

1 **Differences in the importance of mast cells, basophils, IgE and IgG,**
2 **versus CD4⁺ T cells and ILC2 cells, in primary and secondary immunity**
3 **to *Strongyloides venezuelensis***

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22 Running Head: Immune responses to *S. venezuelensis* in mice

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42 **Abstract**

43 There is evidence that mast cells, basophils and IgE can contribute to
44 immune responses to parasites, however, the relative importance of these
45 effector elements in parasite immunity is not fully understood. Previous
46 work in *Il3*-deficient and *c-kit* mutant *Kit*^{W/W-v} mice indicated that
47 interleukin-3 and c-Kit contribute to expulsion of the intestinal nematode
48 *Strongyloides venezuelensis* (S.v.) during primary infection. Our findings in
49 mast cell-deficient *Kit*^{W-sh/W-sh} mice and two types of mast cell-deficient mice
50 that have normal *c-kit*: “Hello Kitty” and MasTRECK mice, confirmed prior
51 work in *Kit*^{W/W-v} mice suggesting that mast cells play an important role in S.v.
52 egg clearance in primary infections. We also assessed a possible contribution
53 of basophils in immune responses to S.v. By immunohistochemistry, we
54 found that numbers of basophils and mast cells, were markedly increased in
55 the jejunal mucosa during primary infections with S.v. Studies in basophil-
56 deficient *Mcpt8*^{DTR} mice revealed a small but significant contribution of
57 basophils to S.v. egg clearance in primary infections. Studies in mice
58 deficient in various components of immune responses showed that CD4⁺ T
59 cells and ILC2 cells, IgG, FcRγ, and to a lesser extent, IgE and FcεRI,
60 contribute to effective immunity in primary S.v. infections. These findings
61 support the conclusion that the hierarchy of importance of immune effector

62 mechanisms in primary *S.v.* infection is: CD4⁺ T cells/ILC2 cells, IgG and
63 FcR γ >mast cells>IgE and Fc ϵ RI>basophils. By contrast, in secondary *S.v.*
64 infection, our evidence indicates that CD4⁺ T cells are critical but mast cells,
65 antibodies, and basophils have little or no non-redundant roles.

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82 Introduction

83 *Strongyloides stercoralis* is endemic mainly in tropical and subtropical areas
84 and is estimated to infect 30-100 million people world-wide (1, 2).

85 *Strongyloides venezuelensis* (S.v.) is a rodent parasite with a similar life
86 cycle that is used as an experimental model of *S. stercoralis* (3). Prior work
87 has implicated mast cells (MCs) (4–15) and IL-3 (5, 7, 12) in immune
88 resistance to primary infections with S.v. IL-3 is essential for the increases in
89 jejunal MCs and blood basophils observed after S.v. infection (7, 12), and
90 *Kit*^{W/W^{-v}} mice (markedly deficient in MCs) (16, 17), that were also *Il3*-
91 deficient exhibited a more pronounced defect in S.v. expulsion during
92 primary infections than did either *Kit*^{W/W^{-v}} or *Il3*-deficient mice, suggesting
93 that basophils also might contribute to S.v. clearance (7).

94 However, the importance of MCs and basophils in S.v. immunity was
95 not proven by such work. For example, the MC-deficient mice used in many
96 of the studies (4, 6, 7, 9, 11, 14), *Kit*^{W/W^{-v}} mice, have several c-Kit-related
97 defects in addition to their MC deficiency (17–26) and, until recently, no
98 basophil-deficient mice were available to assess specifically the roles of
99 basophils in this setting. In the present study, we used basophil-deficient
100 mice and three types of MC-deficient mice to examine the roles of basophils
101 and MCs in primary and secondary infections with S.v. Because it has been

102 reported that IgG1 and IgE antibodies can function in concert to enhance
103 expulsion of *S.v.* during primary infections, but that neither type of antibody
104 had non-redundant function in such clearance (14), we also tested mice
105 deficient in antibodies, IgE, the FcR γ chain, and Fc ϵ RI alpha, as well as
106 mice with deficiencies in T cells and B cells. These experiments revealed
107 that MCs, basophils, IgG and IgE have distinct roles in primary vs.
108 secondary infections with *S.v.*

109

110 **Materials and Methods**

111 **Mice**

112 All animal experiments were carried out following protocols approved by
113 the Stanford University Administrative Panel on Laboratory Animal Care.
114 C57BL/6J (B6J)-*Kit*^{W-sh/W-sh} mice (27, 28) and littermate control B6J-*Kit*^{+/+}
115 mice (wild-type control mice), C57BL/6J-*Cpa3-Cre; Mcl-1*^{fl/fl} “Hello Kitty”
116 mice (29) and their control (*Cpa3-Cre; Mcl-1*^{+/+}) mice, BALB/cJ-*Mcpt8*^{DTR/+}
117 mice (30) and littermate BALB/cJ-*Mcpt8*^{+/+} control mice, BALB/cJ-
118 MasTRECK (“MasTRECK” in this paper means heterozygous: Tg/+) mice
119 (31) and littermate wild-type control mice (+/+; no Tg gene) were all from
120 stocks bred in our laboratory. C57BL/6J wild type, BALB/cJ wild type,
121 C57BL/6J-*Fcer1g*^{-/-} (32), C57BL/6J-*Rag2*^{-/-} (33), C57BL/6J-*Ighm*^{-/-} (34),

122 C57BL/6J-*Rora*^{sg/sg} (35, 36), and C57BL/6J-*Fcer1a*^{-/-} (37) mice were
123 purchased from Jackson laboratory (Sacramento, CA). BALB/cAnNTac-
124 *Igh-J*^{-/-} (38) mice were purchased from Taconic (Germantown, NY).
125 BALB/cJ-*Rag2*^{-/-}*Il2g*^{-/-} (39) mice and BALB/c-*Igh-7*^{-/-} mice (40) (and their
126 control BALB/c-*Igh-7*^{+/+} mice (40) were kindly provided by I. Weissman
127 (Stanford University School of Medicine) and H. Oettgen (Harvard Medical
128 School, Boston, MA), respectively.

129 **Infection with *Strongyloides venezuelensis***

130 As previously described (7, 12), mice were infected by subcutaneous
131 inoculation with 10,000 third-stage infective larvae (L3). For secondary
132 infections, mice were infected by subcutaneous inoculation with 10,000 L3
133 28 or 42 days (as indicated) after the primary inoculation. We monitored
134 infections in individual mice by counting the numbers of eggs (per g feces)
135 excreted daily. Each type of immune cell was identified by flow cytometry
136 except for tissue mast cells, which were counted as described (29).
137 *Mcpt8*^{DTR/+} mice received intravenous injections of diphtheria toxin (DT;
138 Sigma-Aldrich, St. Louis, MO) (200 ng/mouse) every 5 days starting 2 days
139 before infection, and MastRECK mice received intraperitoneal injections of
140 DT (250 ng/mouse) every 5 days starting 2 days before infection. Control
141

142 groups were a combination of DT-treated wild type (*Mcpt8*^{+/+} or littermate
143 wild type control for MasTRECK) mice and PBS-treated *Mcpt8*^{DTR/+} mice or
144 MasTRECK mice, because the two groups behaved similarly (see Fig. S1 in
145 the supplemental material). For CD4 T cell depletion, 100 µg of anti-CD4 or
146 isotype-matched control rat IgG2b, κ antibodies (clones GK1.5 or RTK4530
147 from BioLegend, San Diego, CA) were injected intraperitoneally once a
148 week starting at day -3. For NK cell depletion, 300 µg of anti-NK1.1 or
149 isotype-matched control mouse IgG2a, κ antibodies (clones PK136 or
150 MOPC-173 from BioLegend) were injected intraperitoneally once a week
151 starting at day -3. For depletion of ILC2 cells, 200 µg of anti-Thy1.2 or
152 isotype-matched control rat IgG2b, κ antibodies (clones 30-H12 or
153 RTK4530 from BioLegend, San Diego, CA) were injected intraperitoneally
154 into C57BL/6J-*Rag2*-deficient mice starting at day -1 and then continuing
155 for every 3 days until the end of the experiment. Both male and female mice,
156 which were 6-8 weeks old on the day of infection (day 0), were tested in
157 each of the groups analyzed, and we found no statistically significant
158 differences in the results obtained between male and female mice in any of
159 the experimental groups. By direct observation at least until the time of
160 cessation of *S.v.* egg excretion, all mice appeared to be healthy during the
161 entire course of *S.v.* infection.

162

163 **Antibodies and flow cytometry**

164 All reagents used for cytometry were from BioLegend: mouse IgE-FITC or
165 mouse IgE-biotin (R35-72), CD49b-Alexa647 or FITC (DX5), c-Kit-FITC
166 or PE (2B8), FcεRIα-PE or APC (MAR-1), CD200R3-PE (Ba13), and APC
167 streptavidin. Basophils were identified as CD49b positive and either mIgE,
168 FcεRIα, or CD200R3 positive (depending on the type of mutant mouse).
169 Purified anti-mMCP-8 (TUG8) (for tissue staining) was from Biolegend.

170

171 **Histological Analysis**

172 4-μm sections of Carnoy's-fixed, paraffin-embedded jejunum were stained
173 with 0.5% Alcian Blue. Mast cells and goblet cells were quantified per villus
174 crypt unit (vcu) (41). To visualize tissue basophils, sections were stained
175 with anti-mMCP-8 (10 μg/mL) (29, 42). Images were captured with an
176 Olympus BX60 microscope using a Retiga-2000R QImaging camera run by
177 Image-Pro Plus Version 6.3 software (Media Cybernetics).

178

179 **ELISA**

180 Serum was collected before infections, 14 days after primary infections, and
181 14 days after secondary infections from wild type C57BL/6J mice or

182 C57BL/6J-*Ighm* deficient mice. For detection of *S.v.*-specific IgG1
183 antibodies, *S.v.* antigens were prepared from L3 larvae by an alkaline
184 digestion method (43) and coated onto multi-well plates. Serum was added
185 to the antigen-coated plates, followed by incubation with biotinylated anti-
186 mouse IgG1 (clone: R85-1) and then incubation with TMB as a substrate;
187 the reaction then was stopped with 2N sulfuric acid and the O.D. (405 nm)
188 was measured. Total IgG1 was measured with a standard sandwich ELISA
189 (clone A85-3 for coating, and clone A85-1 for detection, and with TMB as a
190 substrate). Total IgE was measured with a standard sandwich ELISA (clone
191 R35-72 for coating, and clone R35-118 for detection, and with TMB as a
192 substrate).

193

194 **Adult worm count**

195 8 days after inoculation with 10,000 *S.v.* L3 to induce a primary infection,
196 the mice were sacrificed and the small intestines were collected and cut open
197 longitudinally, and then cut into small pieces and incubated in PBS at 37C°
198 for 4 hours and worms were counted under the microscope. By direct
199 observation, none of the mice sacrificed for analysis of adult worm burdens
200 exhibited evidence of adverse effects of such worms, such as weight loss,
201 reduced spontaneous activity, or evidence of discomfort or loss of appetite.

202

203 **Cell transfer**

204 4×10^6 CD4⁺ splenic T cells and 1×10^6 B220⁺ splenic B cells were purified
205 (>98% purity) from wild-type BALB/cJ mice with biotinylated anti-CD4
206 (clone:GK1.5) and anti-B220 (clone:RA3-6B2) antibodies (both from
207 Biolegend), followed by incubation with streptavidin microbeads with the
208 MACS system (Miltenyi Biotec, Germany), and a single wash in PBS, and
209 the purified splenic T and B cells in PBS were injected intravenously into
210 BALB/cJ-*Rag2Il2* double-deficient mice on day -1.

211

212 **Statistics**

213 Data were examined for statistical significance using the unpaired, two-
214 tailed Student's *t* test to compare values at each time point and by 2-way
215 ANOVA for the time course of responses.

216

217 **Results**218 **Mast cells and, to a lesser extent, basophils contribute to expulsion of**219 ***S.v.* in primary infections**

220 Both IL-3 and c-Kit can enhance clearance of a primary infection with
221 *S.v.* (7). The role of c-Kit is consistent with other lines of evidence

222 indicating possible contributions of MCs to host resistance to this nematode
223 (4, 6, 11, 44). While it is well known that MC numbers increase greatly in
224 the jejunum of wild type mice during primary infections with S.v. (6–9, 11),
225 it was not known to what extent basophils might also infiltrate such tissues.
226 Using an anti-mouse mast cell protease 8 (mMCP-8) Ab (42) recognizing
227 the basophil-specific marker mMCP-8, we found that basophils were
228 undetectable by immunohistochemistry in the jejunum of uninfected wild
229 type mice (Fig. 1A, left panel) but many basophils were present 7 days after
230 the first S.v. inoculation (Fig. 1A, middle and right panels).

231 It has been suggested that MCs and basophils might have
232 complementary and/or partially overlapping roles in certain immune
233 responses to parasites (7, 44–47). We used genetic approaches to investigate
234 the relative contributions of MCs and basophils in primary and secondary
235 infections with S.v. MC-deficient WBB6F₁-*Kit*^{W/W^{-v}} mice exhibited delayed
236 expulsion of S.v. in primary infections (6, 7, 11), but such mice have other
237 abnormalities related to their *c-kit* mutations that may influence the response
238 to this parasite by mechanisms unrelated to their MC deficiency (17, 31, 48–
239 50).

240 Therefore, we tested genetically-engineered MC-deficient *Cpa3*-
241 *Cre*;*Mcl-1*^{fl/fl} “Hello Kitty” mice, in which a marked MC deficiency, and also

242 a substantial reduction in basophils, occur because of a mechanism
243 independent of mutations affecting *c-kit* (29). Hello Kitty mice exhibited a
244 13 day delay in achieving cessation of S.v. egg excretion compared to the
245 corresponding control *Cpa3-Cre;Mcl-1^{+/+}* MC- and basophil-sufficient mice
246 (Fig. 1B). Another genetically-engineered MC-deficient mouse (with normal
247 *c-kit*), the MasTRECK (Tg/+) mouse (31), exhibited a 7 day delay in
248 cessation of egg excretion compared to DT-treated littermate wild type
249 control (+/+) or PBS-treated MasTRECK mice (Fig. 1C; also see Fig. S1A
250 in the supplemental material).

251 Beyond a profound MC deficiency, both Hello Kitty mice (29) and, at
252 early times after DT treatment, DT-treated MasTRECK mice (31) have
253 significantly reduced numbers of basophils. We therefore also tested MC-
254 deficient C57BL/6J-*Kit^{W-sh/W-sh}* mice (28) which have Kit-related MC
255 deficiency but have slightly increased numbers of basophils (28). Like Hello
256 Kitty mice, *Kit^{W-sh/W-sh}* mice exhibited a 12 day delay in cessation of S.v. egg
257 excretion compared to the corresponding wild type mice (Fig. 1D). Finally,
258 we tested *Mcpt8^{DTR/+}* mice (30), which can be rendered deficient specifically
259 in basophils. Compared to the corresponding control mice (i.e., DT-treated
260 basophil-sufficient littermate wild type *Mcpt8^{+/+}* mice and PBS-treated
261 *Mcpt8^{DTR/+}* mice: see Fig. S1B in the supplemental material), DT-treated

262 *Mcpt8*^{DTR/+} mice exhibited significantly more eggs in their feces on days 8
263 and 9, and a one-day delay in cessation of egg excretion (Fig. 1E). It has
264 been shown that, during primary infection with *S.v.*, *Kit*^{W/W-v} mice (4-7, 11)
265 and IL-3-deficient *Kit*^{W/W-v} mice (7) harbored more adult worms in the
266 intestines compared to the corresponding control mice, as well as exhibiting
267 a more delayed cessation of egg excretion. We found that basophil-depleted
268 DT-treated *Mcpt8*^{DTR/+} mice also exhibited increased numbers of worms in
269 the intestines at day 8 of primary *S.v.* infection compared to the
270 corresponding wild type mice (Fig. S2A in the supplemental material), in
271 addition to exhibiting increased numbers of eggs in the feces on that day and
272 also a subsequent delay in cessation of egg excretion (Fig. 1E).

273 In addition to monitoring primary responses to *S.v.* by counting egg
274 excretion, we measured changes in numbers of basophils and MCs. The
275 various control MC- and basophil-sufficient mice exhibited substantial
276 increases in blood basophils during primary infections (Fig. 2A; also see Fig.
277 S3 in the supplemental material), peaking around days 10-12. Hello Kitty
278 mice, DT-treated MasTRECK mice, and DT-treated *Mcpt8*^{DTR/+} mice each
279 exhibited slight increases in blood basophils that were detectable ~7-11 days
280 after initiation of *S.v.* infection; however, all but the DT-treated MasTRECK
281 mice maintained a substantial basophil depletion (Fig. 2A; also see Fig. S3

282 in the supplemental material). The temporary basophil depletion we saw in
283 DT-treated MasTRECK mice is consistent with previous observations (31).

284 In S.v.-infected MC- and basophil-sufficient mice, MCs were
285 increased in the jejunal mucosa during the primary infection, and numbers of
286 goblet cells also increased (Fig. 2B, 2C, Fig. 4D). By contrast, while all 3
287 types of MC-deficient mice exhibited increased levels of goblet cells in the
288 jejunum (to levels similar to those in the corresponding MC-sufficient mice),
289 numbers of mucosal MCs and peritoneal MCs were drastically reduced (Fig.
290 2B, 2C, Fig. 4D; also see Fig. S3 in the supplemental material). In S.v.-
291 infected basophil-deficient mice, levels of both mucosal and peritoneal MCs
292 were similar to those in the corresponding control mice (Fig. 2B, 2C; also
293 see Fig. S3 in the supplemental material).

294 These results establish that MC deficiencies were sustained during
295 primary S.v. infections in the three kinds of MC-deficient mice tested, and
296 that the basophil deficiencies are maintained during such infections in both
297 DT-treated *Mcpt8*^{DTR/+} mice and Hello Kitty mice. These findings also
298 indicate that the expansion of MCs observed in this setting appears not to
299 require an important non-redundant contribution of basophils, and that the
300 jejunal goblet cell hyperplasia associated with primary infections with S.v.
301 can occur largely independently of contributions of MCs or basophils.

302 Compared to the consistent basophil depletion observed before day 7
303 in DT-treated *Mcpt8^{DTR/+}* and MasTRECK mice, Hello Kitty mice had a
304 larger variation in the % of blood basophil deficiency and also exhibited
305 more variation in the delay in cessation of S.v. egg production vs. DT-treated
306 MasTRECK and *Mcpt8^{DTR/+}* mice. Notably, in individual Hello Kitty mice,
307 there was a strong correlation between the delay in cessation of S.v. egg
308 excretion and in the magnitude of their blood basophil deficiency (Fig. 2D,
309 left). No such correlation was observed between the delay in S.v. egg
310 clearance and the reduction in peritoneal MCs (Fig. 2D, middle) or between
311 the reductions in blood basophils vs. peritoneal MCs (Fig. 2D, right). Also,
312 all individual Hello Kitty mice remained substantially MC-deficient during
313 S.v. infection. The strong correlation in individual Hello Kitty mice between
314 the extent of their reduction in blood basophils and the length of delay in
315 cessation of parasite egg excretion supports the conclusion that basophils, as
316 well as MCs, contribute to protective immunity during a primary infection
317 with S.v.

318

319 **Minor or no roles of mast cells and basophils in S.v. expulsion during**
320 **secondary infections**

321 We also examined whether MCs and/or basophils contribute to

322 cessation of *S.v.* egg excretion during secondary infections. Several studies
323 indicate that, in secondary nematode infections of wild type mice, the larvae
324 can be trapped in the skin and lungs before reaching the intestines, as
325 reported for *S.v.* (51-53), *Strongyloides ratti* (53), and *Nippostrongylus*
326 *brasiliensis* (54-56). In this study, we assessed fecal egg numbers as an
327 indicator of whether larvae could reach the intestines and then produce eggs.
328 Only 3 of 11 *Cpa3-Cre;Mcl-1^{+/+}* mice (the MC- and basophil-sufficient
329 controls for Hello Kitty mice) and none of the C57BL/6J-*Kit^{+/+}* mice (the
330 MC-sufficient controls for the MC-deficient *Kit^{W-sh/W-sh}* mice) excreted any
331 eggs during secondary infections (Fig. 1B and 1D). Even when eggs were
332 detected, the amounts were ~1,000 fold less than during primary infections
333 and eggs were detected only on day 7 (Fig. 1B). No eggs at all were detected
334 during secondary infections in control (MC- and basophil-sufficient) mice
335 on the BALB/cJ background (Fig. 1C and 1E).

336 Compared to the corresponding control mice, half of the Hello Kitty
337 mice and all of the *Kit^{W-sh/W-sh}* mice exhibited delayed cessation of *S.v.* egg
338 excretion during secondary infections, although the amounts of eggs in the
339 feces were ~1,000 fold less than during primary infections (Fig. 1B and 1D).
340 However, the differences between the MC-deficient and corresponding
341 control mice were not statistically significant. DT-treated MasTRECK mice

342 and the corresponding control mice didn't differ in their response to
343 secondary infection with *S.v.*, in that none of the mice in either group
344 exhibited any eggs in the feces (Fig. 1C). Only 1 of 15 DT-treated basophil-
345 deficient *Mcpt8*^{DTR/+} mice excreted any eggs during the secondary infection,
346 and that was only on day 7 (Fig. 1E).

347 In secondary infections, blood basophils were not increased to the
348 same extent in the various basophil-sufficient control mice as in primary
349 infections (Fig. 2A; also see Fig. S3 in the supplemental material).
350 Moreover, such mice exhibited substantially fewer MCs in the jejunal
351 mucosa than in the primary infections, especially in mice on the C57BL/6J
352 background (Fig. 2B, 2C). Peritoneal MCs were not detectable after
353 secondary infections in Hello Kitty mice or *Kit*^{W-sh/W-sh} mice (Fig. 2B, 2C;
354 also see Fig. S3 in the supplemental material). DT-treated *Mcpt8*^{DTR/+} mice
355 and MasTRECK mice (each of them on the BALB/cJ background) had
356 numbers of mucosal MCs in the jejunums after secondary infections that
357 were similar to those in the corresponding control mice (Fig. 2B, 2C; also
358 see Fig. S3 in the supplemental material). Importantly, we found that DT-
359 treated MasTRECK mice not only had mucosal MCs but also peritoneal
360 MCs after secondary infections, at levels similar to those in the
361 corresponding control mice (Fig. 2C). The fact that DT-based MC depletion

362 was no longer effective in this setting (Fig. 2C) might, in part, explain why
363 no eggs were detected during secondary infections in DT-treated
364 MasTRECK mice.

365 Taken together, these results suggest that MCs play only a minor role
366 (if any) in resistance to *S.v.* during secondary infections in the mice analyzed
367 and that basophils do not provide critical non-redundant function in
368 orchestrating cessation of *S.v.* egg excretion in these mice during the
369 secondary infection.

370

371 **Roles of CD4⁺ T cells, ILC2 cells, and IgG and IgE antibodies, in**
372 **immune responses to *S.v.***

373 The more rapidly effective expulsion of *S.v.* observed in wild type
374 mice in the secondary infection is consistent with prior work indicating that
375 adaptive immunity enhances host resistance to this nematode (51, 52, 57).
376 To investigate the importance of various potential effector mechanisms in
377 orchestrating immune responses to *S.v.*, we examined primary and secondary
378 infections in mice deficient in different components of innate or adaptive
379 immunity. We found that T and B cell-deficient *Rag2*^{-/-} mice exhibited a 20-
380 day delay (i.e., requiring 32 days in total) in cessation of *S.v.* egg excretion
381 during primary infections and a 25-day delay (i.e., requiring 37 days in total)

382 in the secondary infection (vs. no eggs detected during secondary infections
383 in any of the wild type mice) (Fig. 3A), underscoring the importance of an
384 adaptive immune response for effective secondary immune responses to this
385 nematode (8, 11, 13, 14, 53, 58–60). In addition, we confirmed that the
386 number of adult worms in the intestines on day 8 of the primary infection
387 was significantly higher in the *Rag2*^{-/-} mice compared to the corresponding
388 wild-type mice (Fig. S2B in the supplemental material), a finding consistent
389 with the differences observed in egg excretion in these groups (Fig. 3A).

390 To assess contributions of CD4⁺ T-cells, we injected anti-CD4 or
391 isotype control antibodies into wild type C57BL/6J mice. Compared to the
392 control group, during the primary infection mice injected with the anti-CD4
393 antibody exhibited a 27-day delay in cessation of *S.v.* egg excretion (Fig.
394 3B). During secondary infections, no eggs were detected in any of the wild
395 type mice injected with the isotype control antibody but eggs persisted until
396 day 29 in the anti-CD4 antibody-treated mice (Fig. 3B). While both *Rag2*-
397 deficient mice and anti-CD4 antibody-treated wild type mice exhibited
398 elevated % of blood basophils at baseline compared to the corresponding
399 control mice (reflecting the depletion of lymphocytes in the blood of such
400 mice), neither the primary nor secondary *S.v.* infection induced a significant
401 increase in the % of blood basophils (Fig. 4A; also see Fig. S4 in the

402 supplemental material). As previously reported (11), jejunal mucosal MCs
403 were essentially absent in *Rag2*^{-/-} mice at baseline and did not appear in such
404 mice after a primary infection (Fig. 4B and C). The low levels of expression
405 of IgE on the surface of blood basophils in anti-CD4 antibody-treated wild
406 type mice during primary infections with *S.v.* (see Fig. S4 in the
407 supplemental material) are consistent with an important role of CD4⁺ T cells
408 in the induction of an IgE response in this setting (61, 62), with resulting
409 upregulation of surface expression of FcεRI on blood basophils (63–65).
410 However, these *Rag2*-deficient mice and anti-CD4 antibody-treated wild
411 type mice exhibited levels of jejunal goblet cell hyperplasia similar to those
412 in the corresponding control mice (Fig. 4B-D; also see Fig. S5 in the
413 supplemental material). These results confirm that, during *S.v.* infection,
414 CD4⁺ T cells are essential for the expansion of jejunal mucosal MCs (11),
415 elevation of IgE levels, and effective clearance of *S.v.* eggs (14), but are
416 dispensable for jejunal goblet cell hyperplasia (Fig. 4B-4D; also see Fig. S4,
417 S5 in the supplemental material).

418 To investigate the role of B cells in immune responses to *S.v.*, we
419 assessed the infections in *Ighm*-deficient mice (known as μMT mice), which
420 lack conventional B cells due to a deficiency in membrane μ heavy chain
421 and exhibit markedly reduced levels of immunoglobulins (34). Surprisingly,

422 *Ighm*-deficient mice did not exhibit a delay in cessation of *S.v.* egg excretion
423 in the primary infection, and, like the corresponding control mice, exhibited
424 no detectable egg production during secondary infections. However, while
425 *Ighm*-deficient mice had no detectable IgE on the surface of basophils before
426 *S.v.* infection, IgE was detected on basophils on day 10 of *S.v.* infection (see
427 Fig. S4 in the supplemental material), a finding in accord with the previously
428 reported observation of IgE production in these mice when infected with
429 *Heligmosomoides polygyrus*, *Trichuris muris*, and *Schistosoma mansoni*
430 (66). IgE was detected by flow cytometry on the surface of basophils after
431 primary infections in all *Ighm*-deficient mice. On the other hand, with
432 ELISA, which is not as sensitive as flow cytometry, total IgE was detected
433 in 2 out of 6 *Ighm*-deficient mice after secondary infections (see Fig. S6A in
434 the supplemental material). Total IgG1 was elevated after primary infections
435 in all *Ighm*-deficient mice (see Fig. S6B in the supplemental material). *S.v.*-
436 specific IgG1 was detected in 1 out of 6 *Ighm*-deficient mice after secondary
437 infections, but much higher titers were detected in all wild-type mice after
438 primary infections (see Fig. S6C in the supplemental material). Moreover,
439 during primary infection with *S.v.*, *Ighm*-deficient mice and the
440 corresponding wild type mice developed similarly expanded populations of
441 blood basophils, jejunal mucosal MCs, and jejunal goblet cells (Fig. 4A-D;

442 also see Fig. S4 in the supplemental material).

443 We also examined primary and secondary *S.v.* infections in *Igh-J*-
444 deficient mice (also known as *Jh*-deficient mice), which lack all detectable
445 antibodies, from either conventional or non-conventional B cells (38). *Igh-J*-
446 deficient mice exhibited a 20-day delay in cessation of *S.v.* egg excretion
447 during the primary infection, suggesting an indispensable role of antibodies
448 in the response to *S.v.*; however, like the corresponding control mice, *Igh-J*-
449 deficient mice exhibited no detectable egg excretion during the secondary
450 infection (Fig. 4D). Antibodies remained undetectable by ELISA (or by
451 FACS of basophils) after either primary or secondary *S.v.* infection in *Igh-J*-
452 deficient mice (data not shown), but *Igh-J*-deficient mice and the
453 corresponding wild type mice developed similarly expanded populations of
454 blood basophils, jejunal mucosal MCs, and jejunal goblet cells during the
455 primary infection (Fig. 4A-D; also see Fig. S4 in the supplemental material).

456 The important role of antibodies in the primary infection with *S.v.*
457 appears to depend on the binding of antibodies to FcRs that signal via the
458 FcR common γ -chain (8, 10, 14), and in our model, we found that *Fcer1g*-
459 deficient mice exhibited a 19-day delay in cessation of egg excretion (Fig.
460 3E), a delay very similar to that seen in *Igh-J*-deficient mice (Fig. 3D). Like
461 *Igh-J*-deficient mice, *Fcer1g*-deficient mice also exhibited levels of

462 expanded populations of blood basophils, jejunal mucosal MCs, and jejunal
463 goblet cells similar to those of the corresponding wild type mice during
464 primary *S.v.* infections (Fig. 4A-D; also see Fig. S4 in the supplemental
465 material). In secondary *S.v.* infections, 2 of 7 *Fcer1g*-deficient but none of
466 the corresponding wild type mice exhibited some egg excretion, but not after
467 day 8 (Fig. 3E), but these differences were not statistically significant. Other
468 groups showed that the numbers of adult worms in the intestines, as well as
469 *S.v.* egg excretion, were increased in antibody-deficient *Aicda*^{-/-} mice and
470 *Fcer1g*-deficient mice compared to the corresponding wild type mice (8,
471 14).

472 Because FcRγ is a component of receptors for IgG and IgE, we
473 searched for any non-redundant contribution of IgE by testing mice deficient
474 in IgE (i.e., *Igh-7*-deficient mice) or the FcεRI α chain (i.e., *Fcer1a*^{-/-} mice).
475 IgE-deficient and FcεRI-deficient mice each had a 4-day delay to egg
476 clearance in primary infections (Fig. 3F and G) and exhibited expanded
477 populations of blood basophils, jejunal mucosal MCs, and jejunal goblet
478 cells similar to those of the corresponding wild type mice (Fig. 4A-D; also
479 see Fig. S4 in the supplemental material). Neither of these mutant mice
480 exhibited any egg production during secondary infections. These findings
481 confirm prior evidence (14) indicating that both IgG and IgE antibodies can

482 contribute to resistance to *S.v.* during primary infections, and identify for the
483 first time a small but non-redundant role for IgE, functioning via FcεRI, in
484 the cessation of egg excretion in this setting. By contrast, there was no
485 evidence of a non-redundant function of IgE or FcεRIα in egg clearance in
486 secondary infections with *S.v.*

487 We also examined *Rag2* and *Il2g* double-deficient mice, which lack T
488 cells, B cells, NK cells and ILC2 cells (67), and found that they had about a
489 270-day delay in egg clearance during primary infection with *S.v.* (Fig. 3H),
490 as well as significantly increased numbers of adult worms in the intestines
491 on day 8 of the infection (see Fig. S2C in the supplemental material). As in
492 *Rag2*^{-/-} mice, *Rag2* and *Il2g* double-deficient mice exhibited no significant
493 increase in the % of blood basophils and virtually lacked jejunal mucosal
494 MCs (Fig. 4A-C; also see Fig. S4 in the supplemental material). However,
495 unlike *Rag2*^{-/-} mice, *Rag2* and *Il2g* double deficient mice exhibited no
496 increase in jejunal goblet cells during the primary infection (Fig. 4B and
497 4D).

498 Because *Rag2* and *Il2g* double-deficient mice lack NK cells and
499 ILC2s (67), in addition to T and B cells, we examined *Rag2*-deficient mice
500 depleted of NK cells using an anti-NK1.1 antibody (see Fig. S5 in the
501 supplemental material). Such treatment did not influence time to egg

clearance in the primary infection (Fig. 3I). We then tested ILC2-deficient *Rora*^{sg/sg} mice (68). *Rora*^{sg/sg} mice excreted significantly more eggs on day 10 of the primary infection than the corresponding wild type mice, but didn't differ in time to cessation of egg excretion (Fig. 3J). Nor did such *S.v.*-infected *Rora*^{sg/sg} mice differ from the corresponding wild type mice in *S.v.*-induced expansion of blood basophil or jejunal mucosal MC populations, or in exhibiting increases in jejunal goblet cells (Fig. 4A-D; 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 ILC2s on primary infection with *S.v.* First, we injected anti-Thy1.2 antibodies into *Rag2*-deficient mice to deplete ILC2s. 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 33 days) but the Thy1.2 antibody-injected *Rag2*-deficient mice exhibited an additional 71 day delay (to a total of 102 days) (Fig. 3K). Second, we found that, compared to a 20-day delay in *Rag2*-deficient mice (to a total of 33 days), *Rora*^{sg/sg} *Rag2*-deficient mice exhibited an additional 19-day delay (Fig. 3L) (to a total of 52 days). Third, we found that the adoptive transfer of purified (>98%) splenic T cells and B cells from wild-type mice into *Rag2;Il2g* double-deficient mice fully normalized the

time to cessation of egg clearance (to a total of 13 days) (Fig. 3M). Taken together, the results in Fig. 3H-M suggest that NK cells make little detectable difference in the time to cessation of egg excretion in primary S.v. infection (Fig. 3H) but support the conclusion that both T cells and ILC2s can contribute to egg clearance (Fig. 3J-M). However, compared to the extraordinarily long delay in egg clearance seen in *Rag2;Il2g* double-deficient mice (Fig. 3H), the shorter delays seen in mice deficient in T cells and/or ILC2 cells suggest that there might be additional mechanism(s) to contributing to the very long delay in egg clearance in *Rag2;Il2g* double-deficient mice.

Discussion

Strongyloides venezuelensis (S.v.) 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.v. (17–26). To our knowledge, there have been no prior reports investigating the role of basophils in this infection.

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

550 In addition to confirming prior reports indicating that IgG and IgE
551 antibodies (in that report, acting in concert) can contribute to cessation of
552 *S.v.* egg excretion during primary infections (14) and that FcR γ also has an
553 important role in that setting (8, 10, 14), we identified a small but
554 statistically significant contribution of IgE and Fc ϵ RI as well (see Table S1
555 and Fig. S7 in the supplemental material), perhaps because we inoculated
556 our mice with a larger number of L3 *S.v.* than did Matsumoto et al. (14).
557 While it seems likely that parasite-specific IgE and IgG antibodies bind to
558 Fc receptors on effector cells, and then their crosslinking by parasite
559 antigens (especially for IgE), or the activation of effector cells by antigen-
560 IgG-immune complexes, results in expulsion of the parasites (14, 69, 70),
561 the actual roles of parasite-specific and non-specific antibodies in this setting

are still controversial (71, 72). *Ighm*-deficient mice that lack antibodies at baseline were able to produce IgG1 and IgE during primary *S.v.* infections and *S.v.* eggs were cleared on the same day as in wild-type mice (Fig. 3C; also see Fig. S4, Fig. S6 in the supplemental material). Total IgE (detected by flow cytometry) and total IgG1 (measured by ELISA) were elevated in all *Ighm*-deficient mice (see Fig. S4, Fig. S6 in the supplemental material). *S.v.*-specific IgG1 was elevated in all wild-type mice, however, only 1 of 6 *Ighm*-deficient mice had detectable *S.v.*-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.v.* infections, it is possible that some important *S.v.* antigens (e.g., those associated with adults worms but not larvae) were not included our extract from L3 larvae. Similarly, it would be helpful to have more sensitive assays to detect *S.v.*-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 were essential for cessation of *S.v.* 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

582 development of an IgE response, as inferred based on increased levels of IgE
583 on the surface of basophils [see Fig. S4 in the supplemental material] and
584 data not shown). Moreover, our evidence indicates that CD4⁺ T cells are
585 largely if not entirely responsible for orchestrating effective immunity
586 during secondary infections (see Table S1 in the supplemental material).

587 Notably, *Rag2;Il2g* double-deficient mice exhibited a much longer
588 delay in cessation of *S.v.* egg production than did *Rag2*-deficient mice. In
589 addition to lacking cytokine responses requiring *Il2g* (which encodes a
590 component of the receptors for IL-2, 4, 7, 9, and 15), *Rag2;Il2g* double-
591 deficient mice lack NK cells, ILC2 cells, and T cells. Since NK cell
592 depletion in *Rag2*-deficient mice didn't have a detectable effect on *S.v.*
593 expulsion, NK cells are unlikely to contribute substantially to *S.v.* expulsion.
594 However, ILC2 cell-depleted *Rag2*-deficient mice took 102 days to achieve
595 egg clearance (71 days longer than ILC2-sufficient *Rag2*-deficient mice),
596 and *Rag2*-deficient *Rora*^{sg/sg} mice took 51 days to achieve egg clearance (19
597 days more than ILC2-sufficient *Rag2*-deficient mice), suggesting that there
598 is cooperation between T cells and ILC2 cells in achieving cessation of *S.v.*
599 egg production. This result also suggests that additional factors can
600 contribute to the 270-day delay in egg clearance exhibited by *Rag2;Il2g*
601 double-deficient mice, such as effects related to the lack of cytokine

602 signalling dependent on *Il2g*. Our work with *Rora*^{sg/sg} mice, that lack ILC2s,
603 also suggests that ILC2 can have a supportive role in cessation of S.v. egg
604 production, which is in contrast to what appears to be a major role for these
605 cells in immune responses to *Nippostrongylus brasiliensis* (73). However,
606 jejunal goblet cell hyperplasia was observed during primary infections with
607 S.v. in *Rag2*-deficient mice but not in *Rag2;Il2g* double-deficient mice,
608 raising the possibility that mucus production by goblet cells can contribute to
609 cessation of S.v. egg excretion, as it does during immune responses to
610 *Nippostrongylus brasiliensis* (74–76).

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612

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618

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620

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908 **Figure Legends**

909 **FIG 1. Roles of mast cells and basophils in primary and secondary S.v.**
910 **infections.**

911 (A) Immunohistochemical staining with an anti-mMCP8 Ab (DAB
912 substrate) to visualize basophils (some indicated with small black arrows)
913 and Giemsa counterstaining (4 μ m-thick, paraffin-embedded sections) in
914 sections of jejunum from wild type C57BL/6 mice 7 days after inoculation
915 with 10,000 S.v. L3. The left and middle panels are representative images
916 from jejunal villi and the right panel is a representative image from jejunal

917 submucosa. Kinetics of *S.v.* egg excretion after 10,000 *S.v.* L3 inoculation in
918 primary infections (1°) or secondary infections (2°, in mice inoculated with
919 10,000 *S.v.* L3 28 days after the first inoculation) in (B) C57BL/6-Hello
920 Kitty and control mice (n=22 each) for 1° infections (7 experiments),
921 C57BL/6-Hello Kitty (n=12) and control (n=11) mice for 2° infections (4
922 experiments), (C) BALB/c-MasTRECK mice treated with DT (n=5) and
923 control mice (n=4) for 1° infections (2 experiments), BALB/c-MasTRECK
924 mice treated with DT and control mice (n=5 for each) for 2° infections (2
925 experiments), (D) C57BL/6-*Kit*^{W-sh/W-sh} and control mice (n=9 each) for 1°
926 infections (6 experiments), C57BL/6-*Kit*^{W-sh/W-sh} (n=10) and control (n=3)
927 mice for 2° infections (3 experiments), (E) *Mcpt8*^{DTR/+} mice treated with DT
928 (n=26) and control mice (n=27) for 1° infections (9 experiments),
929 *Mcpt8*^{DTR/+} mice treated with DT (n=15) and control mice (n=7) for 2°
930 infections (2 experiments). The numbers of eggs are shown as means + SEM
931 by pooling the data from all the experiments that were performed with each
932 strain. Fraction numbers for 2° infections: numerators indicate the number of
933 mice for which eggs were detected and denominators indicate the number of
934 mice examined. No fraction numbers or lines for 2° infections means none of
935 the mice produced detectable eggs. ****P* < .0001; ***P* < .001; **P* < .05; no
936 asterisks *P* > .05 for comparisons between each mutant mouse and their

937 controls at each time point with the unpaired, two-tailed Student's *t* test, and,
938 as indicated next to the legend for groups of mice, with 2-way ANOVA to
939 compare the time course of the responses.

940

941 **FIG 2. Changes in numbers of basophils, mast cells, and epithelial**
942 **goblet cells in wild type and mast cell and/or basophil-deficient mice**
943 **during primary and secondary S.v. infections.**

944 (A) Blood was collected from each mouse at the indicated points after S.v.
945 infection and stained with anti-IgE and anti-CD49b. % of basophils in live
946 cells was plotted. C57BL/6-Hello Kitty (n=21) and control (n=22) mice were
947 analyzed in both the 1° and 2° infections (separate groups of 21 and 22 mice,
948 for a total of 86 mice). Data for 1° infections include those from 16/21 Hello
949 Kitty mice and all 22 control mice in the same 7 experiments shown in Fig.
950 1, as well as 5 additional Hello Kitty mice with 1° infections which were not
951 used in Fig. 1. For basophil counts in 2° infections, data are shown from all
952 of the Hello Kitty mice (n=11) and control mice (n=12) reported in Fig. 1
953 plus an additional 10 Hello Kitty and 10 control mice with 2° infections.
954 BALB/c-MasTRECK mice treated with DT and control mice (a mixture of
955 DT-treated wild type mice [n=5] and PBS-treated BALB/c-MasTRECK
956 mice [n=5]) (n=10 each for both 1° and 2° infections, for a total of 40 mice),

including, for 1° infections, 5 BALB/c-MasTRECK mice treated with DT
and 4 control mice and, for 2° infections 5 BALB/c-MasTRECK mice
treated with DT and 5 control mice. 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, for 1° infections, 9 C57BL/6- *Kit*^{W-sh/W-sh}
and 9 control mice from the 6 experiments shown in Fig. 1, and, for 2°
infections, 3 C57BL/6- *Kit*^{W-sh/W-sh} and 10 control mice from the 3
experiments shown in Fig. 1. *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 shown in Fig.
1. *** $P < .0005$; ** $P < .005$; * $P < .05$; no asterisks. $P > .05$. Black
asterisks (for 1° infections) or red asterisks (for 2° infections) indicate the
statistical significance of comparisons between each type of mutant mouse
and the corresponding control mice for that time point. (B, C) Jejuna
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 (B) and numbers of mast cells per villus crypt
unit (vcu) are shown as bar graphs in (C) (upper panel, mean + SEM, n=9-10
each; data pooled from 3 experiments). Peritoneal fluid was collected 14

977 days after the start of the 1^o or 2^o infections and mast cells in live cells were
978 assessed using anti-IgE and anti-c-Kit antibodies. % of peritoneal mast cells
979 in live cells are shown as bar graphs in (C) (lower panel, mean + SEM, n=3-
980 10 each; data pooled from 2 or 3 experiments). Wild type values for
981 comparison to those from mutant mice were combined from corresponding
982 controls of the same strain (C57BL/6 or BALB/c). Black asterisks:
983 Statistical significance of comparison of values for uninfected mice vs 1^o or
984 2^o infected mice in the same group (as indicated by bars). Red asterisks:
985 Statistical significance of comparison of values for each type of mutant
986 mouse and the corresponding wild type mice for that condition (i.e.,
987 uninfected, or 1^o or 2^o infected). Actual values are noted (means $\pm$ SEM) for
988 low numbers; no bar or numbers mean none were detected. (D) Left panel:
989 Correlation between (Y axis) the delay in achieving cessation of egg
990 excretion (defined as 0 egg count) in each *Hello Kitty* mouse (compared to
991 the average number of days for cessation of egg excretion in the
992 corresponding wild type mice in each experiment) and (X-axis) the %
993 reduction in the % of blood basophils of individual *Hello Kitty* mice
994 (calculated based on the average % of blood basophils in the corresponding
995 control wild type mice in each experiment) 10-12 days after initiation of 1^o
996 infection with S.v. (e.g., an 80% reduction means that that *Hello Kitty* mouse

1007 had 20% the average % of basophils in the corresponding wild type mice in
1008 that experiment]) (n=16). Middle panel: Correlation between (Y axis) % of
1009 peritoneal mast cells in that Hello Kitty mouse 18-20 days initiation of 1°
1000 infection with S.v. and (X-axis) the % reduction in the % of blood basophils
1001 of individual Hello Kitty mice (defined as in left panel) (n=16). Right panel:
1002 Correlation between (Y axis) the delay in achieving cessation of egg
1003 excretion in each Hello Kitty mouse (defined as in left panel) and (X-axis) %
1004 of peritoneal mast cells in that Hello Kitty mouse 18-20 days after initiation
1005 of 1° infection with S.v. (n=16). r is a correlation coefficient. The data in
1006 each panel are from the same 16 Hello Kitty mice, all of them pooled from
1007 the data for the 16 mice in the 7 experiments shown in Fig. 1 for which data
1008 on basophils and peritoneal mast cells were obtained.

1009

1010 **FIG 3. Roles of T cells, ILC2 cells, antibodies, and antibody receptors in**
1011 **primary and secondary S.v. infections.**

1012 Kinetics of S.v. egg excretion during 1° or 2° infections after inoculation with
1013 10,000 S.v. L3 (second inoculations with S.v. were performed 42 days [A, B,
1014 D, E, I] or 28 days [C, F, G, J] after the first inoculation) in (A) C57BL/6-
1015 *Rag2* deficient (n=13) and control C57BL/6 (n=4) mice for 1° infection (3
1016 experiments), C57BL/6-*Rag2* deficient (n=6) and control C57BL/6 (n=4)

1017 mice for 2° infection (2 experiments), (B) C57BL/6 wild type mice treated
1018 with an anti-CD4 antibody or an isotype-matched control antibody (n=3
1019 each) (2 experiments), (C) C57BL/6-*Ighm* deficient (n=9) and control
1020 C57BL/6 (n=7) mice for 1° infection (4 experiments), C57BL/6-*Ighm*
1021 deficient and control mice for 2° infection (n=5 each) (2 experiments), (D)
1022 BALB/c-*Igh-J* deficient (n=8) and control BALB/c (n=5) mice for 1°
1023 infection (3 experiments), BALB/c-*Igh-J* deficient and control BALB/c mice
1024 (n=5 each) for 2° infection (2 experiments), (E) C57BL/6-*Fcer1g* deficient
1025 (n=7) and control C57BL/6 (n=6) mice for 1° infection (3 experiments),
1026 C57BL/6-*Fcer1g* deficient (n=3) and control C57BL/6 (n=7) mice for 2°
1027 infection (3 experiments), (F) BALB/c-*Igh-7* deficient (n=17) and BALB/c-
1028 *Igh-7* sufficient (n=15) mice for 1° infection (6 experiments), BALB/c-*Igh-7*
1029 deficient (n=11) and control BALB/c (n=3) mice for 2° infection (3
1030 experiments), (G) C57BL/6-*Fcer1a* deficient (n=10) and control C57BL/6
1031 (n=8) mice for 1° infection (3 experiments), C57BL/6-*Fcer1a* deficient and
1032 control C57BL/6 mice (n=5 each) for 2° infection (2 experiments), (H)
1033 BALB/c-*Rag2Il2g* double-deficient (n=12) and control BALB/c (n=10) mice
1034 for 1° infection (2 experiments), (I) C57BL/6-*Rag2*-deficient mice injected
1035 with an anti-NK1.1 antibody or an isotype-matched control antibody (n=3
1036 each) for 1° infection (2 experiments), (J) C57BL/6-*Rora*^{sg/sg} (n=3) and (n=4)

1037 mice for 1° infection (2 experiments), C57BL/6-*Rora*^{sg/sg} (n=4) and control
1038 C57BL/6 (n=3) mice for 2° infection (2 experiments), (K) C57BL/6-*Rag2*-
1039 deficient mice injected with an anti-Thy1.2 antibody or an isotype-matched
1040 control antibody (n=3 each) for 1° infection (2 experiments), (L) C57BL/6-
1041 *Rag2*-deficient mice (n=5) and C57BL/6-*Rag2*-deficient *Rora*^{sg/sg} mice (n=3)
1042 and their corresponding wild type C57BL/6 (n=3) mice for 1° infection (2
1043 experiments), (M) BALB/c-*Rag2Il2g* double-deficient mice treated with
1044 PBS (n=3), BALB/c-*Rag2Il2g* double-deficient mice which received splenic
1045 T and B cells from wild-type BALB/c mice (n=3) and their control BALB/c
1046 mice treated with PBS (n=3) for 1° infection (2 experiments). The numbers
1047 of eggs are shown as means + SEM, with data derived by pooling results
1048 from all experiments performed (as indicated for each strain). Fraction
1049 number in E is displayed as in Fig. 1. ****P* < .0001; ***P* < .001; **P* < .05;
1050 no asterisks *P* > .05 for comparisons between each type of mutant or
1051 antibody-treated mouse, or mutant mouse that received splenic T and B
1052 cells, and their corresponding control mice at each time point with the
1053 unpaired, two-tailed Student's *t* test, and, as indicated next to the legend for
1054 groups of mice, with 2-way ANOVA to compare the time course of the
1055 responses.
1056

1057 **FIG 4. Changes in numbers of basophils, mast cells, and epithelial**
1058 **goblet cells in mice deficient in various components of immune**
1059 **responses during primary and secondary S.v. infections.**

1060 (A) Blood was collected from each mouse during all of the experiments
1061 shown in Fig. 3A-J before infection or 10-12 days after 1° or 2° S.v.
1062 infections and stained with anti-IgE and anti-CD49b. % of basophils in live
1063 cells are shown as bar graphs with means + SEM. *** $P < .0005$; ** $P < .005$; * $P > .05$; no asterisks $P > .05$. Black asterisks indicate the statistical
1064 significance of comparisons between values for uninfected mice and 1° or 2°
1065 infected mice in the same group (as indicated by bars). Red asterisks indicate
1066 the statistical significance of comparisons between each type of mutant
1067 mouse and the corresponding control mice for that condition. (B) Jejuna
1068 were collected 14 days after the start of 1° infections and sections of jejunum
1069 were stained as in Fig. 2B (in the panel showing the histology in BALB/c
1070 wild type mice, some of the mast cells are indicated with filled arrows and
1071 some of the goblet cells are indicated with open arrows), and numbers of
1072 mast cells (C) or goblet cells (D) per villus crypt unit (vcu) are shown as bar
1073 graphs (n=4-12 each; data combined from 2-3 experiments). Wild type
1074 values for comparison to those from mutant mice were combined from
1075 corresponding controls of the same strain (C57BL/6 or BALB/c).









