Robust Serum- and Feeder-Free Expansion of Mouse B Cells In Vitro

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INTRODUCTION

Mouse B cells are used in applications to discover novel therapeutic antibodies or develop therapeutic proteins using genetically engineered B cells. In vitro expansion of B cells offers the opportunity to improve the recovery of antibody sequences and increase the diversity of the B cell repertoire. Achieving robust expansion of B cells in culture can be challenging, as it typically requires the addition of serum, feeder cells, or specialized culture plates. The addition of serum can introduce unknown factors and result in lot-to-lot variability. Therefore, we have developed a culture system that does not require the use of serum, feeder cells, or specialized culture plates to achieve robust in vitro expansion of mouse pan-B cells. Pan-B cells isolated from mouse splenocytes by immunomagnetic enrichment were cultured in serum-free ImmunoCult™-XF B Cell Base Medium supplemented with ImmunoCult™-ACF Mouse B Cell Expansion Supplement for 9 days, starting with seeding densities of 100,000 cells/well and passaging every 3 days. Although pronounced donor variability was observed in the expansion of viable cells, average fold expansions of ~13.2 ± 4.1-fold (mean ± SEM), 151.3 ± 85.8-fold, and 194.7 ± 92.0-fold were observed after 3, 6, and 9 days in culture, respectively (n = 6). The expanded B cells also upregulated expression of activation and differentiation markers such as CD86 (74 ± 10.3%), CD138 (68 ± 2.5%), and CD267 (79 ± 6.4%) at day 9 (n = 6). This first-to-market mouse B cell culture system provides an adept platform for diverse translational research and antibody discovery workflows.

METHODS

Immunomagnetic Isolation of Mouse Pan-B Cells:
Mouse pan-B cells were enriched from mouse splenocytes using EasySep™ Mouse Pan-B Cell Isolation Kit (STEMCELL Technologies) according to the product information sheet. Enriched cells were analyzed by flow cytometry using a CytoFLEX instrument (Beckman Coulter Life Sciences), and enrichment of over 90% was observed.

Culturing Mouse B Cells:
A Complete Mouse B Cell Expansion Medium was prepared by diluting 50X ImmunoCult™-ACF Mouse B Cell Expansion Supplement in ImmunoCult™-XF B Cell Base Medium. On day 0, pan-B cells were seeded at 1 x 10^5 cells/mL in 24-well tissue culture plates. Cultures were incubated at 37°C and 5% CO2 in a humidified incubator. The cell density was adjusted to 1 x 10^5 cells/mL every 3 days by adding fresh Complete Medium.

Analysis:
Cell counting and measurement of viability were performed using a NucleoCounter® NC-250™ instrument (ChemoMetec A/S) according to the manufacturer's instructions. The fold expansion of viable cells was calculated as the fold-change in cell number relative to the number of viable cells seeded on day 0. Flow cytometric analyses were performed with a CytoFLEX instrument to assess changes in the expression of cell surface markers characteristic of B cell activation and differentiation (B220, CD86, CD138, and CD267). Cultures were imaged using an Olympus CKX53 inverted microscope.

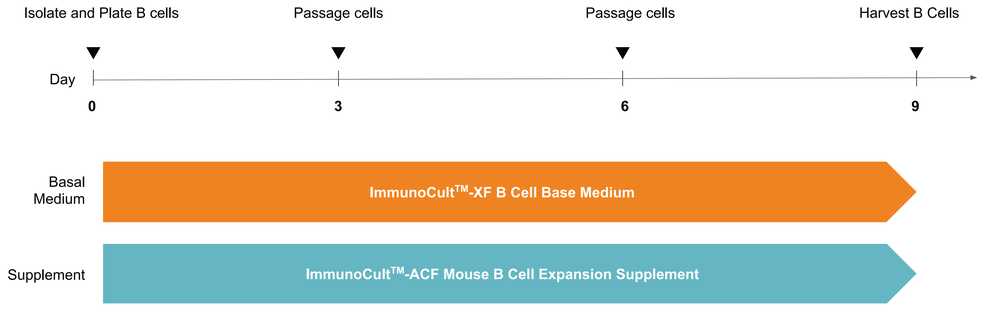

FIGURE 1. Protocol Description for Culturing Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured in Complete Mouse B Cell Expansion Medium as described in Methods. B Cells were harvested on day 9 for analysis. Cells can also be harvested at earlier time points depending on different applications.

RESULTS

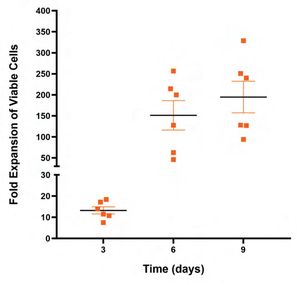

FIGURE 2. Expansion of Mouse B Cells when Cultured with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Methods. Fold expansion of viable cells is shown with bars representing the mean ± SEM (n = 6). B cells expanded 194.7 ± 92.0-fold after 9 days of culture.

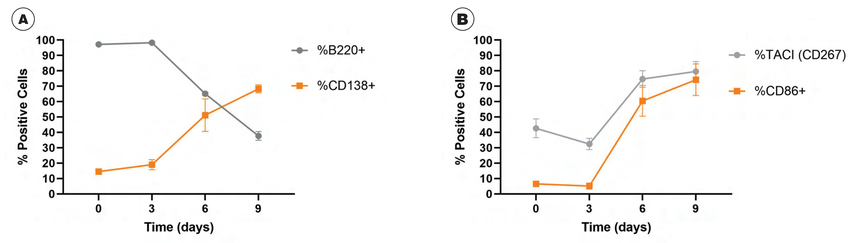

FIGURE 3. Maturation of Mouse B Cells when Cultured with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Methods. **(A)** Expression of B220 and CD138, and **(B)** Expression of TACI (CD267) and CD86 were analyzed by flow cytometry at each timepoint (data represents mean ± SEM, n = 6). An increase in CD86 cell surface expression indicates B cell activation and a decrease in B220 and an increase in CD138 and TACI cell surface expression indicates maturation of B cells to plasmablasts or plasma cells.

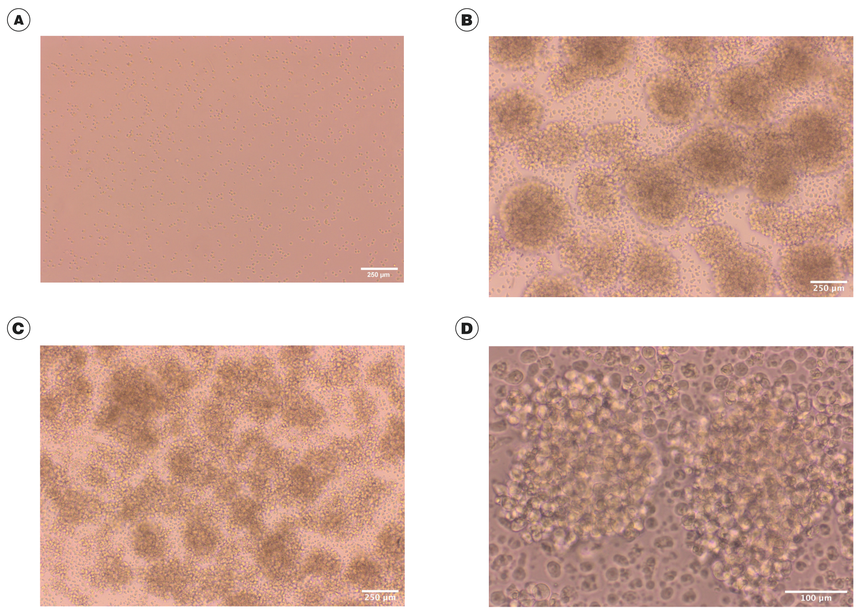

FIGURE 4. Robust Growth of Mouse B Cells when Cultured with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as shown in Figure 1. Cells were imaged at **(A)** 10X magnification on day 0, **(B)** 10X magnification on day 3, **(C)** 10X magnification on day 6, and **(D)** 40X magnification on day 6.

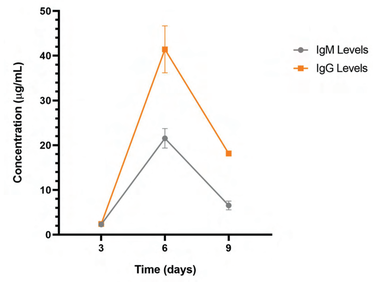

FIGURE 5. IgM and IgG Secretion by Mouse B Cells when Cultured with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as shown in Figure 1. Secretion of IgM and IgG was measured on days 3, 6, and 9 and is shown by line graphs representing the mean ± SEM (n = 2). On day 6, 21.5 ± 2.1 µg/mL of IgM secretion and 41.4 ± 5.2 µg/mL of IgG secretion were observed.

Summary

- This first-to-market product leads to robust in vitro expansion of mouse pan-B cells. An average of 190-fold expansion of viable cells was observed after 9 days of culture
- This expansion of pan-B cells was accompanied by upregulation of the cellular activation and maturation markers such as CD86, CD138, and TACI
- The expanded pan-B cells secrete IgM and IgG antibodies peaking at day 6 of culture
- Expansion of B cells in vitro prior to screening has the potential to increase the redundancy and diversity of antigen-specific clones, which may lead to improved outcomes for antibody discovery campaigns