論文の内容の要旨

論文題目： Integrin CD11b provides a new marker of IgA$^+$ B cells entering germinal centers in murine Peyer's patches

（インテグリンCD11bは、マウスパイエル板の胚中心に移行するIgA$^+$ B細胞の新しいマーカーである）

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Background

The COVID-19 has brought one of the biggest disasters in recent years within human history. The COVID-19 pandemic highlighted the need for the development of safe and effective vaccines. One of the criteria to evaluate the quality of a given vaccine is whether it induces effective antibody immune response to generate high-affinity long-lived PCs and memory B cells via germinal center (GC) reaction. GC reaction is initiated from B cell activation by a specific antigen. Upon antigen stimulation, the newly activated naïve B cells and the activated memory B cells either directly differentiate into extrafollicular short-lived PCs or migrate to the interfollicular (IF) area to establish a stable interaction with the activated T cells. After T-B cell interaction, some B cells differentiate into low-affinity PCs, while others, the pre-GC B cells, migrate into GCs for high-affinity antibody production (Figure 1). To produce high-affinity PCs and memory B cells, B cells in GCs rely on a specific process called somatic hypermutation (SHM) to accumulate many mutations in the immunoglobulin (Ig) variable gene, which encodes the antigen-binding site of the antibody (or Ig). In GC, antigen-activated B cells undergo SHM to change the B cell receptor (BCR) affinities to the specific antigen, which generates many B cell progenies with different affinities to the specific antigen in GCs. B cells with high-affinity BCR are selected for further differentiation into high-affinity PCs and memory B cells.

During immune response as described above, only a small population of activated B cells can develop into pre-GC B cells and enter the GC. However, there is no known specific surface marker for pre-GC B cells. Hence the criteria for pre-GC B cells, and which kinds of activated B cells enter GCs to produce high-affinity BCR against a specific antigen are not well understood.

In the gut, IgA is the main Ig isotype at mucosal surface, maintains the intestinal hemostasis and provides host protection against the invading pathogens. High-affinity IgA$^+$ PCs are derived from the IgA$^+$ B cells in Peyer's patches (PPs). PPs are good sites to study the pre-GC B cells, since the B cells in

PPs are always exposed by the antigens from the gut lumen and GCs in PPs are constitutively active. Therefore, we can find pre-GC B cells that are entering the existing GCs in PPs without immunization.

CD11b, the 165-kDa integrin alpha M, associates with CD18 to form heterodimeric integrin known as macrophage-1 antigen (Mac-1). CD11b is widely considered as a marker for myeloid cells and is known to be involved in cell migration and adhesion. In addition, previous studies demonstrated a complex function of CD11b on B cells. Ding *et al.* have shown that integrin CD11b negatively regulates BCR signaling to maintain autoreactive B cell tolerance (Ding *et al.*, *Nature communications*, 2013). CD11b$^{-/-}$ mice exhibited enhanced antibody production and GC response with autoreactive B cells (Zhou *et al.*, *J Immunol.* 2021). Accordingly, human genetic variations in the *ITGAM* gene (encoding CD11b) strongly associate with risk for systemic lupus erythematosus (SLE) (Faridi *et al.*, *The Journal of clinical investigation*, 2017). Therefore, we thought that CD11b plays a key role in controlling the activated B cells for undergoing beneficial GC reaction but not for the harmful autoreactive response.

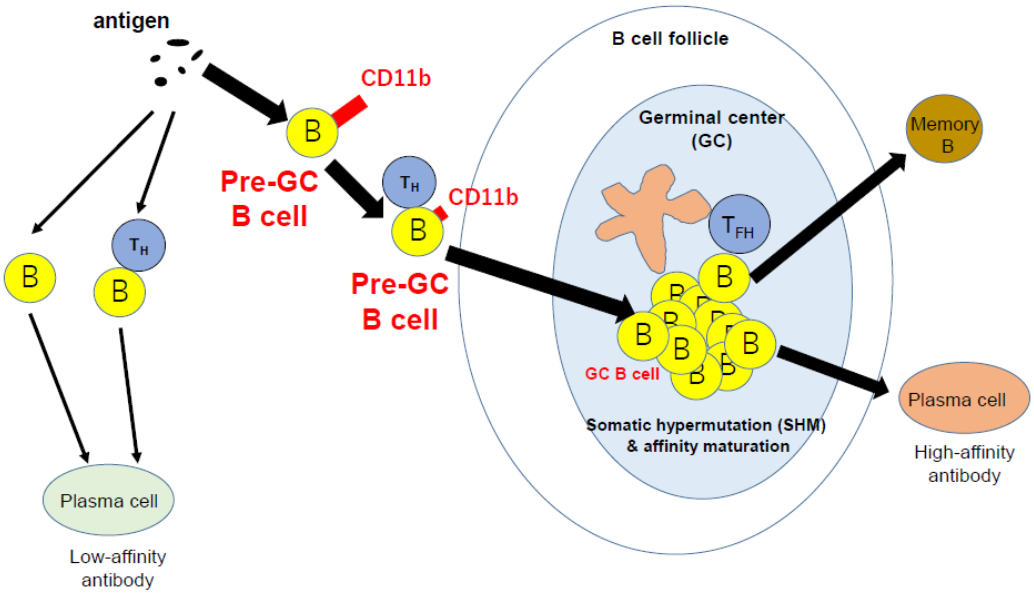

Figure 1 Process of antibody production

Questions

1. Which population of activated B cells is pre-GC B cells entering GCs to produce high-affinity antibodies?

2. How are pre-GC B cells induced in order to enhance the GC reaction?

Results and Discussion

We hypothesized that CD11b$^+$IgA$^+$ B cells are pre-GC IgA$^+$ B cells in PPs. By flow cytometry analysis with PPs, we found a small population of CD11b$^+$IgA$^+$ PP B cells. In order to check whether they are the pre-GC B cells in PPs, we investigated that population from the following three aspects.

1. Localization and migration

(a) We consider that pre-GC B cells are located outside of GCs, so we performed immunohistochemistry to analyze the localization of CD11b$^+$IgA$^+$ PP B cells. We found a few CD11b$^+$IgA$^+$ PP B cells located in the IF area, outside of GCs.

(b) To check whether CD11b$^+$IgA$^+$ PP B cells are pre-GC B cells entering GCs, we labeled the sorted CD11b$^+$IgA$^+$ PP B cells and CD11b$^-$IgA$^+$ PP B cells with dye and injected them to a PP of mice, separately. One hour after injection, most CD11b$^-$IgA$^+$ PP B cells migrated into GCs, indicating that CD11b$^-$IgA$^+$ B cells are GC B cells. However, the injected CD11b$^+$IgA$^+$ PP B cells were located in surrounding GC but not inside GC. Forty hours after injection, the injected CD11b$^+$IgA$^+$ PP B cells had entered into GCs.

Therefore, CD11b$^+$IgA$^+$ PP B cells are B cells located outside of GCs, but they will enter GCs, and we thought that CD11b$^+$IgA$^+$ B cells are pre-GC B cells in PPs.

2. Gene expression

(a) By microarray analysis, CD11b$^+$IgA$^+$ PP B cells express relatively higher levels of non-GC genes and lower levels of GC genes than CD11b$^-$IgA$^+$ PP B cells, indicating that CD11b$^+$IgA$^+$ PP B cells are distinct cell population.

(b) By quantitative PCR (qPCR) analysis, CD11b$^+$IgA$^+$ PP B cells can be distinguished from non-GC PP B cells, since CD11b$^+$IgA$^+$ PP B cells expressed GC specific genes, such as *bcl6*, *s1pr2* and *aicda*, and do not express non-GC genes including *gpr183* and *bcl2*.

(c) Additionally, qPCR results showed that CD11b$^+$IgA$^+$ PP B cells highly express *irf4*, which is known to be expressed in pre-GC B cells.

Gene expression results indicate that CD11b$^+$IgA$^+$ PP B cells are pre-GC B cells.

3. Interaction with $CD4^+$ T cells

Since pre-GC B cells interact with $CD4^+$ T cells before entering GCs, we further confirmed the interaction between $CD11b^+IgA^+$ PP B cells and $CD4^+$ T cells by conjugation analysis of flow cytometry and confocal microscopic imaging.

Taken together, we concluded that $CD11b^+IgA^+$ PP B cells are pre-GC B cells in PPs, and CD11b is a candidate surface marker for pre-GC B cells.

The next question is what stimulation can induce CD11b expression on B cells.

B cells can be activated by dendritic cell (DC)-B, T-B interaction, BCR-crosslinking and Toll-like receptor (TLR) ligands stimulation. Therefore, we sorted the naive spleen B cells from unimmunized mice and culture them *in vitro* with each ligand. We found that only pam3csk4 (TLR2 ligand) and LPS (TLR4 ligand) induced CD11b in naïve B cells *in vitro*.

Since TLR2 and TLR4 are sensors for bacterial antigen, we further check if CD11b expression is induced by bacteria. We selected *E. coli* and *S. enterica* as harmful bacteria, and *Bifidobacterium bifidum* (*B. bifidum*) and *B. breve* as representative beneficial bacteria to stimulate B cells *in vitro*. We found that heat-killed *E. coli* and *S. enterica* induced expression of CD11b, while *B. bifidum* and *B. breve* did not. Therefore, not all bacteria but only specific ones can induce CD11b expression on B cells.

To check whether induced $CD11b^+$ B cells can enter GCs, we then cultured the bacterial antigen-stimulated spleen B cells on the induced GC-like B cells (iGB) culture system to mimic the T-B and DC-B interaction. The CD11b expression on B cells induced by pam3csk4 or heat-killed *E. coli* before T-B or DC-B interaction allowed the intravenously-injected B cells to enter existing GCs in PPs. Without CD11b expression before T-B or DC-B interaction, the intravenously-injected B cells did not enter GCs.

Finally, by oral administration of those bacterial antigens for *in vivo* stimulation, we confirmed that mice orally administered with pam3csk4 or heat-killed *E. coli* increased the number of PP GC B cells within two days, and enhanced the mucosal antigen-specific IgA response.

Taken together, our results demonstrate that the induction of CD11b on B cells is a promising marker to identify pre-GC B cells and to select an effective mucosal vaccine adjuvant.