Cancer research

Stimulation and expansion of human B cells by magnetic bead–based activation of CD4+ T cells

Highlights

- Use of Dynabeads Human T-Activator CD3/CD28 beads to stimulate B cells and differentiate them into antibody-producing plasma cells or memory B cells via autologous stimulation of CD4+ Th cells
- A Dynabeads magnetic bead–based activation method for the study of neoplasms (multiple myeloma and chronic lymphocytic leukemia) and antibody research to expand and proliferate B and plasma cells

Keywords

B cells, Th cells, Dynabeads Untouched Human B Cells, Dynabeads Untouched Human CD4, Dynabeads Human T-Activator CD3/CD28, plasma cells, memory B cells

Introduction

B cells are part of the acquired immune response to diseases. These lymphocytes produce antibodies against a wide range of pathogens and orchestrate pro-inflammatory responses. Only B cells that differentiate into large plasma cells will produce antibodies. In addition, some B cells will differentiate into memory B cells.

The dependency on Th cell activation is not only confined to normal B cell differentiation; it also has been reported in proliferation of certain blood cancers, such as multiple myeloma [1] and chronic lymphocytic leukemia [2]. These reports and findings show Th cell–B cell interaction and crosstalk are not only critical for B cell development but also necessary for the progression of some blood cancers.

Th cell activation and differentiation of B cells to plasma cells is crucial for the collective understanding of many cancers of the blood. Therefore, a method for expansion of B cells *in vitro* is the cornerstone to study mechanisms of drug resistance, proliferation pathways, oncogenes, and neoantigens. Here we describe a method to isolate Th cells and B cells using negative isolation (untouched) to leave important biomarkers free for downstream applications. The use of Gibco™ Dynabeads™ Human T-Activator CD3/CD28 beads is crucial for stimulating Th cells into driving B cell differentiation and proliferation.

Activation of Th cells using Dynabeads CD3/CD28 beads is enough to stimulate proliferation of B cells *in vitro*

To measure cell proliferation, the nuclear protein Ki-67 was used as a biomarker. In addition, forward scatter (FSC) area was used to address cell size for B cells differentiating into plasma cells. Figure 1 illustrates the interaction between a naïve B cell in resting state with an activated Th cell. Th cells are activated *in situ* through the co-stimulatory domains CD3 and CD28 by an antigen presenting cell. In turn, this activates naïve B cells into antibody-secreting plasma cells or memory B cells.

Th experimental design was set up to co-culture a ratio of 1:1 B cells to Th cells. Test cells were cultured with either 200 U/mL IL-2 alone or Dynabeads Human T-Activator CD3/CD28 beads alone, at a ratio of 1:1 beads:cells. Positive controls were incubated with a combination of 200 U/mL IL-2 and Dynabeads Human T-Activator CD3/CD28 beads at a ratio of 1:1 beads:cells. Negative controls were B cells alone without stimulants, Th cells alone without stimulants, and a co-culture of 1:1 B cells:Th cells without stimulants.

$CD4^+$ Th cells and $CD19^+$ B cells were negatively selected, leaving the cells' surfaces untouched by antibodies and beads. This resulted in a purity of ~75% $CD19^+$ B cells and ~80% $CD4^+$ Th cells (Figure 2).

B cells differentiated into plasma cells after Th cells were stimulated using Dynabeads Human T-Activator CD3/CD28 beads (Figures 3 and 4). Light microscopy images in Figure 3 show the differences in cell sizes between resting naïve B cells (Figure 3A) and blasting plasma cells (rounded cells; Figures 3D and 3E). Figure 4 shows a blasting B cell (Figures 4C and 4G) that is proliferating following Th cell stimulation (Figures 4D and 4H).

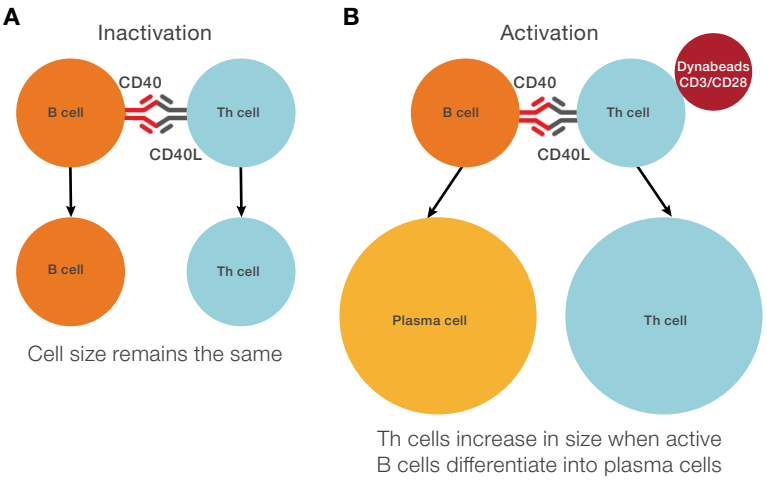

Figure 1. Activated Th cells expand and proliferate B cells to plasma cells. (A) Inactivated Th cells are unable to stimulate naïve B cells into a proliferative state morphology. **(B)** Upon activation of Th cells by Dynabeads Human T-Activator CD3/CD28 beads, Th cells were able to drive naïve B cells into plasma cells.

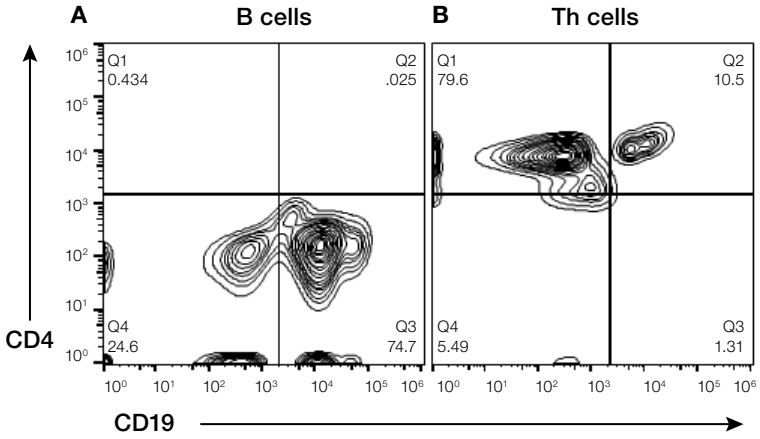

Figure 2. Isolation of $CD19^+$ B cells and $CD4^+$ Th cells using the Invitrogen™ Dynabeads™ Untouched Human B Cells Kit and Dynabeads Untouched Human CD4 T Cells Kit. **(A)** Gated B cells following negative isolation were 74.7% of the total live cell population positive for the biomarker CD19 (Q3). **(B)** Th cells gated on total live cells were 79.6% pure following negative isolation (Q1).

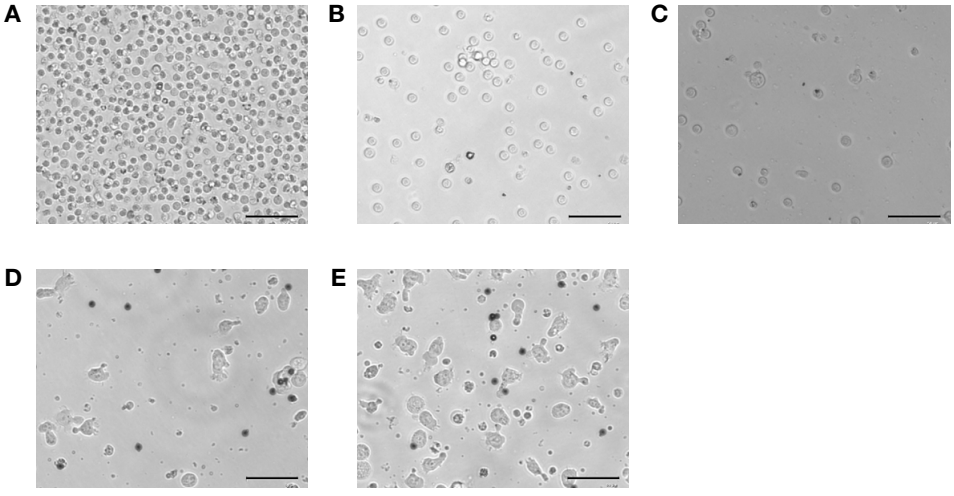

Figure 3. Light images of cells after four days in culture. (A) B cells without stimulants (negative control). **(B)** Th cells without stimulants (negative control). **(C)** Co-culture of both B and Th cells with 200 U/mL IL-2. **(D)** Co-culture of both B and Th cells with Dynabeads Human T-Activator CD3/CD28 beads. **(E)** Co-culture of B and Th cells treated with 200 U/mL IL-2 and Dynabeads Human T-Activator CD3/CD28 beads. Scale bar is 50 µm.

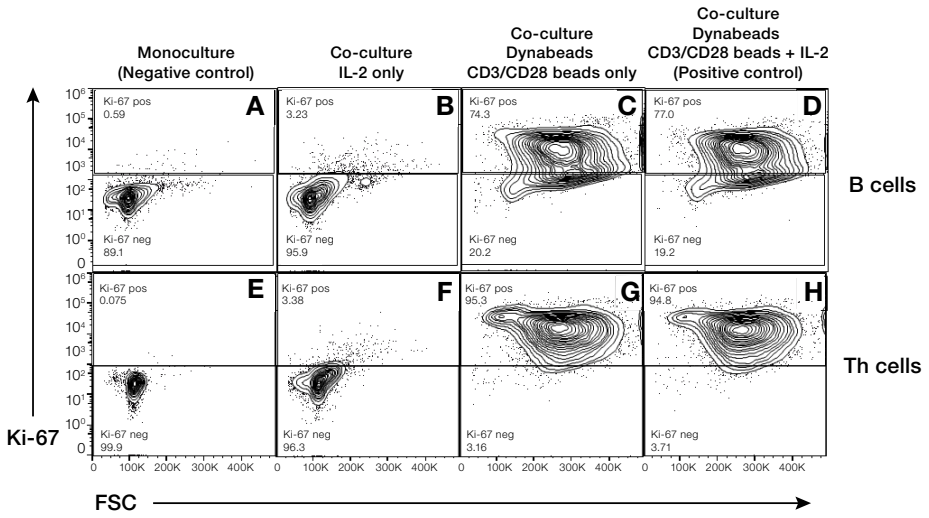

Figure 4. Cell proliferation measured by FSC and Ki-67. Cultures of B cells or Th cells **(A** and **E)** were left unstimulated, and they remained small with no Ki-67 expression, as expected. For cells in co-culture with IL-2 stimulation **(B** and **F)**, minimal Ki-67 expression and blasting of B cells were observed. For cells in co-culture with Dynabeads CD3/CD28 beads **(C** and **G)** and cells in co-culture with Dynabeads CD3/CD28 beads and IL-2 **(D** and **H)**, B cells were able to blast into larger cells after activation of Th cells. These B cells had high Ki-67 expression and increased cell sizes.

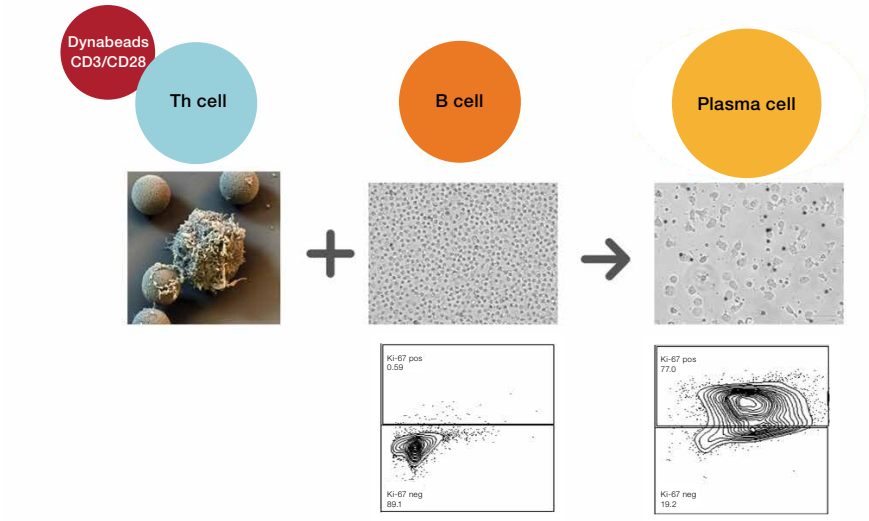

Figure 5. Summary of B cell activation and stimulation to a plasma cell using Dynabeads Human T-Activator CD3/CD28 beads. By activation of Th cells with Dynabeads Human T-Activator CD3/CD28 beads, naïve B cells were able to blast into larger memory B cells or plasma cells.

Summary

The results shown here demonstrate the importance of Th cells stimulation and activation of B cells *in vitro* (Figure 5). In addition, we show that using Dynabeads Human T-Activator CD3/CD28 beads alone were able to stimulate the Th cells into proliferating and activating B cells without IL-2.

This method can be adopted to expand and test cancerous B cells, such as multiple myeloma cells. It can also be used to blast naïve B cells into antibody-producing plasma cells for experimental CAR B cell research. And finally, as CD19 is becoming an important target in cancerous B cells, using the Dynabeads Untouched Human B Cells Kit prohibits CD19 on B cells to be blocked with beads or antibodies.

Authors

Marc McGowan, Anette Kullmann, Axl Alois Neurauter, and Ketil Winther Pedersen, Thermo Fisher Scientific

References

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Ordering information

Product	Cat. No.
Dynabeads Untouched Human B Cells Kit	11351D
Dynabeads Untouched Human CD4 T Cells Kit	11346D
Dynabeads Human T-Activator CD3/CD28	11131D
eBioscience CD4 Monoclonal Antibody, PE	12-0047-42
eBioscience CD19 Monoclonal Antibody, APC	17-0199-42
Ki-67 Monoclonal Antibody, FITC	MHKI6701
SYTOX Blue Dead Cell Stain	S34857

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