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Effective collaboration between IL-4 and IL-21 on B cell activation

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Abstract

Although IL-4 and IL-21 synergistically promote proliferation and differentiation of activated B cells, the mutual role in their collaboration is not known. When splenic B cells were sequentially stimulated with anti-IgM Ab and anti-CD40 Ab plus IL-4 and then with IL-21 at a 2-day interval, proliferation, frequency of class switching to IgG1 and plasma cell differentiation were continuously enhanced until day 5 of culture. Amounts of *AID* and *Blimp1* mRNA in sequentially activated B cells with IL-4 and IL-21 increased more than those in activated B cells without IL-21. However, sequential stimulation of B cells with anti-IgM Ab and anti-CD40 Ab plus IL-21 and then with IL-4 at more than 1-day interval did not display the synergistic effect. Furthermore, sequential stimulation of activated B cells with a low dose of IL-4, which did not induce Ig class switching, at the beginning of culture and with IL-21 or IL-4 on day 2 of culture induced proliferation and differentiation of $CXCR4^-$ or $CXCR4^+$ B cells, respectively. Thus, IL-21 effectively promotes proliferation and differentiation of $CXCR4^-$ B cells pre-activated with anti-IgM Ab and anti-CD40 Ab plus IL-4.
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Keywords: B lymphocytes; Cellular differentiation; Ig class switching; Cytokines

Introduction

Interleukin-21 (IL-21) mainly produced by $CD4^+$ T cells drives differentiation of activated B cells into plasma cells. The differentiation is induced by co-activation of B cells with signals from helper T cells and IL-21 through up-regulation of B-lymphocyte-induced maturation protein 1 (*Blimp1*) mRNA (Ozaki et al., 2004). In addition to *Blimp1* expression, IL-21 also induces expression of a transcriptional repressor

Abbreviation: AID, activation-induced cytidine deaminase; *Blimp1*, B-lymphocyte-induced maturation protein 1; CFSE, carboxyfluorescein diacetate succinimidyl ester; IL-21, interleukin-21; S.D., standard deviation; Synd, syndecan-1; Tfh, T follicular helper.

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factor, *Bcl6*, in these populations (Ozaki et al., 2004), even though *Blimp1* and *Bcl6* are known to repress transcription of the genes encoding each other (Angelin-Duclos et al., 2000; Reljic et al., 2000; Shaffer et al., 2000). The other actions of IL-21 in the generation of antibody responses *in vivo* have been demonstrated using IL-21-transgenic mice (Ozaki et al., 2004). Strikingly, however, the ectopic IL-21 induced an increase in the number of total B cells and a marked increase in the generation of both class switched B cells and plasma cells. The increase in the number of plasma cells in IL-21-transgenic mice correlated with increased concentrations of serum IgM and IgG1 and, correspondingly, with more numbers of cell-surface $IgG1^+$ B cells. The increased concentration of serum IgG1 in these mice is also consistent with *in vitro* analyses of human B cells in which IL-21 was shown to induce class switching to IgG1 and IgG3 (Pene et al., 2004). These results indicate that stimulation of activated B cells with IL-21 induces not only plasma cell differentiation but also cell proliferation and Ig class switching.

IL-21 has a pro-apoptotic effect on freshly isolated B cells. Although the IL-21 receptor contains the common cytokine-receptor γ-chain, which has an anti-apoptotic effect on their target cells (Habib et al., 2003; Ozaki et al., 2002), this pro-apoptotic effect is more marked for B cells that have been stimulated with LPS (Jin et al., 2004; Jin and Malek, 2006; Mehta et al., 2003; Ozaki et al., 2004). When cell-surface IgM or CD40 on primary B cells is cross-linked by anti-IgM Ab or anti-CD40 Ab, IL-21 has a potent co-stimulatory effect on B cell proliferation until 48 h after stimulation (Jin et al., 2004; Ozaki et al., 2004). However, IL-21 induces apoptosis of those activated B cells when those B cells were analyzed 90 h after stimulation (Mehta et al., 2003). Thus, the effect of IL-21 on proliferation of activated B cells is transient.

IL-4 is a class switching factor in activated B cells and augments their proliferation, and collaboration between IL-4 and IL-21 was observed on proliferation of primary B cells activated with anti-CD40 Ab but not with anti-IgM Ab (Mehta et al., 2003; Jin and Malek, 2006; Ozaki et al., 2004). Although IL-4 and IL-21 synergistically promote differentiation of B cells activated with LPS and anti-CD40 Ab (Jin and Malek, 2006), the mutual role of them in differentiation of primary B cells activated with anti-IgM Ab and anti-CD40 Ab has never been reported. Here we show that sequential stimulation of splenic B cells with anti-IgM Ab and anti-CD40 Ab plus IL-4 and then with IL-21 at a 2-day interval strongly enhanced proliferation and differentiation of those activated B cells. However, the sequential stimulation of activated B cells with IL-21 and then with IL-4 at more than 1-day interval did not display the enhancement. Since IL-21 is produced predominantly by T follicular helper (Tfh) cells (Chtanova et al., 2004) which are critical for germinal center formation of activated B cells (Akiba et al., 2005), we examined expression of germinal center B cell markers on those sequentially activated B cells. Surprisingly, re-stimulation of activated B cells with IL-21 or IL-4 on day 2 of culture induced proliferation and differentiation of a distinct subset ($CXCR4^-$ or $CXCR4^+$) of activated B cells, respectively. As CXCR4 expression on germinal center B cells distinguishes centroblasts from centrocytes (Allen et al., 2004), we discuss a role for IL-21 and IL-4 in proliferation and differentiation of activated B cells including germinal center B cells.

Materials and methods

Mice

C57BL/6 mice were purchased from Japan SLC Co. Ltd. (Hamamatsu, Japan). All mice were maintained under specific pathogen free conditions in the animal center of the Graduate School of Medicine, Chiba University. The care of all animals used in the present study was in accordance with Chiba University Animal Care guidelines.

Purification of splenic B cells

Splenic B cells were enriched by depleting non-B lineage cells from spleen cells. In brief, spleen cells were incubated with biotin-anti-mouse CD43 Ab and biotin-anti-mouse IgG1 Ab (BD PharMingen, San Jose, CA). These cells were subsequently reacted with immunomagnetic beads conjugated to streptavidin (Miltenyi Biotec, Gladbach, Germany). Labeled cells were removed by a MACS system (Miltenyi Biotec). The resulting B cell fraction contained >95% of $B220^+$ B cells.

Cell culture

Purified B cells ($5.0 \times 10^5\,ml^{-1}$) were cultured in RPMI medium (Sigma Chemical Co., St Louis, MO) supplemented with 10% FCS (Intergen, New York, NY), 50 μM 2-ME, 10 mM HEPES, 100 μg/ml of streptomycin (Wako Chemical Co., Osaka, Japan) and 100 U/ml of penicillin G potassium (Banyu Pharmaceutical Co., Tokyo, Japan). For B cell activation, purified B cells were stimulated with F(ab)2 fragments of anti-mouse IgM Ab (1.0×10^{-5} μg/ml, Alpha Diagnostic Co., San Antonio) and anti-mouse CD40 monoclonal Ab (1.0 μg/ml, BD PharMingen). These B cells were cultured with rIL-4 (2–20 U/ml) (Karasuyama and Melchers, 1988) in the presence or absence of IL-21 (30 ng/ml, R&D Systems, Inc.) (Jin et al., 2004) in a

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