immune responses to *Nippostrongylus brasiliensis* (64–66).

## MATERIALS AND METHODS

**Mice.** All animal experiments were carried out following protocols approved by the Stanford University Administrative Panel on Laboratory Animal Care. C57BL/6J (B6J)-*Kit$^{W-sh/W-sh}$* mice (37, 67) and littermate control B6J-*Kit$^{+/+}$* mice (wild-type control mice), C57BL/6J-*Cpa3-Cre;Mcl-1$^{fl/fl}$* "Hello *Kitty*" mice (36) and the corresponding control (*Cpa3-Cre;Mcl-1$^{+/+}$*) mice, BALB/cJ-*Mcpt8$^{DTR/+}$* mice (38) and littermate BALB/cJ-*Mcpt8$^{+/+}$* control mice, and BALB/cJ-MasTRECK ("MasTRECK" in this paper means heterozygous: Tg/+) mice (32) and littermate wild-type control mice (+/+; no Tg gene) were all from stocks bred in our laboratory. C57BL/6J wild-type, BALB/cJ wild-type, C57BL/6J-*Fcer1g$^{-/-}$* (68), C57BL/6J-*Rag2$^{-/-}$* (69), C57BL/6J-*Ighm$^{-/-}$* (54), C57BL/6J-*Rora$^{sg/sg}$* (70, 71), and C57BL/6J-*Fcer1a$^{-/-}$* (72) mice were purchased from The Jackson Laboratory (Sacramento, CA). BALB/cAnNTac-*Igh-J$^{-/-}$* mice (56) were purchased from Taconic (Germantown, NY). BALB/cJ-*Rag2$^{-/-}$Il2g$^{-/-}$* mice (73) and BALB/c-*Igh-7$^{-/-}$* mice (74) (and the corresponding control BALB/c-*Igh-7$^{+/+}$* mice) (74) were kindly provided by I. Weissman (Stanford University School of Medicine) and H. Oettgen (Harvard Medical School, Boston, MA), respectively.

**Infection with *Strongyloides venezuelensis*.** As previously described (7, 12), mice were infected by subcutaneous inoculation with 10,000 third-stage infective larvae (L3). For secondary infections, mice were infected by subcutaneous inoculation with 10,000 L3 28 or 42 days (as indicated) after the primary inoculation. We monitored infections in individual mice by counting the numbers of eggs (per gram of feces) excreted daily. Each type of immune cell was identified by flow cytometry except for tissue mast cells, which were counted as described previously (36). *Mcpt8$^{DTR/+}$* mice received intravenous injections of diphtheria toxin (DT; Sigma-Aldrich, St. Louis, MO) (200 ng/mouse) every 5 days starting 2 days before infection, and MasTRECK mice received intraperitoneal injections of DT (250 ng/mouse) every 5 days starting 2 days before infection. Control groups were a combination of DT-treated wild-type (*Mcpt8$^{+/+}$* or littermate wild-type control for MasTRECK) mice and PBS-treated *Mcpt8$^{DTR/+}$* mice or MasTRECK mice, because the two groups behaved similarly (see Fig. S1 in the supplemental material). For CD4 T cell depletion, 100 μg of anti-CD4 or isotype-matched control rat IgG2b κ antibodies (clone GK1.5 or clone RTK4530 from BioLegend, San Diego, CA) was injected intraperitoneally once a week starting at day −3. For NK cell depletion, 300 μg of anti-NK1.1 or isotype-matched control mouse IgG2a κ antibodies (clone PK136 or clone MOPC-173 from BioLegend) was injected intraperitoneally once a week starting at day

−3. For depletion of ILC2 cells, 200 $\mu$g of anti-Thy1.2 or isotype-matched control rat IgG2b $\kappa$ antibodies (clone 30-H12 or clone RTK4530 from BioLegend, San Diego, CA) was injected intraperitoneally into C57BL/6J-*Rag2*-deficient mice starting at day −1 and then every 3 days until the end of the experiment. Both male and female mice, which were 6 to 8 weeks old on the day of infection (day 0), were tested in each of the groups analyzed, and we found no statistically significant differences between male and female mice in the results obtained in any of the experimental groups. By direct observation at least until the time of cessation of *S. venezuelensis* egg excretion, all mice appeared to be healthy during the entire course of *S. venezuelensis* infection.

**Antibodies and flow cytometry.** All reagents used for cytometry were from BioLegend and were as follows: mouse IgE-fluorescein isothiocyanate (IgE-FITC) or mouse IgE-biotin (R35-72), CD49b-Alexa 647 or -FITC (DX5), c-Kit-FITC or -phycoerythrin (PE) (2B8), Fc$\varepsilon$RI$\alpha$-PE or -allophycocyanin (APC) (MAR-1), CD200R3-PE (Ba13), and APC-streptavidin. Basophils were identified as CD49b positive and mIgE, Fc$\varepsilon$RI$\alpha$, or CD200R3 positive (depending on the type of mutant mouse). Purified anti-mMCP-8 (TUG8) (for tissue staining) was from Biolegend.

**Histological analysis.** Sections (4 $\mu$m thick) of Carnoy's solution-fixed, paraffin-embedded jejunum were stained with 0.5% alcian blue. Mast cells and goblet cells were quantified per villus crypt unit (vcu) (75). To visualize tissue basophils, sections were stained with anti-mMCP-8 (10 $\mu$g/ml) (28, 36). Images were captured with an Olympus BX60 microscope using a Retiga-2000R QImaging camera run by Image-Pro Plus Version 6.3 software (Media Cybernetics).

**ELISA.** Serum was collected before infections, 14 days after primary infections, and 14 days after secondary infections from wild-type C57BL/6J mice or C57BL/6J-*Ighm*-deficient mice. For detection of *S. venezuelensis*-specific IgG1 antibodies, *S. venezuelensis* antigens were prepared from L3 by an alkaline digestion method (76) and coated onto multiwell plates. Serum was added to the antigen-coated plates, followed by incubation with biotinylated anti-mouse IgG1 (clone R85-1) and then incubation with TMB (3,3′,5,5′-tetramethylbenzidine) as a substrate; the reaction then was stopped with 2 N sulfuric acid, and the optical density (OD) (405 nm) was measured. Total IgG1 was measured with a standard sandwich ELISA (clone A85-3 for coating and clone A85-1 for detection, with TMB as a substrate). Total IgE was measured with a standard sandwich ELISA (clone R35-72 for coating and clone R35-118 for detection, with TMB as a substrate).

**Adult worm count.** At 8 days after inoculation with 10,000 *S. venezuelensis* L3 to induce a primary infection, the mice were sacrificed and the small intestines were collected and cut open longitudinally and then cut into small pieces and incubated in PBS at 37C° for 4 h and worms were counted under the microscope. By direct observation, none of the mice sacrificed for analysis of adult worm burdens exhibited evidence of adverse effects of such worms, such as weight loss, reduced spontaneous activity, or evidence of discomfort or loss of appetite.

**Cell transfer.** CD4$^+$ splenic T cells (4 × 10$^6$) and B220$^+$ splenic B cells (1 × 10$^6$) were purified (>98% purity) from wild-type BALB/cJ mice with biotinylated anti-CD4 (clone GK1.5) and anti-B220 (clone RA3-6B2) antibodies (both from Biolegend), followed by incubation with streptavidin microbeads with a magnetically activated cell sorting (MACS) system (Miltenyi Biotec, Germany) and a single wash in PBS, and the purified splenic T and B cells in PBS were injected intravenously into BALB/cJ-*Rag2Il2* doubly deficient mice on day −1.

**Statistics.** Data were examined for statistical significance using the unpaired two-tailed Student's $t$ test to compare values at each time point and by 2-way analysis of variance (ANOVA) for the time course of responses.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/IAI.00053-17.

**SUPPLEMENTAL FILE 1,** PDF file, 0.8 MB.

## ADDENDUM IN PROOF

Shortly after our accepted manuscript was posted online, it was brought to our attention that a similar study (M. Reitz, M. L. Brunn, H. R. Rodewald, T. B. Feyerabend, A. Roers, et al., Mucosal Immunol 10:481–492, 2017, https://doi.org/10.1038/mi.2016.56) was posted online about the time we submitted the first version of our manuscript on 13 July 2016. Reitz et al., using c-Kit-independent mast cell deficient-

mice and genetically basophil-deficient mice, detected roles for mast cells and basophils in primary infections with *Strongyloides ratti*. Although Reitz et al. and we used different species of *Strongyloides*, numbers of L3 to establish infection, types of c-Kit-independent mast cell- or genetically basophil-deficient mice, and protocols to establish secondary infections, the findings of the two studies support the following conclusions regarding mast cells and basophils: (i) mast cells can have an important role and basophils a smaller role in host defense against *Strongyloides venezuelensis* or *Strongyloides ratti* in primary infections, and (ii) while mast cells also may contribute to host defense in secondary infections with *Strongyloides*, their role is minor compared to that in primary infections, and the importance of such roles may vary according to the species of *Strongyloides*, the model of secondary infection analyzed, and/or other factors.

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