Evaluating the IgMi mouse as a novel tool to study B-cell biology

The current study aims to provide a detailed characterization of the IgMi mouse as a novel tool to study B-cell biology. The IgMi mouse was firstly introduced in 2007 by Waisman et al., as IgH IgMi mouse was a novel tool to study B-cell biology. The IgMi mouse was stated to have a normal B-cell development, although its B cells only express IgM as a B-cell receptor on the surface. Moreover, IgMi mice cannot produce any soluble antibodies as all constant regions in the IgH chain have been deleted [1]. Thus, as a model to study B-cell biology, the IgMi mouse offers great potential by virtue of its normal B-cell development. However, there have been no subsequent publications providing a detailed description of the IgMi mouse under steady state, other than its inability to secrete antibodies.

We corroborate in the current study that IgMi mice do not secrete any soluble antibodies. Total Ig analysis on the sera using ELISA showed that total Ig was not detected in the sera of IgMi mice with results below the background (Fig. 1A). Furthermore, no IgA antibody was detected in the stool of IgMi mice (Fig. 1B). However, the IgMi mouse showed enlarged secondary lymphoid organs (Fig. 1C) with elevations in B-cell subsets, including B1a, B1b, transitional and marginal zone B cells (Fig. 1D–H). Natural antibodies, especially IgM, have been considered to play an important role in bridging the innate and adaptive immune systems. Thus, as shown in previous study using the AID+/−μS+/− double mutant mouse [3], the current study revealed that secreted antibodies are important in cell extrinsic processes, controlling B-cell subsets, such as B1 and MZ B cells. Interestingly, B-cell development in the IgMi bone marrow was also altered as pre-B cells and immature B cells were significantly higher, while mature B cells was significantly lower, compared to WT mice (Supplementary 1).

B cells are also known to play a role in modulating the maturation and function of DCs [4, 5]. Therefore, we wondered whether the lack of antibodies in IgMi mice would affect the subsets of DCs in IgMi secondary lymphoid organs. Interestingly, IgMi mice had significantly lower MLN CD11c+MHCII+ DCs compared to WT littermates. Previous studies showed that the majority of CD11c+MHCII+ DCs in the MLN under steady state are CD103+ DCs, including CD103+CD11b+ and CD103−CD11b− [6]. Interestingly, IgMi mice had significantly lower numbers of MLN CD103+CD11b+ DCs compared to WT littermates (Fig. 1I–K), while CD103−CD11b− DCs were significantly increased in the spleen (Fig. 1L). Collectively, the data shows that lack of secreted antibody, directly or indirectly, affects B-cell development in the bone marrow and changes the balance of B cell and DC subsets in secondary lymphoid organs. However, further studies are required to investigate the mechanisms behind those alterations in the IgMi mouse.

In contrast to the AID+/−μS+/− double mutant mouse [3], IgMi mice only showed an increase in the relative percentage and number of germinal centre B cells (Fig. 2A–C), and number of GC in the MLNs (Fig. 2D–E). According to Zhang et al. [7], higher affinity antibodies secreted by B cells reenter the GC and negatively regulate GC formation by binding to follicular dendritic cells and limiting the access of B cells to antigens. Thus, in the absence of soluble antibodies, GC B cells in the IgMi mouse may be undergoing more proliferation and less apoptosis. Our study supports this hypothesis as GC B cells from IgMi mice showed increased proliferation and reduced apoptosis (Supplementary 2). We also investigated plasma cell and plasmablast populations in IgMi MLNs. Levels were very low under steady-state conditions and we did not see any significant difference between genotype in any tissue (data not shown). In parallel to the increase in GC, the population of T-follicular helper cells (Tfh) in the MLNs of IgMi mice were also significantly increased (Fig. 2F–G). CD4+PD-1hiCXCR5hiPD-1hi CXCR5hiPD-1hi CD11b+ cells are known as GC Tfh cells due to their ability to enter GCs and maintain stable interactions with B cells. Therefore, the increase in GC seen in IgMi mice correlates well with the increase in GC Tfh. Interestingly, we did not see significant differences in CD4 and CD8 T cells in IgMi mice (data not shown).

B-cell function is not only related to antibody production, but B cells are also able to produce cytokines including IL-10 [8]. Due to its pleiotropic activities, IL-10 is an important regulatory cytokine that is able to act both as an...
Figure 1. The absence of secreted antibodies in the IgMi mouse affects B cell and dendritic cell subsets. WT and IgMi mice were analyzed for (A) serial dilution of total Ig (ng/mL) using ELISA. Total Ig levels were quantified in serum samples of WT and IgMi mice relative to an IgA standard. (B) Faecal samples were stained with IgA using flow cytometry. (C) The comparison of weight of MLNs and spleen between IgMi mice and their age-matched WT littermates. (D) Gating strategy to analyze B-cell subsets in the spleen. B1a was defined as CD19+ B220low/midCD43+CD5+, B1b as CD19+ B220low/midCD43+CD5+, marginal zone (MZ) as CD19+ B220+CD5+CD43-CD23-CD24-CD21+ and transitional B cells as CD19+ B220+CD5-CD43-CD23-CD24+CD21-. (E–H) B-cell subsets in IgMi mice were altered either in secondary lymphoid organs or peritoneal cavity (PNC). (I) Gating strategy for DC subsets. Two main DCs populations: conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Conventional DCs was divided into migratory DCs and resident DCs. Migratory DCs consisted of four subpopulations: CD103+CD11b-, CD103+CD11b+, CD103-CD11b+ and CD103-CD11b-. Resident DCs was consisted of two subpopulations: CD8α+CD11b- and CD8α-CD11b+. Plasmacytoid DCs: CD11c+ lineage-PDCA-1+. (J) Total cell number of CD11c+MHCII+ in MLNs. (K) Total cell number of CD103+CD11b+ in MLNs. (L) Total cell number of CD103+CD11b- in the spleen. Sensitivity of the assay is shown as mean ± SD (dotted black line). (A&B) Data are representative of two separate experiments, n = 4/group, males, 12 weeks old. (C–L) Data are pooled from two separate experiments. All data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 Mann–Whitney test.

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Figure 2. Germinal centers and T-follicular helper cells are significantly increased in the MLNs of IgMi mice. WT and IgMi mice were analyzed by flow cytometry and histology (A) Gating strategy of GC B cells and TFH. GC were defined as CD19+GL7+CD38- and B220-CD4+CXCR5+PD-1high, respectively. (B&C) Relative percentage and total cell number of GCs. (D) Immunohistology staining of MLNs labeled with biotin peanut agglutinin (PNA) from WT and IgMi mice. Scale bar 500 μm. Images are representative from a single experiment with five mice per group. (E) The scores of germinal centers for PNA immunoreactivity based on the number of PNA+ within MLNs. (F&G) Relative percentage and total cell number of TFH. (H–J) MLN and spleen cells were restimulated with LPS in the presence of PMA, ionomycin and monensin (PIM) and IL-10 was examined using flow cytometry. (H) Gating strategy of IL-10 intracellular staining. (I) Representative data of IL-10 producing B cells in MLNs and spleen. (J) Relative percentage of IL-10 produced by B cells in MLNs and spleen. (A–G) Data are pooled from three separate experiments, n = 6, males, 12 weeks. (H–J) Data are pooled from two separate experiments, n = 4, males. All data are expressed as mean ± SEM. *p < 0.05, **p < 0.01 Mann–Whitney test.
immunostimulator and immunosuppres-
sor. A previous study showed that IL-10
producing B cells were essential for GC
development during malaria infection [9].
Thus, the increase in GC cells seen in
the IgMi mouse may reflect in part the
increased ability by IgMi B cells to make
IL-10 (Fig. 2I–J). However, the mecha-
nisms underlying the ability of IgMi B
cells make more IL-10 remains unknown.
Further study is required to investigate
whether this phenomenon is related to the
lack of antibodies in IgMi mice, and also
to address other unanswered questions in
B cells biology, such as whether B cells
secrete antibody and produce IL-10 at the
same time.

It remains controversial whether anti-
bodies are important in regulating the
composition of microbiota or not. There-
fore, we also investigated if the lack of
antibodies in IgMi mice would change the
composition of the gut microbiota com-
pared to their WT littermates. Surprising-
ly, we found that IgMi mice had a similar
gut microbiota composition to their WT
littermates based on microbiome analysis
using DGGE and real-time PCR (Supple-
mentary 3). It is possible that, the insignif-
ican difference in gut microbiota between
the IgMi mice and WT littermates could
be related to maternal immunity [10],
however, although both genotypes were
breast-fed by a heterozygous mother, flow
cytometric analyses of faecal IgA failed to
reveal the presence of any antibody in
the IgMi mouse (Fig. 1B). Furthermore,
in keeping with the absence of any differ-
ences in the gut microbiota in IgMi and WT
mice, there were no significant differences
in expression levels of IFN-γ and IL-17 in
the gut in IgMi mice compared to WT lit-
termates (Supplementary 4).

In conclusion, the IgMi mouse repre-
sents a powerful model system which, in
combination with other B-cell transgenic
mice, can be used to investigate the biology
of B cells. However, it is important to be
aware that steady-state differences beyond
lack of antibodies exist including differ-
ces in B-cell subsets, B-cell propensity
to make IL-10, dendritic cell subsets, germ-
inal centers, and T-follicular helper cells.

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Full correspondence: Rinal Sahputra and Dr.
Kathryn Jane Else, Lydia Becker Institute of
Immunology and Inflammation, Faculty of
Biology, Medicine and Health, Manchester
Academic Health Science centre, The
University of Manchester, Manchester,
United Kingdom of Great Britain and
Northern Ireland
2 Institute of Immunology and Immunotherapy,
University of Birmingham, Birmingham,
United Kingdom of Great Britain and Northern Ireland
3 Institute for Molecular Medicine, University of
Mainz, Mainz, Germany

1 Lydia Becker Institute of Immunology and
Inflammation, Faculty of Biology, Medicine and
Health, Manchester Academic Health Science
centre, The University of Manchester,
Manchester, United Kingdom of Great Britain and
Northern Ireland
2 Institute of Immunology and Immunotherapy,
University of Birmingham, Birmingham, United
Kingdom of Great Britain and Northern Ireland
3 Institute for Molecular Medicine, University of
Mainz, Mainz, Germany

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