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The Prospects of Human CD40L-Activated Antigen-Presenting B Cells

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Review Article

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Abstract

B cells are considered as professional antigen presenting cells (APCs) and being used to induce the activation of cognate T cells. Because the number of primary APC from individuals' blood are limited, in vitro activated and expanded B cells represent a good alternative APC source for studying T cell responses against pathogen or cancer. With the discoveries of T-B collaboration and T cell help for B cell activation, several groups have successfully established CD40L-based methods for generating antigen-presenting B cells. This review aims to overview current representative CD40L-based B cell culture methods with their concomitant characteristics, applications, and significances.

Keywords: *CD40L; Antigen-presenting cells; Human; B cells*

Introduction

Given the fact that antigen-presenting cells (APCs) express MHC molecules and costimulatory factors essential for T cell antigen recognition and activation [1], APCs are frequently used to study T cell functions and design T cell based immunotherapy against pathogens and cancers [2]. Activated B cells are considered as professional APCs, along with dendritic cells and macrophages, as they express high levels of MHC class II molecules and costimulatory molecules [3]. Primary APC numbers isolated from individuals are usually inadequate for comprehensive studies, such as TCR epitope discovery. Since B cells are more frequent than dendritic cells and macrophages in the circulation, several in vitro culture systems have been successfully adapted to promote activation and proliferation of B cells, which can be applied as the potential source of APCs [4-18].

CD40 signaling is essential for B cell activation and is induced upon binding to its ligand CD40L primarily expressed on activated T cells [19,20]. The in vitro CD40L system for B cell activation is intentionally designed to mimic germinal centers where B cells are activated through interactions with T cells [4]. In addition to acting as a CD40L donor, T cells offer a set of B cell activation factors, known as T-cell help, such as cytokines, chemokines, and cell-cell contact [20]. Accumulating evidence reveals that stimulation by CD40L with other activators induces the activation and proliferation of B cells to function as APCs [4-18]. The CD40L-based methods for producing antigen-presenting B cells, therefore, can be considered as a powerful research tool in T and B cell studies, which bypasses the limitations of tetramer-based assays and avoids unwanted effects from using virus transformed lymphocytes or lymphoma cells.

In this review, we overview current CD40L-based culture methods on the subject of generating APCs from human primary, non-malignant, B cells for T cell studies. Following an introduction of a short history of T cell help for B cell activation, including interleukin 4 (IL-4), CD40L, B-cell activating factor (BAFF), and IL-21, recent advances inCD40L-based B cell culture systems are carefully summarized and evaluated for their applications and significances. In sum, this review aims to provide a helpful guide for researchers who are interested in finding and utilizing CD40L-based cultures of human B cells as tools in biomedical research.

T Cell Help for B Cell Activation

T and B cells are the two main cell types in the adaptive immune system. They interact closely and frequently to mount timely and efficient immune responses triggered by pathogens, and avoid unwanted immunologic effects induced by harmless antigens [21,22]. The spatially intimate interface between T and B cells allows the contact of surface molecules required for signal transduction on the partner cells, and vice versa [23].

In 1968, Miller and Mitchell discovered T cell help to B cells [24,25]. They showed that individual cell transfers of T cells or B cells into irradiated mice were not sufficient to induce antibody production upon immunization, whereas transfers of both T and B cells were permissive for antibody production [24]. They further demonstrated that B cells are the source of antibodies, whereas T cells are the providers of essential B cell activators [25]. Afterwards, accumulating studies provided further confirmation by testing many other antigens, which were capable of triggering antibody production [26]. Of note, exceptions exist that B cells could be activated by certain antigens in the absence of T cell help [27].

Interleukin-4

IL-4 is the first-identified help factor from T cells to B cells, discovered in 1982, more than a decade after the discovery of T to B help [28]. This soluble factor secreted from mouse EL4 thymoma cells can induce B cell proliferation in synergic with BCR agonist [28]. The discovery of that soluble growth factors of B cells are derived from other immunocytes further illustrates the importance of soluble cytokines in modulation of immune cell communication. Moreover, the Th1-Th2 paradigm was elegantly demonstrated by the biased production of antibodies in mice lacking IL-4 [29].

CD40L

CD40, a type I trans membrane protein, belonging to the tumor necrosis factor (TNF) receptor super family, was discovered in 1986 [30], whose expression is elevated on many cell types including professional APCs [31]. In 1989, Liu et al showed that CD40-CD40L interaction is an essential T cell help for B cell activation [32]; B cells were activated upon CD40 stimulation to be linked to cell division and differentiation. In 1991, Banchereau's group established the pioneer CD40L-based culture method by using stromal cells expressing human Fc receptor combined with anti-CD40 monoclonal antibodies (the CD40 system), to enumerate B cell numbers in antibody production [4]. Banchereau's group also evaluated the effects of IL-2, IL-4, and IL-10 on cell proliferation and antibody production of activated B cells.

Later, CD40L was cloned in 1992 [33, 34], and CD40L-based methods were largely used to activate primary B cells [31]. As mentioned earlier, activated B cells express high levels of MHC class II and costimulatory factors such as CD80 and CD86, which in return, are capable of activating T cells. The in-depth understanding of CD40-CD40L signaling pathways underlying the interaction of B and T cells allows researchers to gain insight into the biology of both cell types.

BAFF

BAFF is a member of the TNF super family and identified by sequence homology search of the databases in 1999 [35]. BAFF receptors and ligands are important for B cell survival and maturation [36]. B cells bind BAFF through several receptors, such as TACI, BCMA, and BAFF-R, which are expressed on B cells undergoing maturation and differentiation from transitional B cell stage to terminal plasmacytic differentiation [37]. BAFF induces signal transduction through these receptors and promotes cell survival, APC function, and differentiation of B cells [37]. Dysregulation of BAFF expression is linked to the development of several autoimmune diseases reflects the significant roles of BAFF in B cell homeostasis [38].

Interleukin-21

IL-21 is a type I cytokine that binds to receptors composed of common-gamma chain and a private receptor [39]. IL-21 was cloned in 2000, and it is considered as the most potent cytokine capable of inducing B cell proliferation and differentiation [40]. IL-21 drives B cells to express transcriptional factors,

including BCL-6, AICDA, and BLIMP-1, known for their role in hypermutation and plasma cell differentiation [41]. In addition to enhancing proliferation of CD40L-stimulated B cells [13], IL-4 and IL-21 work synergistically to trigger class-switch recombination to IgG and the secretion of IgE from activated B cells [40,41]. It was later demonstrated that follicular helper T cells (T_{FH} cells), a specialized T cell subset in the germinal centers, are able to serve the major T cell help to B cells, e.g., CD40L, IL-21, IL-4, and BAFF [42].

Current CD40L-Based B Cell Cultures

As firstly proposed by Banchereau [4], CD40L-based methods were designed to mimic germinal centers where T_{FH} cells release essential factors to activate B cells. CD40 ligation can be achieved through anti-CD40 monoclonal antibodies [4-6], soluble recombinant CD40L molecules [15-18], or co-culturing with cells expressing CD40L [7-14]. Table 1 presents a summary of the CD40L-based methods that have been globally employed to generate antigen-presenting B cells [4-18]. The representative works with their corresponding features are also presented.

UsingCD40L-expressing cells as the source of CD40L are less expensive while stromal cells are favored because they provide much cell-cell contact than suspension cells in supporting B cell growth [43]. CD40L can be constructed in both membranous and soluble forms [10]. Although the expression levels of CD40L by stromal cells may have different effects on B cell activation and differentiation [44], CD40-CD40L signaling is an active termination signal for germinal center reaction [45]. Moreover, researchers can seek CD40L-expressing human stromal cell lines [10] or stromal free systems [4-6,15-18] if there is much concern about xenograft effects. Agonist anti-CD40 antibodies and/or recombinant CD40L molecules are available and can be used to adoptive T cell transfer for cancer immunotherapy [46].

Year	Authors (ref.)	CD40L source	Supplements	B cell populations	Efficiency (B cell number increase in culture)	Culture duration	APC phenotype	T cell function tests	Significances
1991	Banchereau et al. [4, 5]	Anti-CD40 mAb with hFcRII/CDw32 expressing mouse fibroblasts	IL-4	Splenic or tonsillar B cells	400 fold increase in 5 weeks	10 weeks	ND	ND	Pioneer CD40-ligation culture
1995	Schultze et al. [7]	t-CD40L cells (NIH3T3-transfectd with hCD40L)	IL-4	Splenic B cells	ND	ND	MHC I and II, B7-1, B7-2, ICAM-1, LFA-3	(a) Alloreactivity: proliferation and IL-2 production.	First cell-line based CD40L system
1997	Schultze et al. [8]	t-CD40L cells (NIH3T3-transfectd with hCD40L)	IL-4, CsA	Whole PBMC	10^3 fold increase in 16 days; 10^5 fold in 61 days	61 days	MHC I and II, CD54, CD58, CD80 and CD86	(a) Alloreactivity. (b) T cell proliferation, cytokine production, and cytotoxicity. (c) Halplo-mismatched T-B coculture.	Long-term culture up to 61 days. Haplo-mismatched in vitro assay

2002	von Bergwelt-Baildon et al. [15]	GMP-grade trimeric soluble CD40L	IL-4, CsA	Whole PBMC	10^3 fold increase in 16 days	50 days	CD80, CD58, MHC I and II, Ag loading	CTL expansion and lytic effects in response to viral and tumor Ags.	Stromal cell free system
2004	Palena et al. [16]	Soluble non-covalent trimeric CD40L	ND	B cells infected with rF-TRICOM	ND	ND	ND	(a) Alloreactivity. (b) IFN_Y production in response to influenza and HPV peptides.	Consistent expression of B7-1, ICAM-1, and LFA-3 on B cells
2005	Ivanov et al. [10]	293-CD40L-sCD40L cell line	IL-4, IL-10	$CD20^+$ B cells isolated from pre-SCT BM samples of patients with hematologic malignancies	10^5 fold increase in 25 days	25 days	CD80, CD86, MCH I and II	Alloreactivity, IFN_Y production.	Human stromal cell line that expresses both membraneous and soluble CD40L molecules
2005	Yoon et al. [11]	CD40L-expressing Drosophila cell line	IL-4	Total B cells	3 fold increase in 6 days	6 days	CD86, MHC II,	(a) Alloreactivity. (b) CMV Ag presentation: IFN_Y and IL-2 secretion.	hCD40L transduced and expressed on a non-mammal cell line.
2008	Wiesner et al. [12]	CD40Lig-L mouse fibroblast cell line [ref. 9]	IL-4, CsA	PBMC	300 fold increase in 27 days	up to 1600 days	MHC I and II, CD80, CD86	(a) 693-day-old CD40-stimulated B cells as APCs. (b) Expansion of CD8 T cells specific to viral or melanoma antigen.	Long-term culture beyond two years
2009	Carpenter et al. [6]	Agonist CD40 monoclonal antibody CP-870893	+/- CpG	Total $CD19^+$ B cells; $CD9^+$ $CD27^+$ memory B cells; $CD19^+$ $CD27^-$ naive B cells	ND	48 hours	CD86, CD54, MHCI and II, CD70	Alloreactivity: proliferation, IFN_Y and IL-2 secretion.	Clinical trial medication

2013	Naito et al. [17]	Recombinant CD40L-Tri	IL-4	$CD19^+$ B cells	30 fold increase in 14 days; 18 fold increase in day 28	28 days	CD80, CD86, CD83	(a) Alloreactivity. (b) CD8 T cells response (proliferation and cytokine IFN_Y production) to influenza peptide.	Another trimeric CD40L. Stromal cell free system
2014	Garcia-Marquez et al. [18]	Multimerized rCD40L	IL-4	$CD19^+$ B cells	200 fold increase in 14 days	14 days	CD80, CD86, HLA-DR, CD62L	Alloreactivity	Stromal cell free system
2016	Su et al. [13]	MS40L mouse stromal cells	IL-2, IL-4, IL-21, BAFF	Naïve B cells; Memory B cells; TT-specific memory B cells	10^6 fold increase in 16 days.	16 days	MHCII, CD80, CD86	(a) Alloreactivity. (b) T cell proliferation against microbial Ag (HA, TT). (c) TT-specific CD4 T-cell repertoire.	The most efficient system. Expanded TT-specific memory B cells as APCs
2016	Zhang at el. [14]	293-CD40L-sBAFF cell line	IL-2, IL-4, IL-10, CsA, CpG	B cells from PBMC	21 fold increase in 35 days	35 days	CD80, CD86, CD70, CD275	(a) Alloreactivity. (b) CTL response to HIV and melaloma antigens: proliferation, IFN_Y secretion, cytotoxicity.	Soluble BAFF secreting cell line

Table 1: Description of CD40L-based cultures, cytokines, efficiency, readouts, and significances.
CsA, cyclosporin A; Ag, antigen; TT, tetanus toxoid; ND, not determined

Cytokines and Supplements

Among the cytokines and their related supplements, IL-4 is widely used in almost all of CD40L-based cultures since its discovery [28]. Cyclosporin A is applied in B cell culture when B cells are not directly isolated from PBMC [8,12,15]. Several TLR agonists, such as CpG, can be supplemented to induce B cell activation through a CD40L-independent manner [47]. IL-21 was discovered more than a decade ago and it has been recognized as the most efficient cytokine among soluble T cell help factors [39,48]. Indeed, the addition of IL-21 was shown to promote generating large number of B cells that are capable of acting as APC [13]. The introduction of BAFF to the CD40L-based culture seems to produce beneficial effects on cell survival but not cell proliferation [13,14].

Characteristics and Applications

Functional characteristics of B cells derived from CD40L-based cultures to be used as APCs are determined

by certain representative surface markers and their ability to uptake and to present antigens to induce T cell activation. Alloreactivity assay is relatively easy to perform and is commonly used to score APC function of cultured-expanded B cells. However, since the mechanism of alloreactivity is not entirely understood [49], APC function of cultured B cells should be further confirmed by antigen presentation to autologous T cells. Meanwhile, appropriate (positive and negative) controls should always be included in testing APC function and T cell responses to facilitate accurate interpretation.

The B cell sources for culture can be whole PBMC, isolated $CD19^+$ B cells, or B cell subsets (e.g., naïve B cells, memory B cells, or selected antigen memory B cells) according to the study designs of the culture system. With the generation of enormous amounts of antigen antigen-presenting B cells, a considerable number of T cell studies have been conducted (not listed). For example, CD4 T cell differentiation and repertoire can be evaluated [13]. The application of adoptive CD8 T cells transfer is proposed as anti-HBV immunotherapy [50]. Furthermore, cognate interactions between T and B cells specific to antigens of interest can be assessed [51].

Concluding Remarks

T cells are the primary sources of membranous and soluble factors known to be essential for B activation. The CD40L-based in vitro culture system resembles in vivo germinal centers where T_{FH} cells tightly react with B cells [41]. This review summarizes current representative CD40L-based culture methods for generating antigen-presenting B cells and these methods are widely used in many T cell studies. Moreover, the adapted culture systems can be further used to enhance APC function in order to fit the specific needs whenever necessary. Finally, with the ability to culture antigen-specific memory B cells, it is conceivably expected that cognate T and B interactions can be more easily scrutinized and to be considered as part of precision medicine.

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