A standardized and reproducible method for expansion and activation of human primary B cells

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Introduction

For several decades, activation and proliferation of B cells *in vitro* have been achieved through CD40-Ligand (CD40L)-expressing feeder cell layer systems, continuously improving over the years[1-3]. Many studies have proven the efficacy of such systems for studying diverse B cell functions[2,4,5]. Despite their unarguable usefulness, the systems share a common drawback which is their dependence on CD40L-expressing feeder cell layers. Use of feeder cells impedes standardization of the activation and proliferation process and introduces a variable into the protocol. Considering that B cells are becoming a promising target for cell–based therapeutic approaches, systems that fulfill GMP requirements for B cell stimulation are highly advantageous.
Here we show that using the newly developed B Cell Expansion Kit, human the expansion of B cells reaches an up to 10-fold increase after 14 days of culture *in vitro*. In addition, we describe the phenotype and characteristics of the expanded B cells and examine the effect on these characteristics when using human IL-21, premium grade (Miltenyi Biotec) instead of IL-4 during expansion.

Methods

1 The working principle

Cross-linking of CD40 expressed on B cells leads to cell activation and proliferation. Here we introduce our newly developed B Cell Expansion Kit consisting of a recombinant CD40L multimer, a cross-linking antibody, and IL-4 or IL-21. Combining CD40L together with IL-4, which is a known growth factor for activated B cells, has been shown to be crucial for effective activation and proliferation of B cells[1,2]. Likewise, IL-21 has also been reported to be an effective stimulus of B cell activation and proliferation.[6] However, additionally, IL-21 drives B cell maturation towards a plasma cell phenotype.[6] Notably, this system is able to achieve an activation and proliferation outcome which is comparable to the classical, feeder cell–based NIH3T3/tCD40L protocol[7].

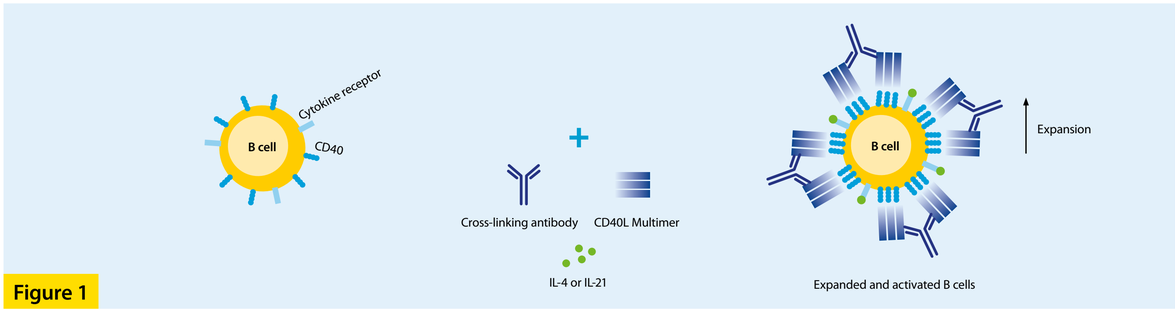

Figure 1

Results

1 Expansion of CD19⁺ B cells

Due to the low frequency of B cells in human peripheral blood, it is hard to obtain sufficient numbers of B cells for research purposes. Therefore, B cell expansion is an important step in human B cell research.
Applying the low-density protocol provided with the B Cell Expansion Kit an increase in the expansion rate of up to 10-fold could be achieved after 14 days of culture. A slight increase in expansion was already detected on days 7 and 10. This effect was further enhanced by replacement of IL-4 with IL-21. The modified procedure resulted in an earlier onset of expansion compared to the standard procedure of the B Cell Expansion Kit. To facilitate expansion, B cells were isolated on day 0 from human buffy coat using CD19 MicroBeads, human after performing a density gradient.

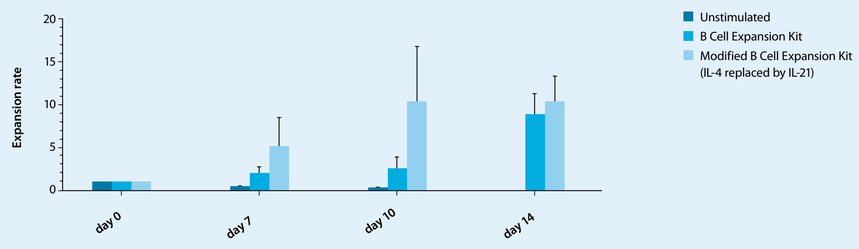

Figure 2

2 Subset distribution of expanded CD19⁺ B cells

To determine the phenotype of isolated B cells and differentiation on day 14 of culture, a gating strategy was developed for analysis of cells by flow cytometry.
After debris and doublets were excluded, viable CD19⁺ cells were selected and analyzed for the proportions of naive and memory cells according to their CD27 and IgD expression (fig. 3A). Viable plasma cells were determined as CD138⁺CD38⁺⁺ cells (fig. 3B). Both graphs show dot blots of unstimulated B cells as examples.
The amounts of naive B cells decreased under all culture conditions on day 14. This is in line with reports describing that naive B cells have a shorter survival rate.[8] In contrast, the amounts of non-switched memory B cells increased in both the unstimulated culture and the culture expanded by the B Cell Expansion Kit and IL-4 indicating a greater survival capacity of this subset. Interestingly, B cells cultured with the kit and IL-4 seemed to resemble untreated B cells in their subset distribution, while B cells cultured with the kit and IL-21 instead of IL-4 varied from cells cultured under the other conditions. In the presence of IL-21, a significant increase in the plasma cell fraction (up to 20% of all B cells) was observed while switched memory B cells doubled from 22 to 45%. This suggests that the culturing condition provided by the B Cell Expansion Kit + IL-21 drives B cell differentiation towards a matured, switched status.

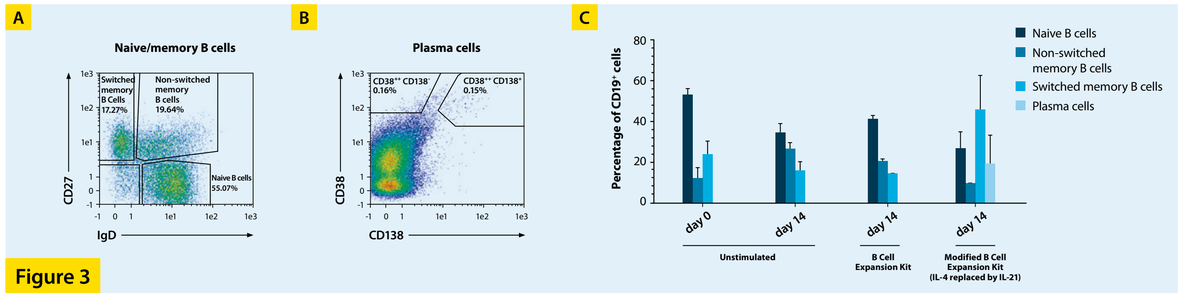

Figure 3

3 Activation state of expanded CD19⁺ B Cells

Expanded B cells were analyzed for their activation status based on the surface expression of the activation markers CD86 and MHCII by flow cytometry. Cells obtained from three healthy donors were cultured with or without the B Cell Expansion Kit (with either IL-4 or IL-21) and pre-gated for viable CD19⁺ cells based on 7-AAD staining.
Cells that were cultured with the kit displayed an increased expression of both markers (fig. 4 A, B). Hence, the B Cell Expansion Kit enables a standardized and reproducible activation and proliferation of B cells *in vitro*. In contrast, use of the B Cell Expansion Kit in combination with IL-21 resulted in the highest expression level of MHCII on day 7, which decreased on day 14. Expression of CD86 was also slightly increased on day 7, but dropped down to base level on day 14. This indicates that IL-4 and IL-21 induced different activation kinetics. IL-4 was described to be important for B cell survival[9], while IL-21 is considered a driver for plasma cell differentiation[6]. These features could be a reason for the different expression of activation markers.

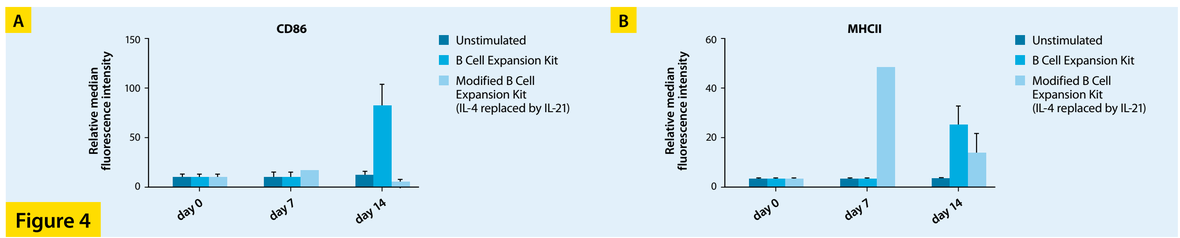

Figure 4

4 IL-4, IL-6, and IL-10 secretion during B cell expansion

IL-6 is a pro-inflammatory cytokine and is secreted by activated B cells, whereas the immune regulatory cytokine IL-10 is secreted with a delay in time. IL-10 is suggested to counterbalance the inflammatory properties of IL-6 and TNF-α during the course of an inflammation process[10]. IL-4–producing B cells are found after immunization or infection, but little is known about the signals regulating this process. It is suggested that IL-4–producing B cells develop in response to interaction with TH2 cells and potentially amplify the TH2 response by activating new naive CD4⁺ T cells[11,12]. In line with this, our data from the B Cell Expansion Kit (with IL-4) demonstrate a high secretion level of IL-6 on day 7 which decreased on day 14 when IL-10 secretion increased. IL-4 could be detected on days 7 and 14, but as this cytokine was added to the culture, further experiments have to be performed to clarify its origin.
In comparison to the B cells cultured with the kit and IL-4, the B cells cultured with the kit and IL-21 displayed only a slight secretion of IL-6 on day 7, but a high secretion level of IL-10. The boost of IL-10 secretion from IL-21–stimulated B cells has been reported in the literature in the context of plasma cell differentiation[13] and further strengthens the observation that different cytokine supply strongly effects the outcome of the B cell fate (fig. 5A-C).

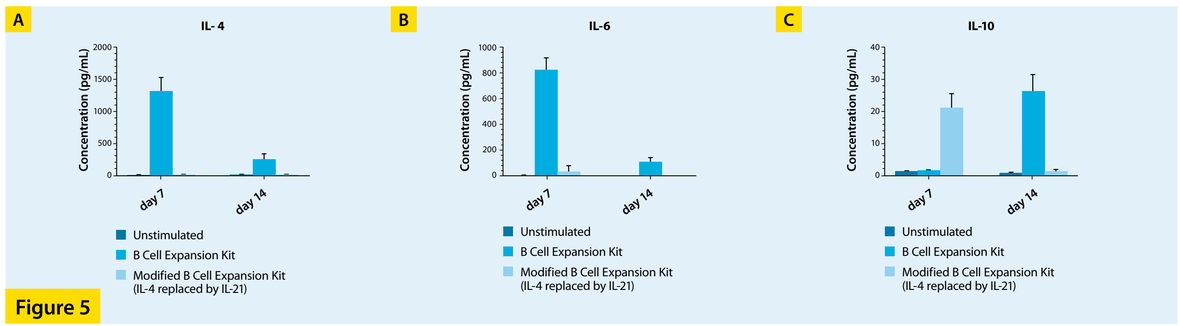

Figure 5

Conclusion

- The combination of CD40L, cross-linking antibody, and IL-4 included in the B Cell Expansion Kit allows an up to 10-fold expansion of B cells *in vitro*.
- Expanded B cells display an activated phenotype with a distribution of memory and plasma cells similar to the original fraction.
- The cytokine secretion pattern of B cells cultivated with the B Cell Expansion Kit shows a peak activation on day 7 and IL-10 counterbalancing from day 7 onward to day 14.
- The B Cell Expansion Kit is a standardized and reproducible system that can be used in future for clinical processes requiring GMP standards.
- B cells expanded in the presence of IL-21 contain a larger number of switched memory cells and plasma cells compared to cells expanded in the presence of IL-4. Additionally, an earlier onset of expansion and an altered cytokine secretion profile could be observed.

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