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Toll-like receptor 7 cooperates with IL-4 in activated B cells through antigen receptor or CD38 and induces class switch recombination and IgG1 production

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ABSTRACT

IL-4 and 8-mercaptoguanosine (8-SGuo) stimulation of CD38-activated B cells induces μ to γ1 class switch recombination (CSR) at the DNA level leading to a high level of IgG1 production. Although some of signaling events initiated by IL-4 in activated B cells have been characterized, the involvement of TLR/MyD88 and Btk pathway in IL-4-dependent μ to γ1 CSR has not been thoroughly evaluated. In this study, we characterized receptors for 8-SGuo and differential roles of 8-SGuo and IL-4 in the induction and μ to γ1 CSR and IgG1 production. The role of TLR7 and MyD88 in 8-SGuo-induced AID expression and μ to γ1 CSR was documented, as 8-SGuo did not act on CD38-stimulated splenic B cells from *Tlr7*$^{-/-}$ and *Myd88*$^{-/-}$ mice. CD38-activated B cells from Btk-deficient mice failed to respond to TLR7 ligands for the AID expression and CSR, indicating that Btk is also indispensable for the system. Stimulation of CD38-activated B cells with 8-SGuo induced significant AID expression and DNA double strand breaks, but IL-4 stimulation by itself did not trigger μ to γ1 CSR. Intriguingly, the μ to γ1 CSR in the B cells stimulated with CD38 and 8-SGuo totally depends on IL-4 stimulation. Similar results were obtained in the activated B cells through BCR and loxoribine, a well-known TLR7 ligand, in place of 8-SGuo. *In vivo* administration of TLR7 ligand and anti-CD38 antibody induced the generation of CD138^{+} IgG1^{+} cells. These results indicate that TLR7 is a receptor for 8-SGuo and plays an essential role in the AID and Blimp-1 expression; however it is not enough to complete μ to γ1 CSR in CD38-activated B cells. IL-4 may be required for the induction of DNA repair system together with AID for the completion of CSR.

1. Introduction

During immune responses, B cells encounter Ags, and respond to them within the context of cytokines and microbial components, with or without T-cell help. The net result is to drive B cells to proliferate and differentiate into Ab-secreting plasma cells. These processes are highly regulated by signals delivered via B cell antigen receptors (BCR), cytokine receptors (e.g., IL-4R and IL-5R), transmembrane molecules (e.g., CD40 and CD38), and receptors for microbial or viral products, such as the toll-like receptors (TLRs). This causes up-regulation of critical enzymes and transcriptional factors, such as activation-induced cytidine deaminase (AID) and B lymphocyte-induced maturation protein (Blimp-1). AID is a crucial factor for class switch recombination (CSR), a complex process of genomic DNA recombination between switch regions in gene clusters of BCR (Muramatsu et al., 1999, 2000). This process includes DNA double strand breaks (DSBs) and DNA repair. Blimp-1 is required for terminal differentiation of B cells to Ig-secreting cells (Lin et al., 2002; Shaffer et al., 2002, 2004). The underlying molecular mechanisms are being intensively investigated, but little is known about how B cells integrate signals for CSR and differentiation into Ig-producing cells.

T cells deliver signals to the B cell via surface CD40 ligand and cytokines such as IL-4, IL-5 and IL-6 that are indispensable for B

Abbreviations: 8-SGuo, 8-mercaptoguanosine; loxoribine, 7-allyl-8-oxoguanosine; 8-BrGuo, 8-bromoguanosine; 6-SGuo, 6-mercaptoguanosine; 8-BrA, 8-bromoadenosine; Blimp-1, B lymphocyte maturation protein 1; AID, activation-induced cytidine deaminase; RP105, radioprotective 105; HPRT, hypoxanthineguanine phosphoribosyltransferase.

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cell proliferation and differentiation. In the mouse, IL-5 supports B-1 cell proliferation, survival, and IgM production (Mizoguchi et al., 1999; Horikawa et al., 2001). IL-5 also acts to increase CSR frequency and differentiation of conventional B (B-2) cells in activated stages to antibody-secreting plasma cells in STAT5-dependent manners (Mizoguchi et al., 1999; Horikawa et al., 2001). When murine spleen B cells are stimulated with IL-5 in combination with CD38 ligation by agonistic anti-CD38 antibody (αCD38), AID expression and μ to γ1 CSR are elicited and IgG1 production is induced along with Blimp-1 expression (Mizoguchi et al., 1999). IL-4 is both a survival factor for B cells and a prominent inducer of CSR primarily to IgG1 and IgE in the presence of CD40 ligand or lipopolysaccharide (LPS) (Snapper et al., 1991; Shapira et al., 1992; Siebenkotten et al., 1992). Intriguingly, IL-4 stimulation of αCD38-stimulated B cells does not induce μ to γ1 CSR and IgG1 production, indicating that a costimulus is required for μ to γ1 CSR (Mizoguchi et al., 1999; Horikawa et al., 2001).

A group of synthetic nucleoside analogs have the ability to activate immunocytes including B cells (Goodman and Weigle, 1983a,b, 1985, 1986; Goodman et al., 1995; Pope et al., 1995). Among these compounds, the C8-substituted guanosine analogs have been shown to have potent adjuvant activities. An 8-mercaptoguanosine (8-SGuo), a C8-substituted analog is a potent activator for B cells (Goodman and Weigle, 1983a,b, 1986) and supports CSR to IgE in B cells stimulated with αIgM plus IL-4 (Hikida et al., 1996). We reported that the addition of 8-SGuo with IL-4 to the culture of αCD38-activated B cells can induce μ to γ1 CSR and IgG1 production (Tsukamoto et al., 2005). Although 8-SGuo by itself induces AID and Blimp-1 expression in CD38-activated B cells, it does not induce μ to γ1 CSR at the DNA level (Tsukamoto et al., 2005). We imply that 8-SGuo gives us a useful tool for investigating signals required for the CSR induction and terminal differentiation of activated B cells.

TLRs are pattern-recognition receptors that sense well-conserved microbial components (Akira et al., 2006). For example, TLR4 recognizes LPS in combination with MD-2 (Shimazu et al., 1999; Nagai et al., 2002). Nucleic acid-containing antigens are also recognized via intracellular TLRs such as TLR9 and TLR7. TLR9 recognizes bacterial CpG DNA (Hemmi et al., 2000), while TLR7 recognizes small antiviral compounds such as imidazoquinolines, imiquimod, and R-848 (Hemmi et al., 2002). TLR7 also recognizes guanosine analogs such as 7-allyl-8-oxoguanosine (loxoribine) (Lee et al., 2003). As TLR4 stimulation augments IgE and IgG1 production in B cells together with IL-4 (Snapper et al., 1991; Siebenkotten et al., 1992), TLR9 ligand stimulation induces IgG2a, IgG2b, and IgG3 production in mouse B cells activated with CD40 ligand and IL-4 (Lin et al., 2004) and in human B cells stimulated with BCR (Fischer, 2004). It inhibits, however, IgE and IgG1 production in mouse B cells (Liu et al., 2003). It is uncovered how TLR signals regulate CSR and B cell differentiation into antibody-forming cells.

In the present study, we extended our approach to clarify roles of 8-SGuo and IL-4 in the μ to γ1 CSR and IgG1 production and analyze receptors for 8-SGuo. Here we report that 8-SGuo induces the AID expression and DSBs through TLR7/MyD88/Btk in B cells activated via CD38 or BCR. IL-4 is indispensable for the induction of μ to γ1 CSR at the DNA level and IgG1 production. Our results indicate that signals delivered via TLR7 in B cells are integrated with those from CD38 or BCR and have previously unsuspected roles in CSR and antibody production in B cells.

2. Materials and methods

2.1. Mice

C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan) and CLEA Japan, Inc. (Tokyo, Japan), and were used at 7–10 weeks of age. C57BL/6, *Myd88*$^{-/-}$ (Adachi et al., 1998), *Tlr7*$^{-/-}$ (Hemmi et al., 2002), *Btk*$^{+/Y}$, and *Btk*$^{-/Y}$ mice were maintained in microisolator cages under specific pathogen-free conditions, and maintained in the animal facility of the Institute of Medical Science, The University of Tokyo. All experiments were performed according to the guidelines for the care and treatment of experimental animals at the Institute of Medical Science, the University of Tokyo.

2.2. Reagents

Recombinant mouse IL-4 was purchased from R and D Systems (Mineapolis, MN). Lipid A from *Salmonella minnesota* and 8-mercaptoguanosine (8-SGuo) were purchased from Sigma–Aldrich (St. Louis, MO). Loxoribine (7-allyl-8-oxoguanosine) was purchased from InvivoGen (San Diego, CA). CpG-B was purchased from Hycult biotechnology (Uden, The Netherlands). Carboxyfluorescein diacetate succinimidyl ester (CFSE) was purchased from Molecular Probes (Carlsbad, CA). Streptavidin-conjugated magnetic beads was purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Mouse IL-5 was purified according to previously described procedures (Mita et al., 1989).

2.3. Antibodies and flow cytometry

Purified anti-mouse CD38 mAb (clone CS/2) was prepared as previously described (Yamashita et al., 1995). Anti-mouse CD40 mAb (clone LB429) was kindly gifted from Dr. Nobuo Sakaguchi (Kumamoto University). Anti-mouse IgM F(ab′) fragment was purchased from Jackson Immunoresearch (West Grove, PA).

The following antibodies for flow cytometry were purchased from BD Pharmingen (San Diego, CA): biotinylated anti-CD43 (clone S7), biotinylated anti-CD138 (clone 281-2), biotinylated anti-IL-4Rα (CD124; clone mIL4R-M1), biotinylated anti-CD38 (clone 90), and PE-conjugated anti-mouse IgG1 (A85-1).

The following antibodies for flow cytometry were purchased from eBioscience (San Diego, CA): FITC-conjugated anti-CD45R/B220 (clone RA3-6B2), APC-conjugated anti-CD45R/B220 (clone RA3-6B2), PE-conjugated anti-CD86 (clone GL1). For analyzing cultured cells by flow cytometry, 7-amino-actinomycinD (Sigma Fine Chemical Co., St. Louis, MO) to exclude dead cells.

The cells (1×10^5) were incubated with purified anti-mouse FcγR (clone 2.4G2) to block binding of the labeled Abs to FcγR. After 15 min, the cells were stained with predetermined optimal concentrations of the respective Abs. Flow cytometry analyses were conducted on a FACSCalibur (Becton Dickinson and Co., Mountain View, CA), and the data were analyzed with Flowjo software (Treestar, San Carlos, CA).

2.4. B cell preparation and proliferation in vitro

Spleen cells were prepared from 7 to 10-week-old mice, and their B cells were isolated by magnetic depletion of other cells with CD43-specific magnetic microbeads (Miltenyi Biotec). The purified B cells contained >95% B220$^+$ cells, as assessed by fluorescence analysis using the FACSCalibur (Becton Dickinson).

For ^{3}H-Thymidine uptake, B cells were cultured with their respective stimuli in 96-well plates at a density of 5×10^5 cells/ml. After 72 h of culture, B cells were pulse-labeled with [^{3}H]thymidine (0.2 μCi/well, Amersham Life Science, Little Chalfont, UK) and cultured for additional 6 h. After the culture, the cells were harvested with Cell Harvester (Inotech, Rockville, MD) and the [^{3}H]thymidine incorporated into cells were measured by 1450 Microbeta Jet (PerkinElmer, Wallesley, MA) following the manufacture's instructions.

For the measurement of cell division, B cells were stained with CFSE. Cells were suspended in 1% FCS-RPMI containing 1 μM CFSE,