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1 **The essential role played by B cells in supporting protective immunity against *Trichuris***
2 ***muris* infection is dependent on host genetic background and is independent of antibody**

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11 **Abstract**

12 This study investigates the role of B cells in immunity to *Trichuris muris* (*T. muris*)
13 infection in two genetically distinct strains of mouse, using anti-CD20 monoclonal antibody
14 (mAb) (Genentech-clone 5D2) to deplete B cells. Data is presented for the mouse strains:
15 C57BL/6 and BALB/c, which mount mixed Th1/Th2, and highly polarised Th2 immune
16 responses to *T. muris*, respectively. C57BL/6 mice receiving anti-CD20 treatment prior to
17 and during, or anti-CD20 treatment that commenced two weeks post infection (p.i.), were
18 susceptible to *T. muris*. Parasite-specific IgG1 antibodies were absent and Th2 type cytokines
19 produced by mesenteric lymph nodes cells from mice receiving α-CD20 mAb treatment were
20 significantly lower than produced by cells from isotype control treated mice. T follicular
21 helper cells were also significantly reduced. Importantly, and in complete contrast, BALB/c
22 mice were still able to expel *T.muris* in the absence of B cells, revealing that the essential role
23 played by B cells in protective immunity was dependent on genetic background. To explore
24 whether the important role played by the B cell in the protective immune response of
25 C57BL/6 mice was in enabling strong Th2 responses in the presence of IFN-γ, IFN-γ was

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26 blocked using anti-IFN-γ mAb post B cell depletion. Depleting IFN-γ, in the absence of B
27 cells restored worm expulsion in the absence of parasite-specific IgG1/IgG2c and partially
28 rescued the *T. muris* specific IL-13 response. Thus, our data suggest an important, antibody
29 independent role for B cells in supporting Th2 type immune responses in mixed IFN-γ-rich
30 Th1/Th2 immune response settings.

31

32 **Author summary**

33 How B cells contribute to protective immunity against parasitic nematodes remains
34 unclear, with their importance as accessory cells under-explored. This study reveals that, on
35 some genetic backgrounds, B cells are important for the expulsion of *T. muris* by acting as
36 accessory cells, supporting Th2 immune responses.

37

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38 **Introduction**

39 Infecting over two billion people around the world, mostly in resource-limited
40 countries, the ability of parasitic helminths to maintain long standing chronic infections
41 makes them a major health care issue (1). *Trichuris trichiura* (*T. trichiura*) is one of the most
42 common gastrointestinal nematodes, infecting approximately 465 million people worldwide
43 (2), primarily children. In infected children, trichuriasis is strongly associated to malnutrition,
44 growth stunting, and intellectual retardation; whereas in pregnant adults it is related to
45 anaemia and low birth weight babies (3).

46 For decades, *T. muris* in the mouse has provided a useful and relevant model system
47 with which to explore immunity to *T. trichiura* in man. Infection of mice with intestinal
48 nematode parasite *T. muris* drives polarized T helper cell (Th) responses which associate with
49 resistance (Th2) or susceptibility (Th1). However, the key cellular contributions which
50 support Th2 cell polarization during *T. muris* infection are still not well understood. One of
51 the cells thought to be important is the B cell. It is well established that B cell function is not
52 only related to antibody production, but B cells can also act as antigen presenting cells
53 (APCs) due to the expression of MHC class II molecules and several co-stimulators,
54 including CD40, CD80, and CD86 on its surface (4-6), and as accessory cells, acting as a
55 cellular source of multiple cytokines (7).

56 B cells are well placed to act as accessory cells as they are able to influence CD4 T
57 cells polarisation. For example, previous studies have shown that CD4 T cells produce
58 significantly more IL-4 when stimulated with antigen presented by B cells compared to
59 macrophages (8). In addition, B cells have been shown to produce either Th1 or Th2 type
60 cytokines *in vivo* depending on the type of parasite. Thus, for the Th1 driving parasite
61 *Toxoplasma gondii*, B cells produce IFN-γ and IL-12p40 (9); whilst in the context of the Th2
62 polarising parasite *Heligmosomoides polygyrus* (*H. polygyrus*), B cells produce IL-4 and IL-2

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63 (10, 11). Recent studies reported that IL-4 producing B cells during early infection of
64 *Schistosoma mansoni* (*S. mansoni*) are critical for Th2 polarisation, protecting the host
65 against *S. mansoni*-induced pathology (12). B cells can also influence T cell polarisation
66 towards Th2 response by providing co-stimulatory molecules. Linton *et al*., (13) showed that
67 co-stimulation via OX40L expressed by B cells is essential for T cell polarisation towards
68 Th2 cells. Likewise, both CD80 and CD86 co-stimulatory molecules expressed by B cells, as
69 well as other APC, are required for the Th2 response during *H. polygyrus* infection (14).

70 Recently, it was suggested that B cells, together with T cells and DCs are required not
71 only to optimize the development of Th2 type responses, but also to maintain T follicular
72 helper (T_{FH}) and memory Th2 cells development following *H. polygyrus* infection (15). Thus,
73 Leon *et al.* (15) showed that the deletion of CXCR5 on either DC or CD4 T cells, during *H.*
74 *polygyrus*-infection of C57BL/6 mice, impaired the development of T_{FH} and Th2 cells by
75 impairing the migration of CXCR5+ cells towards the B cell follicle in response to CXCL13.
76 Interestingly, B cell depletion also impaired Th2 responses (15).

77 Previous studies suggested that B cells and antibody are not important in mediating
78 resistance following a primary *T. muris* infection (16-18). Since then, many different studies
79 have been performed to show the importance of CD4 T cells in mediating resistance against
80 *T. muris* (19-21). By contrast, the role of B cells in immunity to *T. muris* remains largely
81 unexplored. *T. muris*-infected μMT mice on a C57BL6 background develop Th-1 type
82 responses, resulting in the susceptibility to *T. muris* infection (22). These susceptible *T.*
83 *muris*-infected μMT C57BL6 mice produced high IFN-γ, without any Th2 cytokines
84 production. Furthermore, when these mice were treated with B cells from naive C57BL6 or
85 with anti-IL-12 antibody, resistance to infection was restored (22). This suggests that B cells
86 are important in either inhibiting Th1 development or supporting Th2 type immune

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87 responses. However, given the importance of B cells in the development of lymph nodes and
88 tissue organisation (23, 24), data from μMT mice is difficult to interpret.
89 This study therefore investigates the role of B cells and antibodies in immunity to *T.*
90 *muris* infection using anti-CD20 monoclonal antibody (mAb) to deplete B cells from mice of
91 two distinct genetic backgrounds, C57BL/6 and BALB/c. The benefit of using anti-CD20
92 mAb is that it allows depletion of CD19+ cells either prior to or post infection and avoids the
93 complicating consequences of B cell deficiency during embryonic development. We
94 demonstrate that B cells are important in the protective immune response to *T. muris*; that the
95 role played by the B cell is antibody-independent; and that the importance of the B cell varies
96 with genetic background of the host. Thus the B cell plays an essential role in supporting Th2
97 type immune responses only in mixed Th1/Th2 IFN-γ rich settings, as seen in C57BL/6 mice,
98 and is redundant in the highly Th2 polarised, IFN–γ-deficient environment of the BALB/c
99 mouse post *T. muris* infection.
100
101

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102 **Results**

103 2.1 Depletion of B cells throughout infection requires two injections of anti-CD20 mAb

104 As a transmembrane calcium channel, CD20 is important in B cell activation,
105 proliferation, and differentiation (25). The CD20 molecule is normally expressed on the
106 surface of B cells during the late pre-B cell. Therefore, the injection of anti-CD20 mAb will
107 deplete all B cells, except early pre-B cells and plasma cells (26). The vast majority of IgM^+
108 or $B220^+$ cells in the spleen, lymph nodes, peritoneal cavity, and blood express CD20, whilst
109 in bone marrow the expression of CD20 increases with B cell maturation (27). Previous
110 studies showed that anti-CD20 effectively depleted B cells either via complement-mediated
111 lysis of target cells (28) or by inducing FcγR-mediated clearance (Ab-dependent cell
112 mediated cytotoxicity) (29). Furthermore, a single dose of anti-CD20 treatment prior to
113 lymphocytic choriomeningitis virus infection depleted B cell populations for up to 45 days
114 (30). However, the current study showed that a single anti-CD20 injection failed to ablate B
115 cells for the full duration of *T. muris* infection, with a clear CD19+ population re-emerging in
116 the MLNs, spleen, and blood by day 35 p.i. (Fig. 1A&B). Therefore, a second anti-CD20
117 injection was administered at day 10 p.i. in order to maintain B cell depletion in the blood
118 and secondary lymphoid organs throughout infection (Fig. 1A&B). $CD19^+$ cells were still
119 present in the bone marrow after anti-CD20 treatment (Fig. 1B), however these $CD19^+$ cells
120 were pro-B cells (Fig. 1C) as has been shown previously (26).

121

122 2.2 C57BL/6 mice treated with anti-CD20 mAb fail to expel *T. muris* by d35 p.i., correlates
123 with an absence of class switched antibodies and a significant decrease in Th2 cytokines

124 To investigate whether B cells are important in resistance against *T. muris* during
125 primary infection of C57BL/6 mice, mice were injected with anti-CD20 mAb 7 days prior to
126 infection and 10 days post infection. Chronic infection, characterised by persisting adult stage

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127 parasites from day 32 p. i. defines susceptibility (22). Thus, autopsies were performed beyond
128 this time point. The experimental design is shown in Fig. 2A. Previous studies have shown
129 that C57BL/6 mice take up to 35 days to completely expel the parasite (31). As shown in Fig.
130 2B, C57BL/6 depleted of B cells were significantly more susceptible to infection than control
131 treated mice.

132 It has previously been shown that IgG1 antibodies are associated with resistance to *T.*
133 *muris* infection, whilst IgG2c antibodies are related to susceptibility (32). Therefore, the
134 levels of parasite specific antibodies in the sera of anti-CD20 treated mice and isotype control
135 treated mice were compared. Mice depleted of B cells using anti-CD20 mAb failed to secrete
136 IgG1 and IgG2c parasite specific antibodies, in contrast to control treated mice (Fig. 2C&D).
137 Further, the levels of *T. muris* specific IgM antibodies in the sera of anti-CD20 treated mice
138 were significantly lower than in the sera of the isotype control-treated mice (Fig. 2E). These
139 effects of B cell depletion on antigen-specific IgG and IgM antibodies are consistent with
140 reports in other model systems (33).

141 In common with other nematode parasites, hosts resistant to *T. muris* mount Th-2
142 immune responses characterised by the production of IL-4, IL-5, IL-9 and IL-13; in contrast,
143 mice susceptible to *T. muris* mount a Th-1 type response, dominated by the release of IFN-γ
144 (32, 34, 35). B cell depleted mice on a C57BL/6 background produced significantly lower
145 Th2 cytokines, including IL-5, IL-9, and IL-13 compared to mice treated with the isotype
146 control (Fig. 2F-H). Interestingly, the production of IFN-γ and IL-17 was similar in both
147 groups (Fig. 2I&J) suggesting that B cells do not affect Th1 development but rather boost
148 Th2 responses. These data are in keeping with the previous study by Leon, *et al.*, which
149 revealed that B cell depletion impaired the development Th2 in mice against *H. polygyrus*
150 infection (15).

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152 2.3 B cell depletion reduced the T_{FH} population and altered DC subsets in the MLN

153 The importance of B cells for the formation of T_{FH} has been shown previously in both
154 mouse (15) and man (36). In keeping with these studies, the MLN T_{FH} population of anti-
155 CD20 treated mice in C57BL/6 genetic background was significantly reduced compared to
156 isotype control treated mice (Fig. 3A-C). Gating strategy was shown in Supplementary Fig. 1.
157 In contrast, T_{FH} development in the spleen, distal to the site of infection, was not significantly
158 affected by B cell depletion (Supplementary Fig.2), although the lack of statistical
159 significance may be due to the high variability in the control mice.

160 Previous studies have suggested that CD11b$^+$ DCs in MLNs might be important for
161 promoting Th2 immune responses against *T. muris* infection (37). Because Th2 cytokines
162 were significantly reduced in the MLNs from anti-CD20 treated mice during *T. muris*
163 infection, DC subsets in MLNs were analysed (Fig. 3D-H). The gating strategy is shown in
164 Supplementary Fig. 3. Total cell number of CD103$^-$CD11b$^+$ DCs (Fig. 3F) and CD103$^+$
165 CD11b$^+$ DCs (Fig. 3H) were significantly reduced in MLNs of anti-CD20 treated mice
166 compared to isotype control treated mice.

167

168 2.4 B cell depletion from day 14 post infection also altered the worm burden

169 As depletion of B cells throughout the course of infection of C57BL/6 mice lead to
170 susceptibility to *T. muris*, we wondered whether B cell depletion from day 14 p.i. would also
171 alter the phenotype. Previous studies on the expulsion kinetics of *T. muris* have shown that
172 the onset of worm expulsion occurs after day 12 p.i., with T cell activation occurring after the
173 first 7 days (38). Therefore, B cells were depleted 2 weeks post *T. muris* infection to see if
174 this still impacted on the ability to expel the parasite. The experimental design is shown in
175 Fig. 4A. Surprisingly, the depletion of B cells from day 14 p.i. also impaired worm expulsion
176 with significantly more parasites present at day 35 p.i. than seen in control-treated mice (Fig.

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177 4B). T_{FH} cells and Th2 type cytokines, including IL-5, IL-9, and IL-13 were also significantly
178 reduced in the absence of B cells (Fig, 4C-H). Interestingly, TNF-α was significantly
179 increased in B cell depleted mice (Fig. 4I), whilst IFN-γ and IL-17A remained the same
180 between groups (Fig. 4J&K). These data suggest that the B cell plays an important role in
181 maintaining Th2 responses.
182
183 2.5 Anti-IFN-γ treatment partially restored IL-13 production in B cell depleted mice and
184 rescued worm expulsion
185 In order to investigate whether the impaired resistance to infection in the absence of B
186 cells was due to the reduced Th2 immune response or loss of parasite specific antibodies, B
187 cell depleted mice were injected with anti-IFN-γ. Anti-IFN-γ treatment is a common strategy
188 to promote resistance in susceptible mice (39). The experimental design for anti-IFN-γ
189 injection plus B cell depletion is shown in Fig. 5A. As shown in Fig. 5B, anti-IFN-γ treatment
190 restored resistance to *T. muris* in B cell depleted mice.
191 As expected, blocking IFN-γ in the absence of B cells did not rescue the *T. muris*
192 specific IgG1 response (Fig. 5C). T. muris specific IgG2c antibodies were not detected in the
193 sera of anti-IFN-γ treated mice with intact B cells (Fig. 5D), consistent with a highly
194 polarised Th2 immune response. In contrast, the isotype control mice produced both IgG1
195 and IgG2c antibodies (Fig. 5C&D). Consistent with our previous data, mice treated with anti-
196 CD20 mAb produced significantly lower Th2 cytokines, such as, IL-13, IL-4, IL-5 and IL-9
197 compared to isotype control treated mice (Fig. 5D-G) with the Th1 response unaffected (data
198 not shown). Importantly, blocking IFN-γ significantly increased IL-13 production in mice
199 depleted of B cells (Fig. 5D), although levels were still significantly lower than in isotype
200 control treated mice (Fig. 5D). Although no significant difference was noted in the
201 production of other Th2 type cytokines, there were trends towards increased *T. muris* specific

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202 IL-4, IL-5, and IL-9 after anti-IFN-γ treatment in B cell depleted mice compared to mice
203 depleted of B cells but not IFN-γ (Fig. 5F-H). Taken together, these results show that in the
204 absence of IFN-γ, B cells and IgG1 parasite specific antibodies are not important for *T. muris*
205 expulsion on a C57BL/6 genetic background; thus without any competing IFN-γ partial
206 restoration of the Th2 immune response is sufficient to render animals resistant to infection

207 Consistent with data in Fig 4, B cell depletion reduced the T_{FH} population in MLNs
208 (Fig. 5I&J). T_{FH} cells have been associated with Th2 immunity due to their ability to secrete
209 IL-4, a Th2 signature cytokine (33, 40, 41). Given that IL-13 production persisted in B cell
210 depleted mice after anti-IFN-γ injection, we wondered whether the T_{FH} population was also
211 altered. Anti-IFN-γ treatment did not restore the T_{FH} population in MLNs of anti-CD20
212 treated mice at day 21 p.i. (Fig. 5I&J), however it remains a possibility that the few TfH
213 remaining were sufficient to induce the partial Th2 response.

214

215 2.6 B cells are essential in supporting Th2 immune responses against *T. muris* only in an IFN-
216 γ-rich environment.

217 In the current study we found that when C57BL/6 mice were depleted of IFN-γ, the
218 immune response became more highly polarised towards Th2 mice, evidenced by parasite-
219 specific IgG1 antibodies, in the absence of IgG2c. In this more polarised Th2 environment
220 the depletion of B cells did not prevent worm expulsion. These data suggest that the
221 important role played by B cells in supporting Th2 immune responses against *T. muris* is only
222 necessary in IFN-γ-rich environments. Therefore, we decided to deplete B cells in BALB/c
223 mice, which are naturally very resistant to *T. muris* and mount highly polarised Th2 immune
224 responses in the absence of IgG2a/c and IFN-γ (31). The experimental design is shown in
225 Fig. 6A. CD19+ cells in MLNs, spleen, and PerC of BALB/c mice were not detected after
226 anti-CD20 mAb injection (Fig. 6B). In complete contrast to B cell depleted C57BL/6 mice,

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227 BALB/c mice were still able to expel the parasite in the absence of B cells (Fig. 6C). IgG1
228 and IgG2c parasite specific antibodies were undetectable in B cell depleted mice (Fig.
229 6D&E), and levels of *T. muris* specific IgM were significantly lower than in the sera of
230 isotype control treated mice (Fig. 6F). As shown previously (31, 39), isotype control treated
231 mice on BALB/c genetic background mounted a strong parasite specific IgG1 response but
232 did not secrete *T. muris*-specific IgG2a/c (Fig. 3K), indicative of a highly polarised Th2
233 immune response.

234 Collectively, our data reveals that the important role played by the B cell in promoting
235 resistance to *T. muris* infection in mixed Th1/Th2 cytokine settings is in supporting the
236 development and maintenance of the Th2 immune response and is not related to antibody
237 production. Importantly, the essential role played by the B cell in the protective immune
238 response varied with genetic background and the degree of Th cell polarisation of the host.
239 Thus, if IFN-γ is depleted from mixed Th1/Th2 settings, or the Th2 immune response is
240 dominant, the B cell becomes redundant in the protective immune response.

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242 **Discussion**

243 *T. muris* infection drives different Th cell responses in different strains of mice (34).
244 BALB/c, BALB/k, and NIH mice are very resistant to *T. muris* infection, expelling the
245 worms by around day 18 post infection, while AKR mice are susceptible to infection, unable
246 to expel the parasite and harbour patent chronic infections (42). C57BL6 and C57BL10 mice
247 are also resistant to infection, but they expel the worms more slowly, between day 18 and 35
248 p.i. (43). Susceptible hosts mount predominantly Th-1 immune responses associated with the
249 presence of IFN-γ and IL-12, whilst very resistant host strains mount strong Th-2 type
250 polarised immune response characterised by cytokines such as IL-4, IL-5, IL-9 and IL-13,
251 and very low levels of IFN-γ. The quality of the Th cell response in C57BL6 mice is less
252 clearly polarised with the slower expulsion kinetic associated with a mixed Th1/Th2
253 phenotype and presence of IgG1 and IgG2c. Despite a good understanding of the Th cell
254 response during *T. muris* infection, the key cellular contributions which support T helper
255 polarization are still not well understood, nor how genetic background impinges on this.

256 This study aimed to investigate the role of B cells in immunity to *T. muris* during
257 primary infection by using anti-CD20 mAb to deplete B cells. In order to understand whether
258 the contribution of the B cell varied with the nature of the protective immune response, we
259 used mice of two different genetic backgrounds: C57BL/6 and BALB/c. CD20 is specifically
260 expressed on the surface of B cells from the pre-B cells stage to immature B cells, but then
261 disappear when B cells differentiate to plasma cells (28). Adding antibody against CD20
262 inhibits the progression of B cells from the G1 phase into S/G2+ M stages (44), resulting in
263 the inhibition of B cell differentiation, antibody production, and inducing B cell apoptosis
264 (45). Using anti-CD20 mediated depletion of B cells, antigen specific class switched
265 antibodies are ablated and IgM antibodies are significantly reduced (41, 46). These finding

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266 are in keeping with the finding of current study and enable an assessment of the importance
267 of parasite-specific IgG1 antibodies in the expulsion of *T. muris*.
268 Our data reveal that antibody is not essential for the expulsion of *T. muris*, with both
269 B cell depleted BALB/c mice and B cell depleted C57BL/6 mice also depleted of IFN-γ, able
270 to clear infection. These data also exemplify that the mechanism of immunity to *T. muris* in
271 BALB/c mice is entirely B cell independent. However, our data do identify an important and
272 significant antibody-independent role for the B cell in promoting immunity to infection in
273 mouse strains, such as C57BL/6, which are not highly polarised towards a Th2 immune
274 response. Thus, the importance of the B cell in resistance to infection is dependent on genetic
275 background, and represents a key consideration when interpreting data from other
276 experimental models. Further, given that protective immunity was lost when B cells were
277 depleted both throughout the time course of infection and only after the first two weeks of
278 infection, our data suggest that the role played by the B cell is in supporting both the
279 development and maintenance of Th2 immune responses.
280 Mechanistically, B cell depletion correlated with a significant reduction in
281 CD4+CXCR5+PD-1^{high} cells. CD4+CXCR5+PD-1^{high} cells are recognised as T_{FH} and are
282 known to be able to produce the Th2-type signature cytokine; IL-4 (47). Thus, these data are
283 consistent with previous studies showing that B cells are important in regulating Th2 immune
284 responses (10, 14, 15, 48-51) and maintaining T_{FH} development (15, 52). Interestingly, T_{FH}
285 populations in both MLN and spleen of BALB/c mice were not affected after B cell
286 depletion. Further, the presence of T_{FH} has been associated with Th2-type immune responses
287 in *H. polygyrus* infection (15). Although in the current study, depletion of B cells in mice also
288 treated with anti- IFN-γ restored expulsion of *T. muris* without rescuing the T_{FH} response, the
289 few T_{FH} cells remaining may be sufficient to support the partially restored Th2 response.

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290 Thus it remains possible that T_{FH} cells are essential in building strong Th2 responses in the
291 absence of IFN-γ.

292 The importance of B cells in immunity to *T. muris* has previously been proposed
293 using µMT mice (22) on a C57BL/6 background which are susceptible to infection unless
294 Th1 responses are inhibited using anti-IL-12. These data are keeping with the current study
295 supporting a role for B cells as accessory cells promoting and maintaining Th2 responses
296 rather than antibody producers. Antibody independent expulsion of *T. muris* is also evidenced
297 by the fact that primed CD4+ T cell transfer to SCID mice is sufficient to support worm
298 expulsion in the absence of antibody (18).

299 More broadly, the role of B cells in immunity to gastro intestinal nematodes in
300 general has been debated at length (53-56). Proposed mechanisms include production of
301 antibody (57), promoting and maintaining of primary and memory Th2 cells (10, 14) via their
302 ability to produce cytokines, especially IL-4 (11, 53, 58), and/or by expressing co-stimulatory
303 molecules, including OX40L and CD40 (13, 59). A variety of experimental approaches have
304 been used including the use of transgenic B cell deficient mice (10, 22, 57, 60), FcγR
305 deficiency (61, 62), passive immunization (22, 63, 64) and maternal antibody transfer (54,
306 65); and a variety of conclusions have been drawn. IgG1 antibodies were shown to be
307 essential for worm expulsion against *H. polygyrus* infection based on previous studies using
308 AID mice, which retain a secretory IgM response, on C57BL/6 genetic background (57).
309 Antibody was shown to be essential for worm expulsion against *H. polygyrus* infection via
310 antibody-dependent cell-mediated cytotoxic (ADCC) mechanisms (66). However, ADCC
311 mechanisms do not play a critical role in immunity to *T. muris* infection as FcγR deficiency
312 mice are still able to expel the parasite (62).

313 More recently, two studies have strongly suggested a role for B cells in directing T
314 cell polarisation towards Th2 at the time of antigen presentation (15, 67). Leon *et al.*, showed

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315 that B cell depletion in *H. polygyrus*-infected C57BL/6 mice prevented the migration of DCs
316 into B cell areas and impaired the Th2 immune response (15). Lymphotoxin, produced by B
317 cells, is important in the control of CXCL13 expression (15), a chemokine expressed by
318 follicular dendritic cells (68) and marginal reticular cells in the peri and interfollicular regions
319 between B cell follicles (69) and that is essential to attract CXCR5-expressing cells towards
320 the B cell area (15). Thus by treating C57BL/6 mice with anti-CXCL13, Leon *et al.,* (15)
321 also revealed that CD4 T cells and DCs accumulated in the T cell area rather than in
322 perifollicular region and that IL-4+ Th2 cells in mesenteric lymph nodes (MLNs) were
323 significantly reduced. In support of a mechanism involving the movement of DCs and CD4 T
324 cells to the B cell area to allow CD4 T cells to be educated by B cells towards a Th2, recent
325 studies have revealed that transgenic mice in which CXCR5 depletion was specifically
326 restricted to DCs become susceptible to *T. muris* with very few DCs detected in the B cell
327 area compared to control mice (67).

328 In addition to the proposed role for B cells in educating the DC-CD4 T cell
329 conversation suggested by Leon *et al.,* (15) and Bradford *et al.,* (67), the importance of
330 CD11b+ DCs in MLNs for Th2 polarisation has been shown in previous studies, against both
331 *T. muris* (37) and *S. mansoni* (70) infection. In this context, in the current study we observed
332 reduced numbers of CD11b+ DCs in B cell depleted C57BL/6 mice and this may contribute
333 to the reduced Th2 immune response and increased susceptibility of anti-CD20 treated mice
334 to *T. muris* infection. Although previous studies have indicated the importance of B cells in
335 regulating the development of DC subsets in secondary lymphoid organs (50, 71, 72), further
336 studies are required to investigate how the absence of B cells reduced the number of CD11b+
337 DCs in the MLN.

338 Overall, we present a study showing that, irrespective of host genetic background,
339 antibody is not essential in the expulsion of *T. muris*. However, our data do identify an

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340 essential role played by the B cell on certain genetic backgrounds where IFN-γ rich, mixed
341 Th1/Th2 cell responses are observed, and strongly suggest that the B cell is able to adopt
342 multiple functions in inducing and maintaining protective Th2 responses during gastro-
343 intestinal nematode infection.
344

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345 **Material and Methods**

346 1. Animals

347 C57BL/6 and BALB/c mice were purchased from Envigo, UK and were maintained
348 in ventilated cages in the Biological Services Facilities (BSF) of the University of
349 Manchester. Mice were housed in the facility at least 7 days prior to experimentation and
350 were infected at 6-8 weeks old with *T. muris* by oral gavage.

351

352 2. B cells depletion and infection

353 To assess the importance of B cells for worm expulsion in different strains, C57BL/6
354 and BALB/c mice were split into 2 groups of 4-5 mice: anti-CD20 mAb and isotype control
355 treated mice. C57BL/6 or BALB/c mice were treated with 100µg in 200µl PBS of anti-CD20
356 mAb (5D2, Genentech) or isotype control (Rat IgG2a, Biolegend) i.v. injection via tail vein.
357 Mice were infected with approximately 200 T. muris eggs at day 7 post injection. Mice were
358 re-injected with anti-CD20 mab or isotype control at day 10 p.i. C57BL/6 mice were
359 autopsied at day 35 p.i., whilst BALB/c mice were autopsied at day 42 p.i. For anti-IFN-γ,
360 C57BL/6 mice were split into 4 groups of 5 mice: isotype control of anti-CD20 mAb +
361 isotype control of anti-IFN-γ mAb (rat Ig), anti-CD20 mAb + isotype control of anti-IFN-γ
362 mAb, isotype control of anti-CD20 mAb + anti-IFN-γ mAb, anti-CD20 mAb + anti-IFN-γ
363 mAb. C57BL/6 mice were treated with 100µg in 200µl PBS of anti-CD20 mAb (5D2,
364 Genentech) or isotype control (Rat IgG2a, Biolegend) i.v. injection via tail vein. Mice were
365 infected by oral gavage with approximately 200 *T. muris* eggs at day 7 post injection. Mice
366 were re-injected with anti-CD20 mab or isotype control at day 10 p.i. 1 mg of α-IFN-γ
367 antibody or rat IgG1 (as a control) antibody was given at day 0, day 7, and day 14 p.i. Mice
368 were euthanised by CO2 followed by autopsy at day 21 or day 35 p.i..

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369 3. *Trichuris muris* maintenance and the preparation of E/S proteins

370 All protocols to maintain the parasite and to prepare the E/S were as previously
371 described (73). Briefly, the parasite was passaged through SCID mice that are susceptible to
372 *T. muris* infection. SCIDs received a high dose of approximately 300 *T.muris* embryonated
373 eggs and at approximately day 35 p.i. the large intestine was collected to produce adult E/S.
374 Guts were collected and then longitudinally split open before washed in warmed 5x
375 penicillin/streptomycin in complete RMPI 1640 medium (500U/ml penicillin, 500µg/ml
376 streptomycin, 500ml RMPI 1640 medium). Adult worms were then carefully pulled from the
377 gut using fine forceps and transferred to a 6 well plate containing 4ml warmed 5x pen/strep in
378 RMPI 1640 medium. Plates were incubated in a moist humidity box for 4 hours at 37°C to
379 collect 4 hr E/S. For overnight E/S, adult worms were then split into 2 wells containing fresh
380 medium and incubated again in a humidity box at 37°C overnight. Supernatant from 4 hour
381 and 24 hour incubations was collected and centrifuged at 2000g for 15 minutes. *T. muris* eggs
382 from adult worms were resuspended in 40ml deionised water and filtered through a 100µm
383 nylon sieve before transferring to a cell culture flask. To allow embryonation, eggs were
384 stored in darkness for approximately 8 weeks and then stored at 4°C. SCID mice were
385 subsequently infected with a high dose infection to determine infectivity of each new batch of
386 eggs. Thus, larvae were counted at around d14 p.i. and the number of larvae counted
387 expressed as a % over the number of eggs given to determine the infectivity of the egg batch.
388 All E/S supernatant was filter sterilised through a 0.2µm syringe filter (Merk). E/S was
389 concentrated using an Amicon Ultra-15 centrifugal filter unit (Millipore) by spinning at
390 3000g for 15 minutes at 4°C. E/S was dialysed against PBS using Slide-A-Lyzer Dialysis
391 Cassettes, 3.500 MWCO (Thermo Science) at 4°C. The concentration of E/S was measured
392 using the Nanodrop 1000 spectrophotometer (Thermo Fisher Science) and aliquoted before
393 storing at -80°C.

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394 For high dose *Trichuris muris* infection, approximately 3-4 ml of egg suspension was
395 transferred to a universal tube and topped up with deionised water before centrifuging for 15
396 minutes at 2000g. Pelleted eggs were washed with deionised water, resuspended and only
397 embryonated eggs were counted. Eggs were concentrated or diluted with deionised water,
398 depending on the egg count. For example, eggs with 100% infectivity, approximately 50 eggs
399 per 50µl were counted. Mice were then infected with 200µl by oral gavage to infect mice
400 with approximately 200 eggs.
401
402 4. Worm burden of *Trichuris muris*
403 During autopsy, the caecum and proximal colon were collected and stored at -20°C
404 before analysis. Before worm count, the intestine was thawed at room temperature and cut
405 longitudinally using blunt ended scissors and the epithelium was scraped using curved
406 forceps in a petri dish. Worms were counted blindly under a dissecting microscope (Leica).
407
408 5. Preparation of single cell suspensions for fluorescence activated cell sorting (FACS)
409 Mesenteric lymph nodes (MLNs), spleen, blood and bone marrow were collected and
410 prepared for FACS staining. Lymph nodes and spleen were squeezed through a 70µm nylon
411 cell strainer (Fisher Scientific) and cells were pelleted by centrifugation at 1500 rpm for 5
412 minutes. The supernatant was removed and the pelleted cells were resuspended in 500µl to 1
413 ml of Red Blood Cell Lysing Buffer Hybri-Max™ (Sigma-Aldrich) for 30 seconds to 1
414 minute before adding 10 ml 1xPBS. Cells were pelleted by centrifugation at 1500 rpm for 5
415 minutes and resuspended in 1 ml of complete RPMI 1640 medium. Cells were counted on a
416 CASY cell counter (Scharfe System).
417 Approximately 50µl of blood were placed into 1.5 ml eppendorf tube containing 50µl
418 0.5M EDTA and stored on ice before analysis. 500 µl of Red Blood Cell Lysing Buffer

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419 Hybri-Max™ (Sigma-Aldrich) were added and samples were incubated for 5 minutes at room
420 temperature. 1 ml of 1xPBS were added and cells were pelleted by centrifugation at 1500 rpm
421 for 5 minutes. Red blood cell lysis process was repeated twice before cells were resuspended
422 in 1 ml of complete RPMI 1640 medium.

423 Femurs and tibias were collected at autopsy and after removing any remaining tissue
424 without damaging the bone integrity, the bone was placed on ice until ready to process. Bone
425 was transferred to 70% ethanol for 2-3 minutes and then rinse in 3 changes of 1xPBS. In a
426 petri dish, both ends of bone were cut and the bone was flushed gently with 1xPBS using a
427 3cc syringe and a 23 ga needle. To break up the clumps, the marrow was sucked up and was
428 gently pushed back. Single cells suspension was filtered through 100μm nylon cell strainer
429 (Fisher Scientific) and cells were pelleted by centrifugation at 1500 rpm for 5 minutes and
430 resuspended in 1 ml of 1xPBS. The supernatant was removed and the pelleted cells were
431 resuspended in 500μl of Red Blood Cell Lysing Buffer Hybri-Max™ (Sigma-Aldrich) for 1
432 minute before adding 10 ml 1xPBS. Cells were pelleted by centrifugation at 1500 rpm for 5
433 minutes and resuspended in 1 ml of complete RPMI 1640 medium.

434

435 6. Cell surface markers

436 Cells from MLNs, spleen, blood and bone marrow were stained for live dead (Zombie
437 UV, Biolegend) and Fc block (eBiosciences) prior to cell surface cellular markers staining.
438 Samples were read on a BD LSR Fortessa flow cytometer (BD Biosciences) and data was
439 analysed using FlowJo X (Tree Star, Inc).

440 Cell surface markers: anti-B220 (RA3-6B2); anti-CD19 (6D5); anti-CD3ε (17A2);
441 anti-CD4 (RM4.5); anti-CD8α (53-6.7); anti-CD279 (PD-1) (29F.1A12); anti-
442 CD185/CXCR5 (L138D7); anti-CD93 (AA4.1); anti-CD25 (3C7); and anti-CD117/c-kit
443 (2B8) were purchased from Biolegend. Anti-CD43 (S11); anti-CD103 (2E7); anti-CD11c

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444 (N418); anti-CD317/PDCA-1 (927); anti CD11b (M1/70); anti-CD64 (X54-5/7.1); anti-I-A/I-
445 E (M5/114.15.2); and anti-CD23 (B3B4) were purchased from BD Biosciences. Anti-CD45
446 (30-F11); anti-ly6G (RB6-8C5); anti-NK.1 (PK136); anti-Ter119 (Ter-119) purchased from
447 eBiosciences.
448
449 7. Quantification of parasite specific IgG1, IgG2a/c and IgM
450 To detect IgG1, IgG2a/c and IgM specific *T. muris*, an enzyme linked immunosorbant
451 assay (ELISA) was completed. Blood was collected from mice at autopsy and serum was
452 isolated by centrifuging samples for 10 minutes at 15,000g at room temperature. 96 well
453 immunoGrade plates (BrandTech Scientific, Inc) were coated with 5µg/ml *T. muris* diluted in
454 0.05M carbonate/bicarbonate buffer and incubated on plates overnight at 4°C. Plates were
455 washed using a Skatron Scan Washer 500 (Molecular Devices, Norway) 3 times with 0.05%
456 Tween 20 (Sigma) in PBS (PBS-T). Non-specific binding were blocked with 100µl 3%
457 bovine serum albumin (BSA) (Melford Laboratories)/ PBS at 37°C for 45 minutes. Plates
458 were washed 3 times with PBS-T and 50µl of double diluted serum in PBS (1:20, 1:40, 1:80,
459 1:160, 1:320, 1:640, 1:1280, 1:2560) was added to plates and incubated for 60 minutes. Plates
460 were then washed 3 times with PBS-T and 50µl of either biotinylated rat anti-mouse IgG1
461 (1:500, BD Bioscience), rat anti-mouse IgG2a (1:1000, BD Bioscience) or rat anti-mouse
462 IgM (1:500, BD Bioscience) was added to wells and incubated for 60 minutes. C57BL/6 mice
463 do not make IgG2a, but IgG2c. However, the rat anti-mouse IgG2a antibody also recognises
464 mouse IgG2c, the IgG2a equivalent. Plates were then washed with PBS-T 3 times before the
465 plates were incubated with 75µl Streptavidin peroxidase (1:1000, Sigma) for 60 minutes.
466 Plates were washed 3 times with PBS-T before the colour were developed using 100µl 0.03%
467 hydrogen peroxidase activated ABTS (10% 2,2'azino 3-thyl benzthiazoline in 0.045M citrate
468 buffer). Plates were read at 450nm on a VersaMax Microplate reader (Molecular Devices).

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469 8. Cytokine analysis

470 During autopsy, mesenteric lymph nodes were isolated and collected in complete
471 RPMI 1640 medium. The tissue were squeezed through a 70µm nylon cell strainer (Fisher
472 Scientific) and cells were pelleted by centrifugation at 1500 rpm for 5 minutes. The
473 supernatant was removed and the pelleted cells were resuspended in 500µl to 1 ml of Red
474 Blood Cell Lysing Buffer Hybri-Max™ (Sigma-Aldrich) for 30 seconds to 1 minute before
475 adding 10 ml 1xPBS. Cells were pelleted by centrifugation at 1500 rpm for 5 minutes and
476 resuspended in 1 ml of complete RPMI 1640 medium. Cells were counted on a CASY cell
477 counter (Scharfe System) and then diluted to a concentration of $1x10^7$ cells/ml. 100µl of cells
478 were plated out into a 96 well flat bottom plate. Cells were restimulated with 100µl 4 hr *T.*
479 *muris* E/S at 100µg/ml (to give a final concentration of E/S in the cell culture of 50µg/ml).
480 Cells were incubated for 48 hours at 37°C and 5% CO2. To collect the supernatant, plates
481 were centrifuged at 1400g for 5 minutes and the supernatant was stored at -20°C.

482 The cytokines IL-4, IL-5, IL-6, IL-9, IL-10, IL-17, IL-13, TNF and IFN-γ were
483 detected in supernatant by Cytometric bead assay (CBA). 12.5µl of supernatant from *T. muris*
484 E/S stimulated cells was added to a 96-well round bottom plate. A capture bead cocktail (BD
485 Bioscience), containing beads for each cytokine, was diluted in capture diluent (BD
486 Bioscience) and 12.5µl was added to each well before incubating on a rocker at room
487 temperature for 1 hour. 12.5µl of detection beads (BD Bioscience), diluted in detection
488 reagent (BD Bioscience) was added to each well and incubated again on a rocker at room
489 temperature for 1 hour. Plates were washed and resuspended in 70µl wash buffer (BD
490 Bioscience). Cytokines were measured on a MACSQuant Analyser (Miltenyi Biotec) and
491 analysed using the FCAP array software in reference to a standard curve.

492

493

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494 9. Statistical analysis

495 Statistical analysis was performed using Prism4 (Graph-Pad software Inc., La Jolla,
496 CA). The significant differences between two groups ($P<0.05$) were analysed with the t-test
497 or Mann Whitney test, depend on the n-size and the distribution of samples. For multiple
498 groups, the significant differences were analysed by Anova.

499

500 10. Ethics statement

501 All experiments were approved by The University of Manchester Local Ethical
502 Review Committee and were performed in accordance with the UK Home Office Animals
503 (Scientific Procedures) Act 1986, under the home Office project licence number 70/8127

504

505 **Acknowledgment**

506 This study was funded by the Indonesian endowment fund for education. We would like to
507 thank Rebecca Dookie for helping us with i.v. injection, Genentech (San Francisco, CA) for
508 their gift of purified anti-CD20 mAb (clone-5D2), and Gareth Howell from Flow cytometry
509 facility at the University of Manchester.

510

511 **Conflict of interests**

512 The authors declare no commercial or financial conflict of interest

513

514

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517 **Figure legend**

518

519 **Fig. 1. Two injections of anti CD20-mab are required to ensure B cells are depleted**
520 **occurred throughout infection.** C57BL/6 mice were treated with anti CD20 mAb or Rat
521 IgG2a isotype control at 100 µg in 200 µl PBS by i.v. injection via tail vein. Mice were
522 infected with approximately 150 *T. muris* eggs at day 7 post injection. Some mice were only
523 injected once with anti CD20/ rat IgG2a isotype control, whilst some mice were re-injected
524 i.v. with anti CD20 mAb or Rat IgG2a at 100 µg in 200 µl PBS on day 10 p.i. Mice were
525 autopsied at day 35 p.i. (A&B) CD19+ cells were assessed in MLNs, spleen, blood,
526 peritoneal cavity (PerC), and bone marrow (BM) using flow cytometry. (C) Representative
527 flow cytometric analysis of CD19+ cells in BM of anti-CD20 treated mice. Both immature
528 and mature B cells are IgM+, whilst pro/pre-B cells are IgM-. Pro-B cells are defined as
529 CD19+B220^{low}IgM-CD93+CD43+CD25-ckit+. Data shown is mean ± SEM, representative
530 of 2 independent experiments for Rat IgG2a and α-CD20 2x, from 1 experiment for α-CD20
531 1x, n=5, males. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Anova.

532

533 **Fig. 2. B cell depleted C57BL6 mice were susceptible to *T. muris*, correlating with an**
534 **absence of class switched antibodies and a significant decrease in Th2 cytokines.**
535 C57BL/6 mice were treated with anti CD20 mAb or Rat IgG2a isotype control at 100 µg in
536 200 µl PBS by i.v. injection via tail vein prior to infection. Mice were infected with
537 approximately 150 *T. muris* eggs. Mice were autopsied at day 35 p.i.. (A) Diagram of
538 experimental design. (B) Worm burdens were assessed blindly after autopsy. (C-E) Sera were
539 analysed using ELISA for parasite specific antibodies. (F-J) MLN cells were re-stimulated
540 with parasite E/S antigen for 48 hours and cytokines in supernatants were determined using
541 cytokine bead array (CBA). Data shows mean ± SEM, pooled from 3 independent

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542 experiments (B&F-K), representative of 3 independent experiments (C-E), males, *p<0.05,
543 **p<0.01 ***p<0.001, Student's t-test.
544
545 **Fig. 3. B cell depletion alters the T_{FH} population and DC subsets in MLNs.** C57BL/6 mice
546 were treated with anti CD20 mAb or Rat IgG2a isotype control at 100 µg in 200 µl PBS by
547 i.v. injection via the tail vein. Mice were infected with approximately 150 *T. muris* eggs at
548 day 7 post injection. Mice were re-injected with anti CD20 mAb or isotype control at 100 µg
549 in 200 µl PBS by i.v. injection via the tail vein at day 10 p.i.. Mice were autopsied on day 0,
550 day 10 and day 35 p.i. for T_{FH} and on day 35 p.i. for DC subsets. (A) Gating on
551 CD4+CXCR5+PD-1^{high} to define T_{FH} cells in MLNs. (B&C) Relative % and total cell
552 number of T_{FH} in MLNs, respectively. (D-H) DC subsets in MLNs. (E&F) Relative
553 percentage and total cell number of CD103-CD11b+ DCs, respectively. (G&H) Relative
554 percentage and total cell number of CD103+CD11b+ DCs, respectively. Data shows mean ±
555 SEM, from 1 experiment, n=3-5, males, *p<0.05, **p<0.01, Mann Whitney test.
556
557 **Fig. 4. B cell depletion from day 14 p.i. also leads to susceptibility to *T. muris* infection.**
558 C57BL/6 were treated with anti CD20 mAb or Rat IgG2a isotype control at 100 µg in 200 µl
559 PBS by i.v. injection via the tail vein 2 weeks post infection (p.i.). Mice were re-injected with
560 anti CD20 mAb or isotype control at 100 µg in 200 µl PBS by i.v. injection via the tail vein
561 on day 24 p.i. Mice were autopsied on day 35 p.i. (A) Diagram of the experiment design for
562 B cells depletion 2 weeks post-infection. (B) Worm burdens were assessed blindly after
563 autopsy. (C) Gating on CD4+CXCR5+PD-1^{high} to define T_{FH} cells in MLNs. (D&E) Relative
564 % and total cell number of T_{FH} in MLNs, respectively. (F-K) MLN cells were re-stimulated
565 with parasite E/S antigen for 48 hours and cytokines in supernatants were determined using

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566 cytokine bead array (CBA). Data show mean ± SEM from 1 experiment, n=5, males,
567 *p<0.05, **p<0.01, Mann Whitney test.
568
569 **Fig. 5. Anti-IFN-γ treatment restored resistance to *T. muris* infection and partially**
570 **rescued the IL-13 response in B cell depleted mice in the absence of *T. muris* specific**
571 **IgG1 antibodies and without preserving T_{FH} population.** C57BL/6 mice were treated with
572 anti CD20 mAb or isotype control at 100 µg in 200 µl PBS by i.v. injection via the tail vein.
573 Mice were infected with approximately 150 *T. muris* eggs at day 7 post injection. Mice were
574 re-injected with anti CD20 mAb or isotype control at 100 µg in 200 µl PBS i.v. injection via
575 tail vein on day 10 p.i.. 1 mg of α-IFN-γ antibody or Rat Ig (as a control) was given on day 0,
576 day 7, and day 14 p.i.. Mice were autopsied on day 21 p.i. and day 35 p.i.. (A) Diagram of the
577 experimental design. (B) Worm burdens were assessed blindly after autopsy at day 35 p.i..
578 (C&D) *T. muris* specific IgG1 and IgG2c antibodies in the sera by day 21 p.i., respectively.
579 (E-H) Cytokine analysis of re-stimulated MLN cells day 21 p.i.. (I) Gating on
580 CD4+CXCR5+PD-1^{high} to define T_{FH} cells in MLNs day 21 p.i.. (J) Total numbers of T_{FH}
581 cells in MLNs day 21 p.i.. Data shows mean ± SEM, from 1 experiment, n=5, males,
582 *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Anova.
583
584 **Fig. 6. BALB/c mice were able to expel *T. muris* in the absence of B cells.** BALB/c mice
585 were treated with anti CD20 mAb or Rat IgG2a isotype control at 100 µg in 200 µl PBS by
586 i.v. injection via tail vein prior to infection. Mice were infected with approximately 150 *T.*
587 *muris* eggs and were autopsied at day 42 p.i.. (A) Diagram of experimental design. (B)
588 CD19+ cells were assessed in MLNs, spleen, and peritoneal cavity (PerC) using flow
589 cytometry. (C) Worm burdens were assessed blindly after autopsy. (D-F) Sera were analysed

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590 using ELISA for parasite specific antibodies. Data shows mean ± SEM, from 1 experiment,

591 n=5, males. *p<0.05, ***p<0.001 ****p<0.0001, Student's t-test.

592

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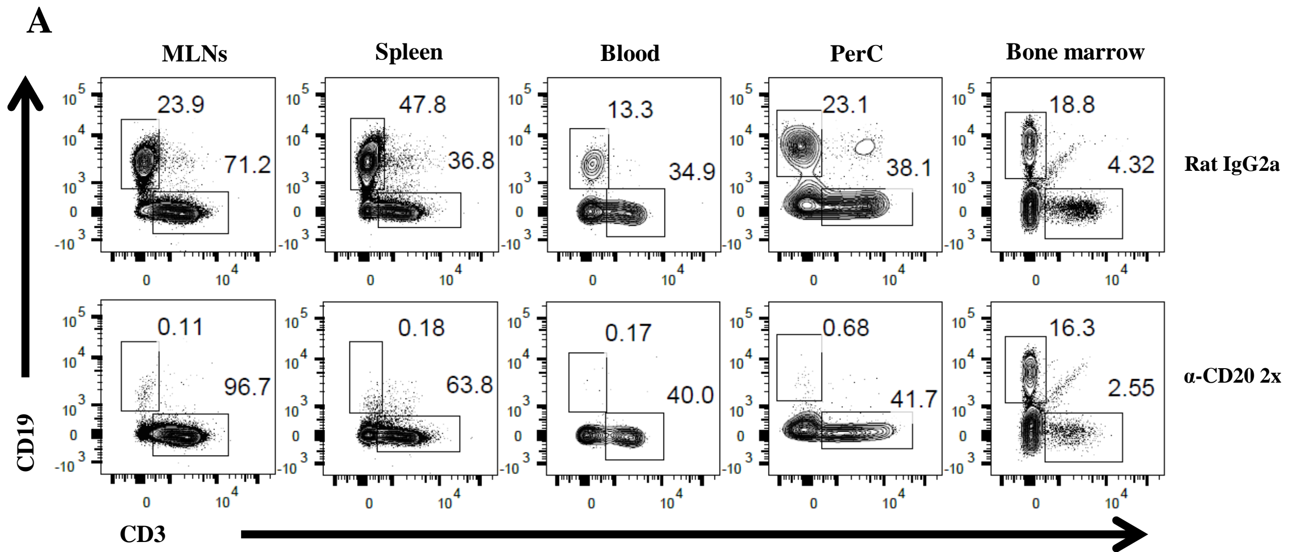
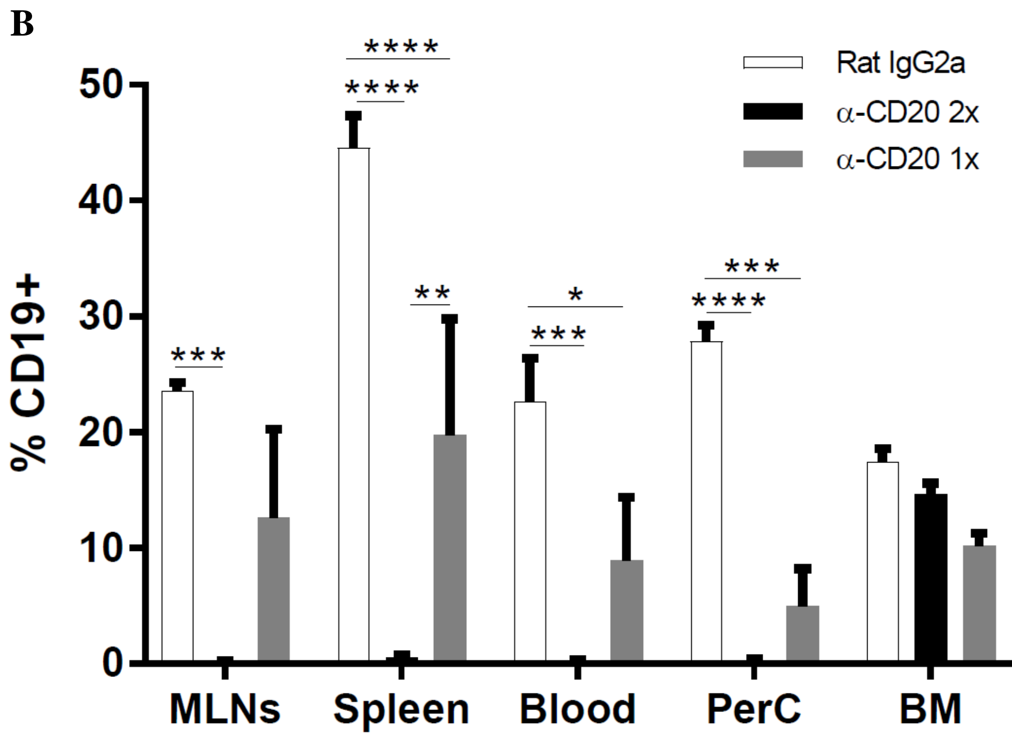
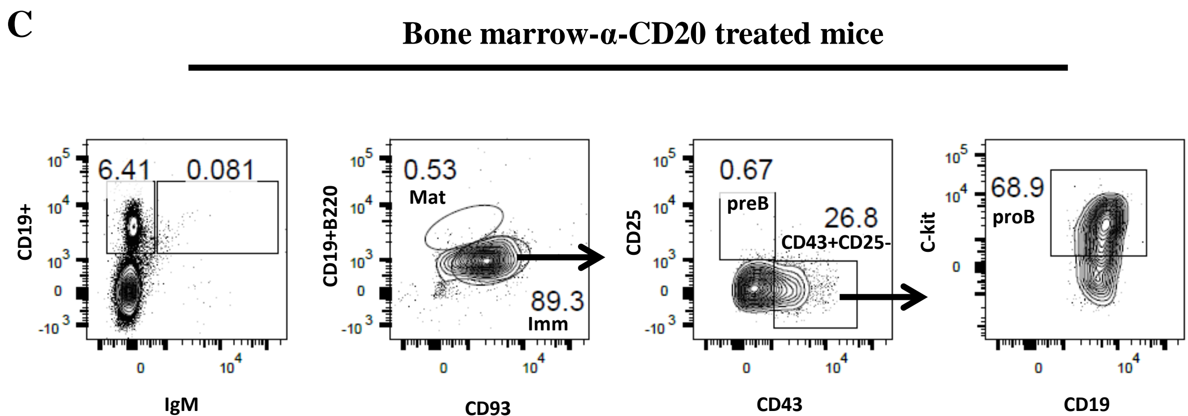
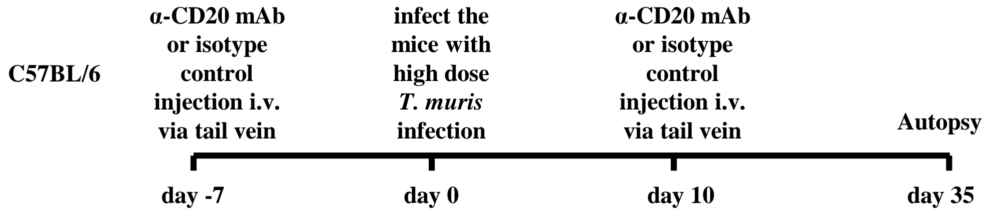

A
MLNs
Spleen
Blood
PerC
Bone marrow
23.9
71.2
47.8
36.8
13.3
34.9
23.1
38.1
18.8
4.32
Rat IgG2a
0.11
96.7
0.18
63.8
0.17
40.0
0.68
41.7
16.3
2.55
α-CD20 2x
CD19
CD3


B
Rat IgG2a
α-CD20 2x
α-CD20 1x
% CD19+
MLNs
Spleen
Blood
PerC
BM


C
Bone marrow-α-CD20 treated mice
6.41
0.081
CD19+
IgM
0.53
Mat
89.3
Imm
CD19+B220
CD93
0.67
preB
26.8
CD43+CD25-
CD25
CD43
68.9
proB
C-kit
CD19

A



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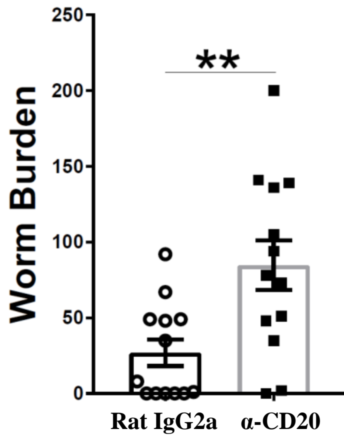

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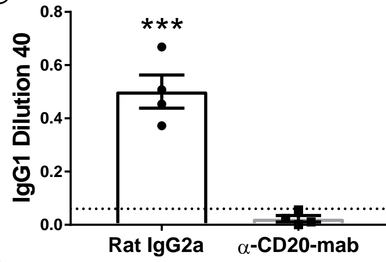

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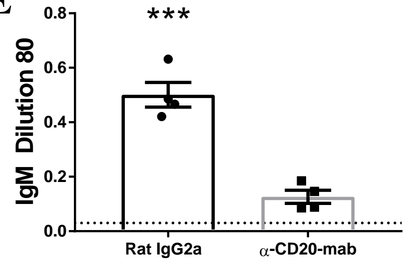

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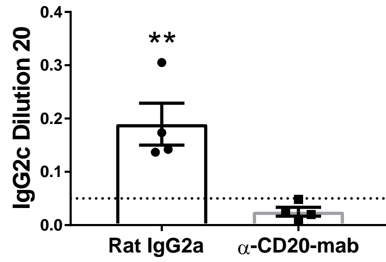
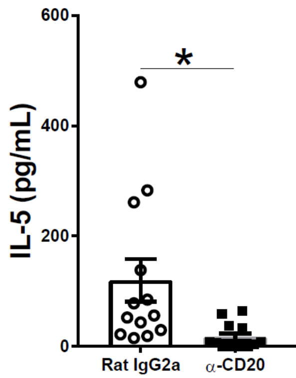


F



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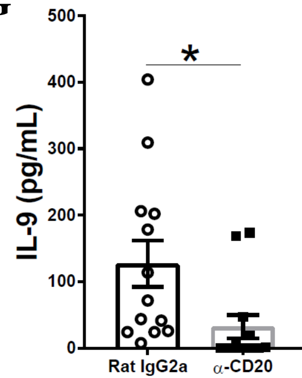

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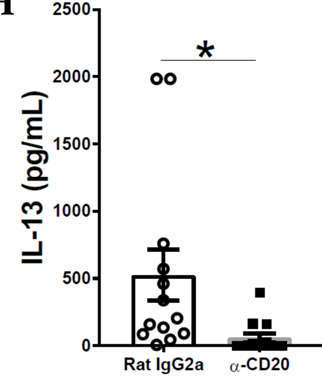

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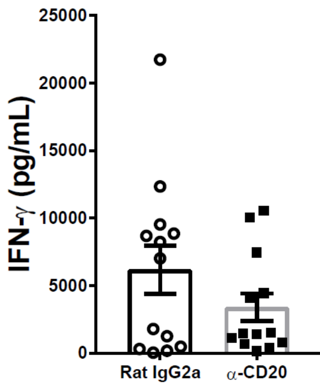

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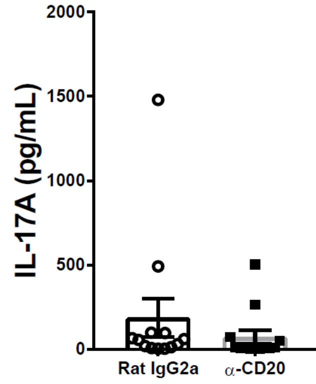
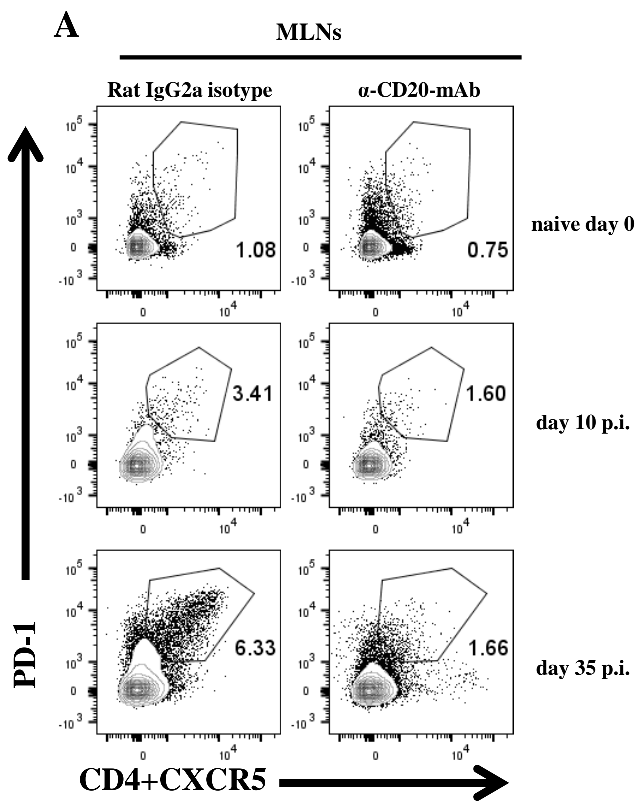
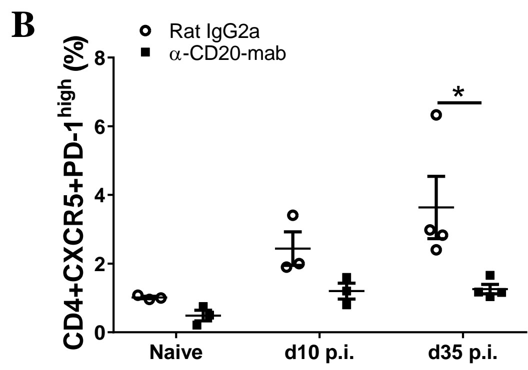
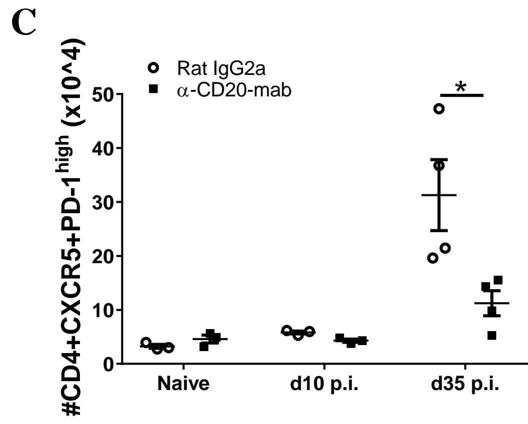
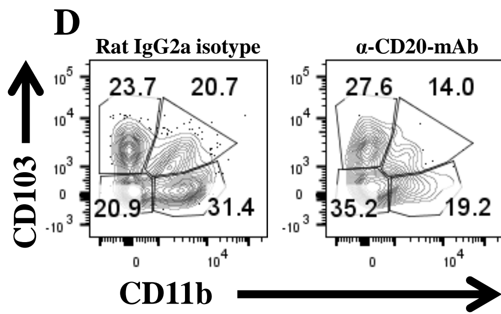
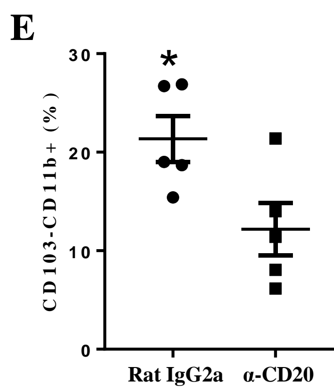


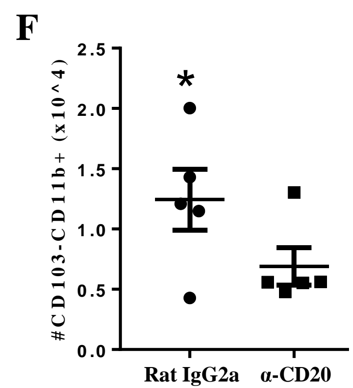

A
MLNs
Rat IgG2a isotype
α-CD20-mAb
1.08
0.75
naive day 0
3.41
1.60
day 10 p.i.
6.33
1.66
day 35 p.i.
PD-1
CD4+CXCR5

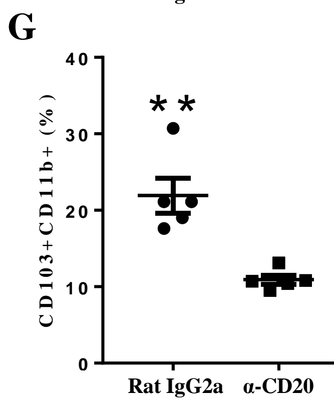

B
Rat IgG2a
α-CD20-mab
CD4+CXCR5+PD-1high (%)
Naive
d10 p.i.
d35 p.i.

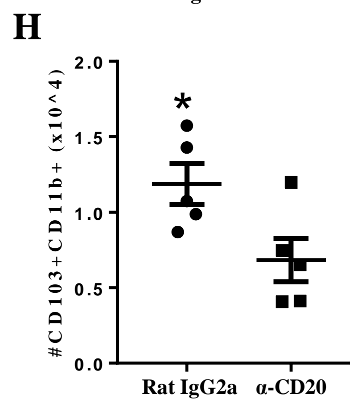

C
Rat IgG2a
α-CD20-mab
#CD4+CXCR5+PD-1high (x10^4)
Naive
d10 p.i.
d35 p.i.

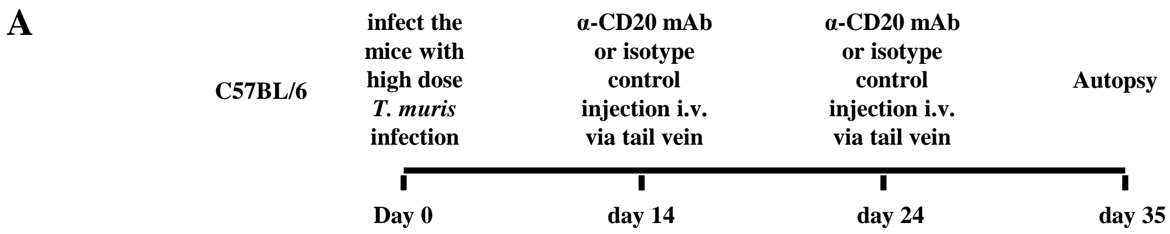

D
Rat IgG2a isotype
α-CD20-mAb
23.7
20.7
20.9
31.4
27.6
14.0
35.2
19.2
CD103
CD11b

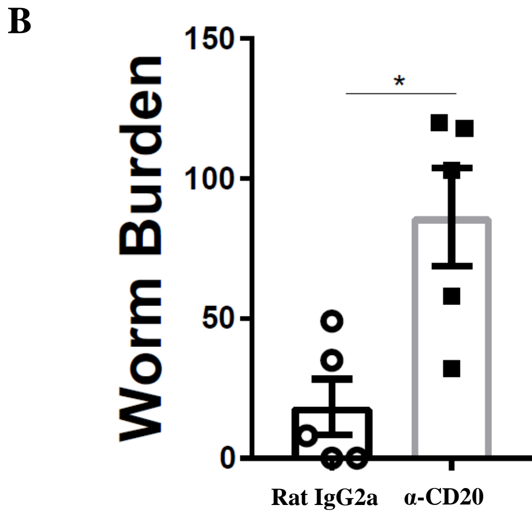

E
CD103-CD11b+ (%)
Rat IgG2a
α-CD20

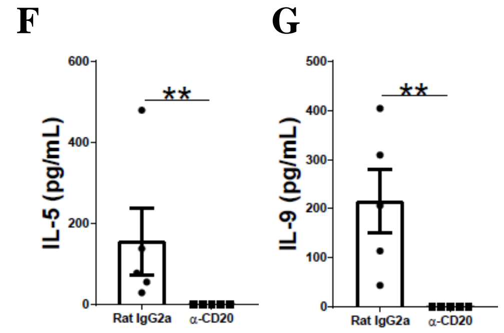

F
#CD103-CD11b+ (x10^4)
Rat IgG2a
α-CD20

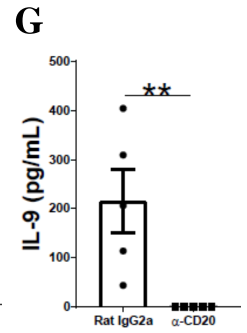

G
CD103+CD11b+ (%)
Rat IgG2a
α-CD20

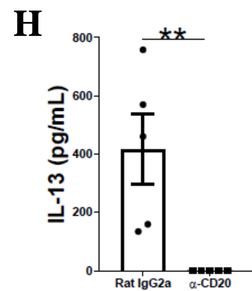

H
#CD103+CD11b+ (x10^4)
Rat IgG2a
α-CD20

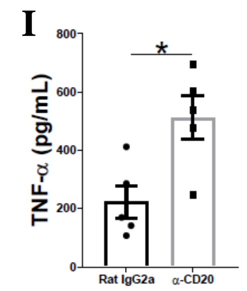

A
C57BL/6
infect the mice with high dose *T. muris* infection
α-CD20 mAb or isotype control injection i.v. via tail vein
α-CD20 mAb or isotype control injection i.v. via tail vein
Autopsy
Day 0
day 14
day 24
day 35

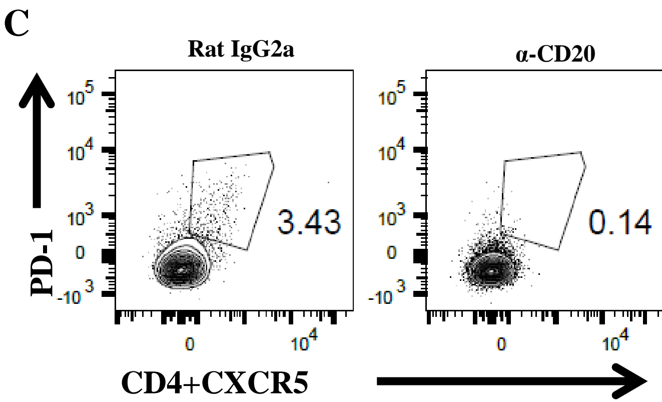

B
Worm Burden
Rat IgG2a
α-CD20

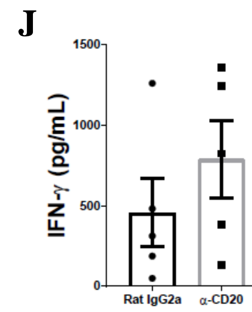

F
IL-5 (pg/mL)
Rat IgG2a
α-CD20

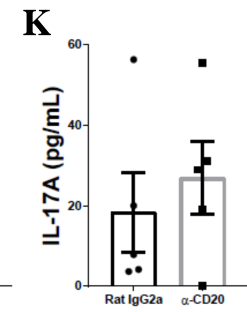

G
IL-9 (pg/mL)
Rat IgG2a
α-CD20

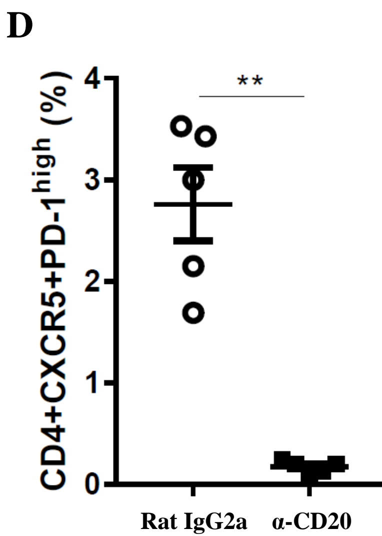

H
IL-13 (pg/mL)
Rat IgG2a
α-CD20

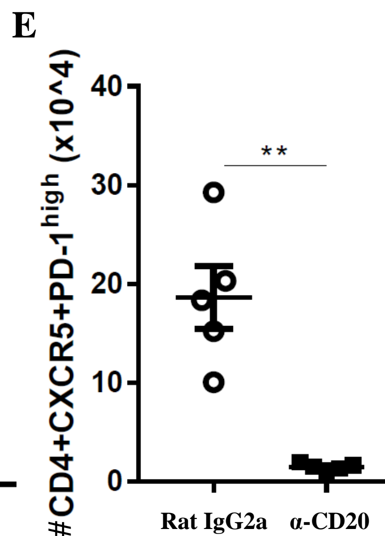

I
TNF-α (pg/mL)
Rat IgG2a
α-CD20

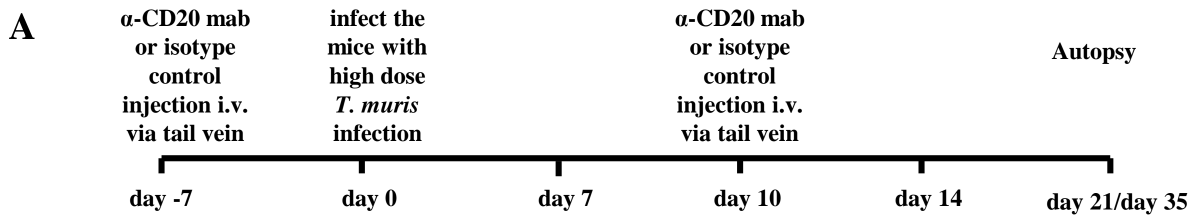

C
Rat IgG2a
α-CD20
3.43
0.14
PD-1
CD4+CXCR5

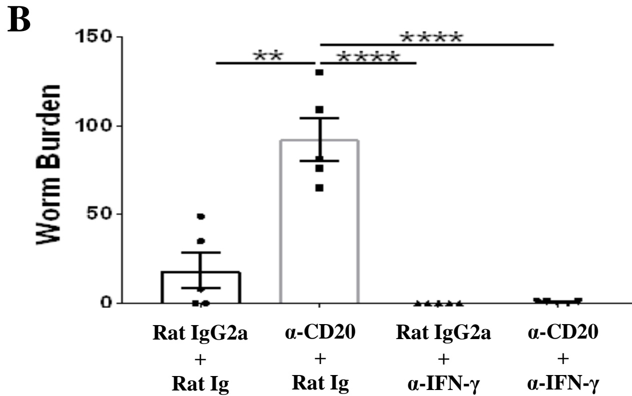

J
IFN-γ (pg/mL)
Rat IgG2a
α-CD20

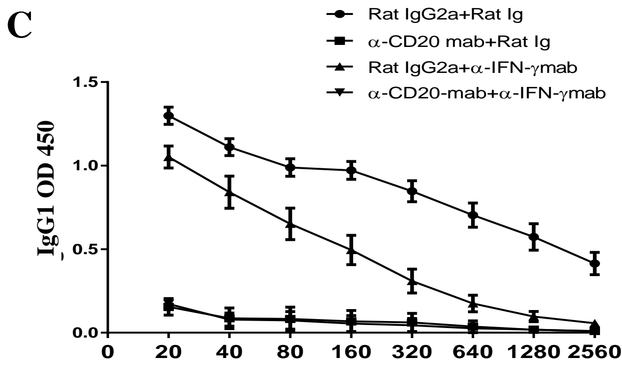

K
IL-17A (pg/mL)
Rat IgG2a
α-CD20

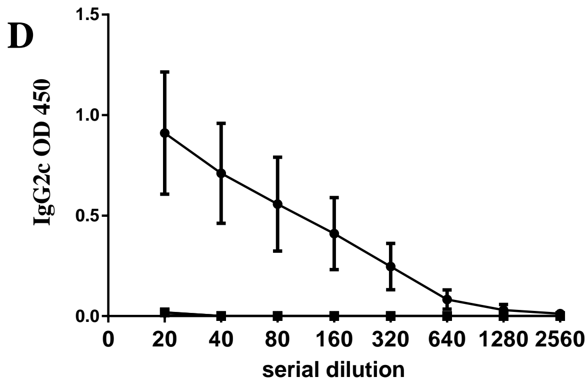

D
CD4+CXCR5+PD-1high (%)
Rat IgG2a
α-CD20

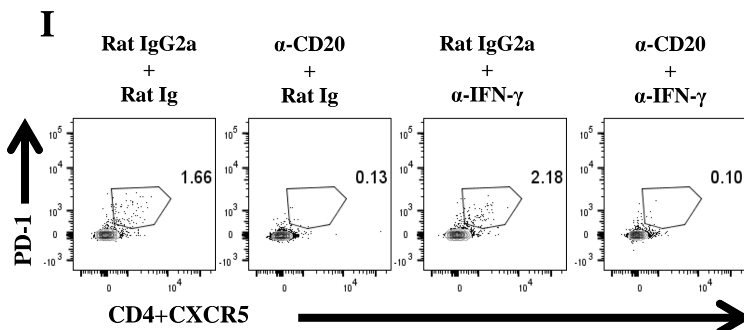

E
#CD4+CXCR5+PD-1high (x10^4)
Rat IgG2a
α-CD20

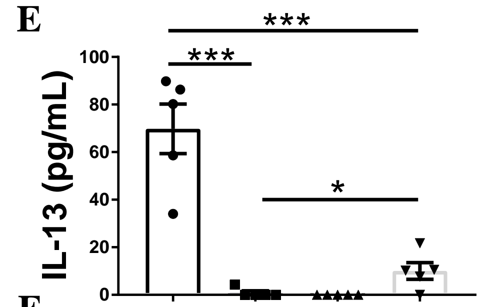

A
α-CD20 mab or isotype control injection i.v. via tail vein
infect the mice with high dose T. muris infection
α-CD20 mab or isotype control injection i.v. via tail vein
Autopsy
day -7
day 0
day 7
day 10
day 14
day 21/day 35
α-IFNγ mAb or isotype control injection i.p.
α-IFNγ mAb or isotype control injection i.p.
α-IFNγ mAb or isotype control injection i.p.

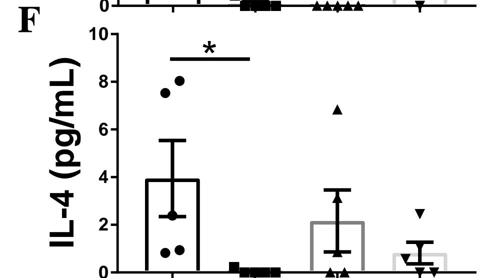

B
Worm Burden
**
****
****
Rat IgG2a + Rat Ig
α-CD20 + Rat Ig
Rat IgG2a + α-IFN-γ
α-CD20 + α-IFN-γ

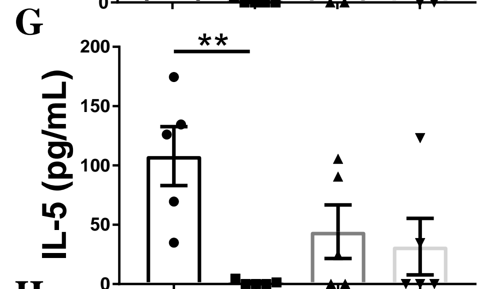

C
Rat IgG2a+Rat Ig
α-CD20 mab+Rat Ig
Rat IgG2a+α-IFN-γmab
α-CD20-mab+α-IFN-γmab
IgG1 OD 450

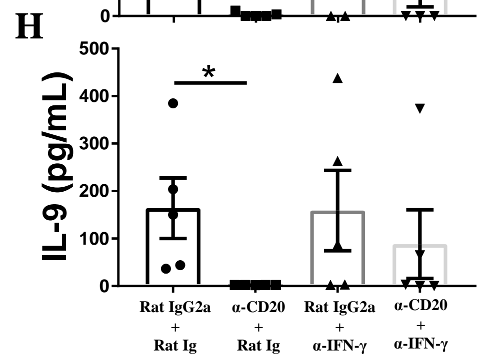

D
IgG2c OD 450
serial dilution

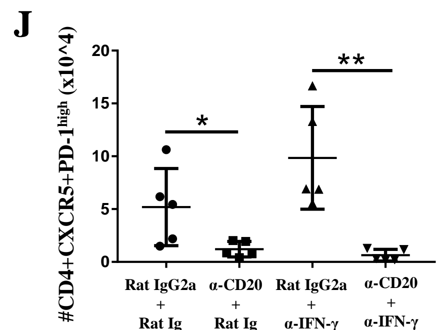

I
Rat IgG2a + Rat Ig
α-CD20 + Rat Ig
Rat IgG2a + α-IFN-γ
α-CD20 + α-IFN-γ
1.66
0.13
2.18
0.10
PD-1
CD4+CXCR5


E
IL-13 (pg/mL)
***
***
*

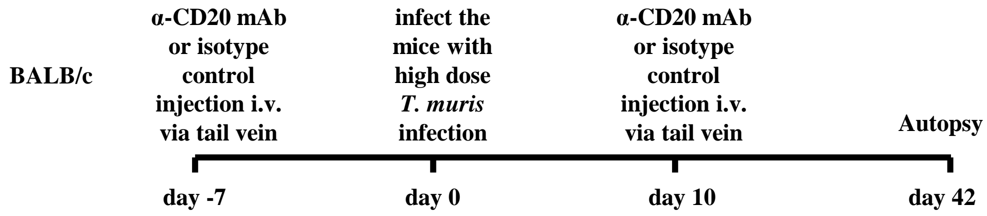

F
IL-4 (pg/mL)
*


G
IL-5 (pg/mL)
**

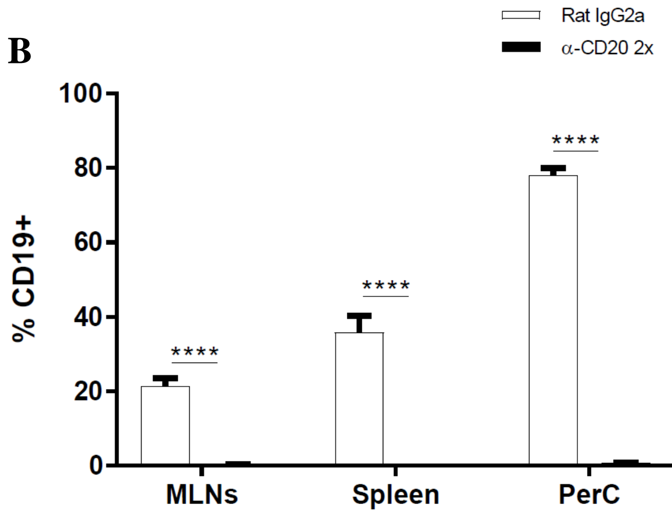

H
IL-9 (pg/mL)
*
Rat IgG2a + Rat Ig
α-CD20 + Rat Ig
Rat IgG2a + α-IFN-γ
α-CD20 + α-IFN-γ


J
#CD4+CXCR5+PD-1high (x10^4)
*
**
Rat IgG2a + Rat Ig
α-CD20 + Rat Ig
Rat IgG2a + α-IFN-γ
α-CD20 + α-IFN-γ

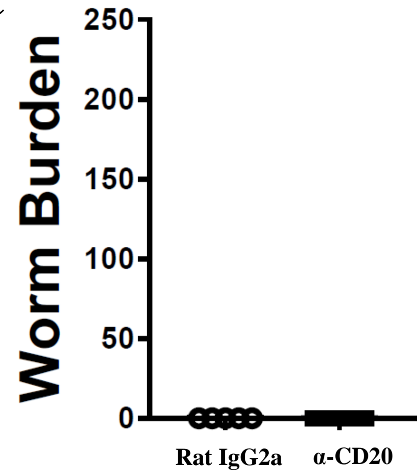
A



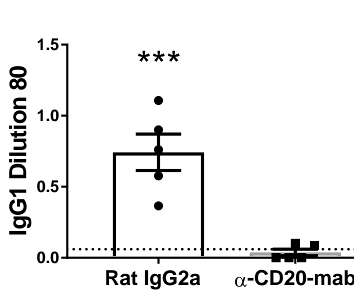
B



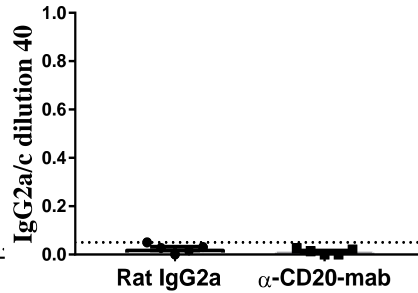
C



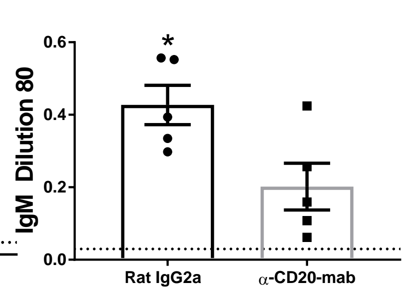
D

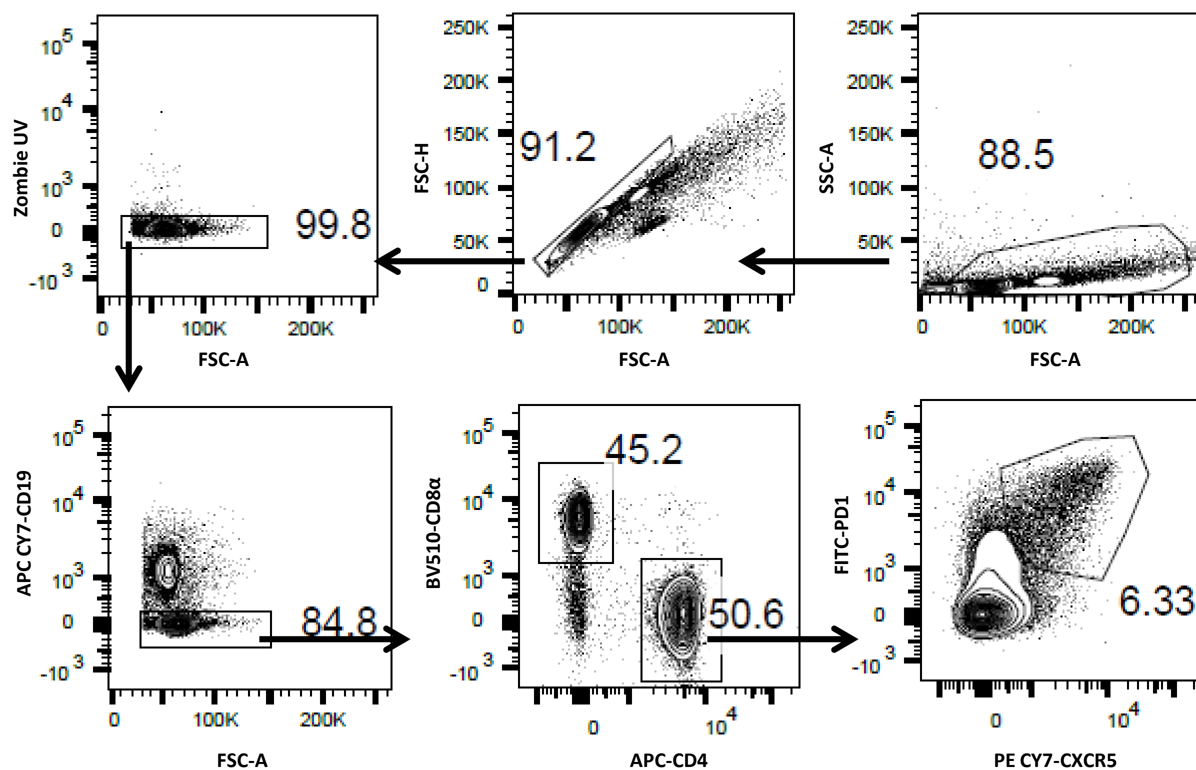


E

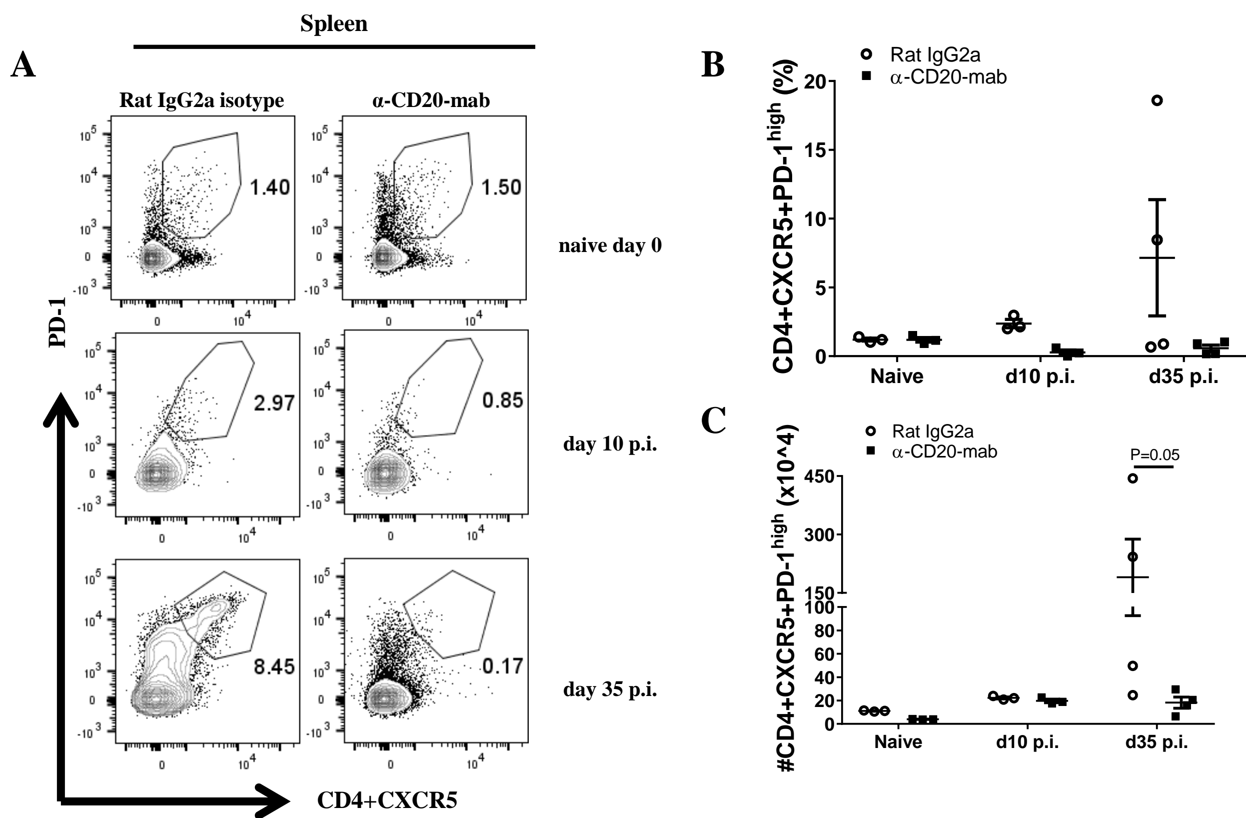


F

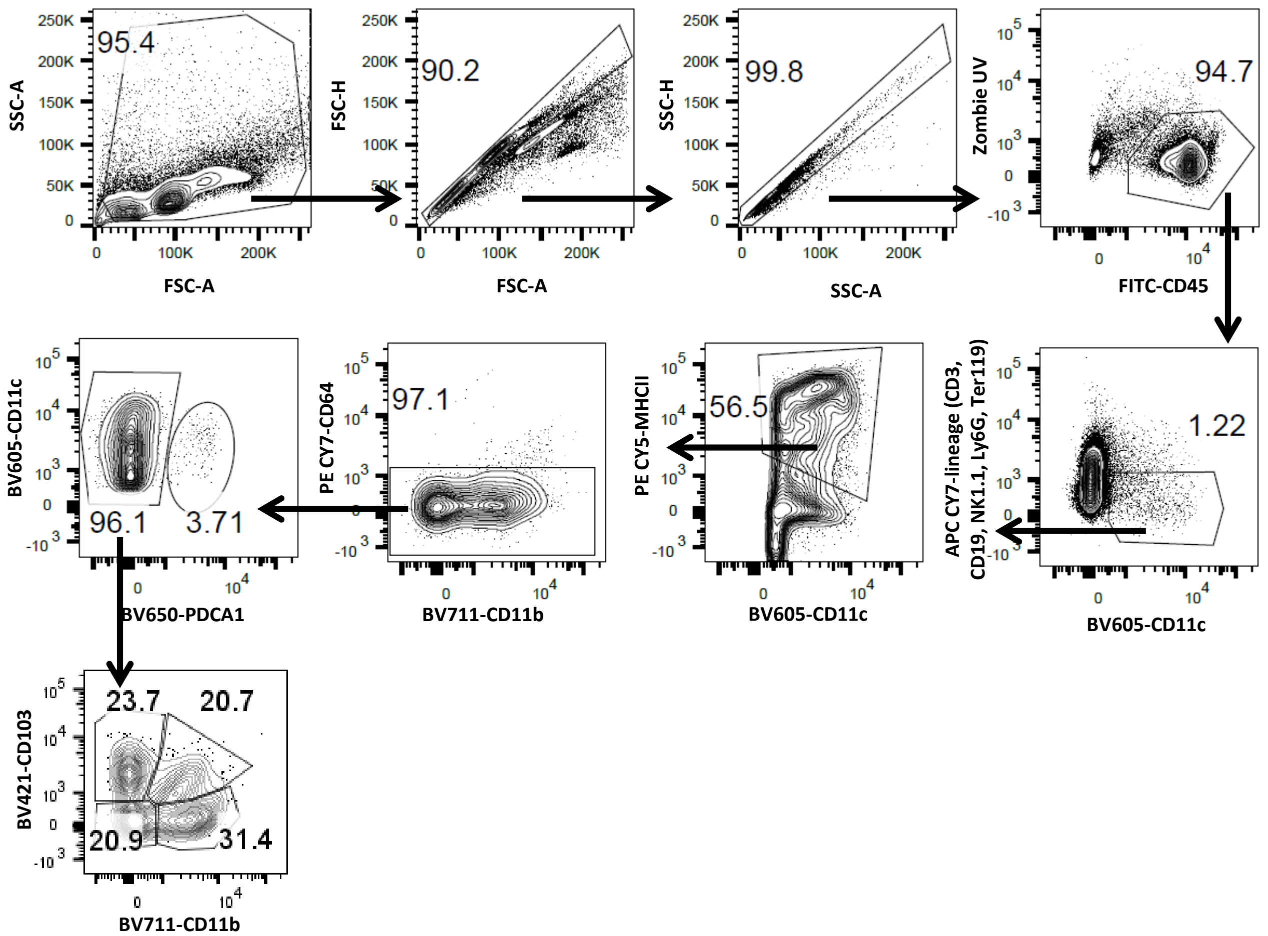




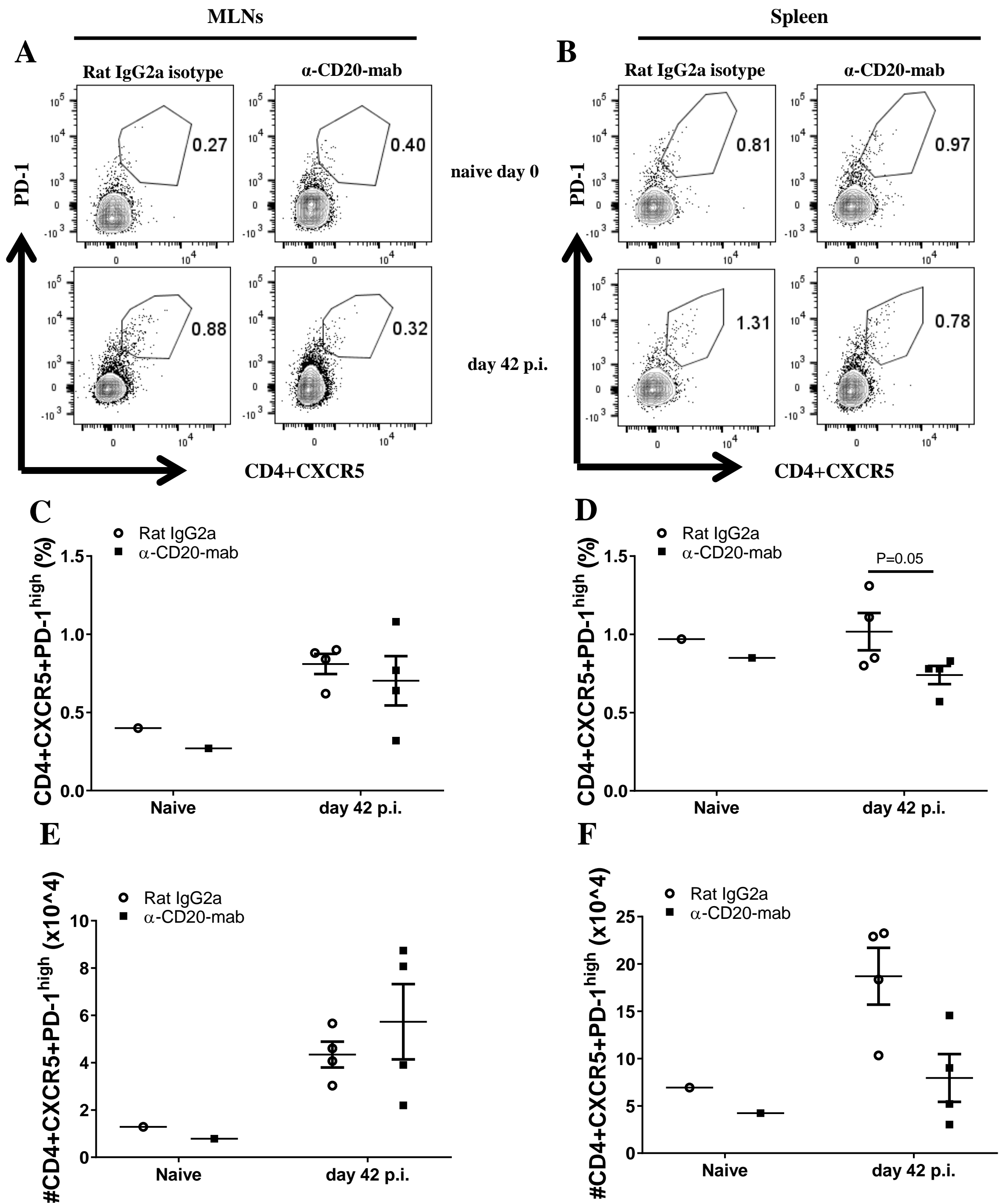
Supplementary Fig.1. Flow cytometry gating strategy for T_{FH}. T_{FH} cells are defined as CD4+CXCR5+PD-1^{high}



Supplementary Fig. 2. T_{FH} population in the spleen of anti-CD20 mAb treated mice. C57BL/6 mice were treated with anti CD20 mab or isotype control 100 µg in 200 µl PBS i.v. injection via tail vein. Mice were infected with approximately 150 *T. muris* eggs at day 7 post injection. Mice were re-injected with anti CD20 mab or isotype control 100 µg in 200 µl PBS i.v. injection via tail vein by day 10 p.i. Mice were autopsied by day 0, day 10 and day 35 p.i. Gating on CD4+CXCR5+PD-1^{high} to define T_{FH} cells in spleen (A). (B&C) Relative % and total cell number of T_{FH} in the spleen, respectively. Data show mean±SEM, from 1 experiment, males., *p<0.05, Mann whitney U test



Supplementary Fig.3. Flow cytometry gating strategy for DC subsets. DCs are defined as CD45+lineage-CD11c+MHCII+CD64-.Two main DCs populations: conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Conventional DCs (CD45+lineage-CD11c+MHCII+CD64-PDCA1-) were divided into migratory DCs and resident DCs. Migratory DCs was consisted of 4 subpopulations: CD103+CD11b-, CD103+CD11b+, CD103-CD11b+ and CD103-CD11b-. Plasmacytoid DCs:CD45+lineage-CD11c+MHCII+CD64-PDCA1+



Supplementary Fig. 4. Tfh population in the MLNs and spleen of anti-CD20 mab treated mice on a Balb/c genetic background were not affected. Balb/c mice were treated with anti CD20 mab or isotype control 100 µg in 200 µl PBS i.v. injection via tail vein. Mice were infected with approximately 150 *T. muris* eggs at day 7 post injection. Mice were re-injected with anti CD20 mab or isotype control 100 µg in 200 µl PBS i.v. injection via tail vein by day 10 p.i. Mice were autopsied by day 0 and day 42 p.i. Gating on CD4+CXCR5+PD-1^{high} to define Tfh cells in MLNs (A) and spleen (B). (C&D) Relative % of Tfh in MLNs and spleen, respectively. (E&F) Total Tfh in MLNs and spleen, respectively. . Data show mean±SEM, from 1 experiment, males.