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Research paper

# Interferon-gamma and B-cell Activating Factor (BAFF) promote bovine B cell activation independent of TLR9 and T-cell signaling

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ABSTRACT

We previously reported that CD21$^+$ B cells purified from bovine blood do not respond to CpG-ODN stimulation unless either CD14$^+$ monocytes or B-cell Activating Factor (BAFF), a cytokine produced by activated monocytes, are present. In this report, we present evidence that CD14$^+$ monocytes are critical for CpG-specific lymphocyte proliferation within the peripheral blood mononuclear cell (PBMC) population but that this response is not mediated by soluble factors produced by CpG-activated monocytes. We further determine that bovine monocytes stimulated with IFN-γ induce expression of the BAFF gene and that recombinant IFN-γ and BAFF induced robust B cell activation when cultured in the absence of CpG ODN. These data suggest that CpG-stimulated monocytes may indirectly promote B cell activation by promoting release of cytokines and/or other soluble factors from accessory cells which in turn act on CpG-stimulated B cells to promote antigen-independent and T cell independent B cell activation. Understanding the T cell independent signals that induce B cell activation has important implications for understanding B cell development in locations where T cells are limited and in understanding polyclonal B cell activation that may contribute to autoimmune diseases.



## 1. Introduction

The innate immune system employs receptors such as Toll-Like Receptors (TLRs) which recognize highly conserved structures present within many microorganisms (Janeway, 1989; Janeway and Medzhitov, 1998) to induce and influence the magnitude of adaptive immune responses. TLR9 recognizes hypomethylated CpG motifs which are characteristic of bacterial, viral and protozoal DNA and which can be mimicked by synthetic, hypomethylated oligodeoxynucleotides (ODNs) (Hemmi et al., 2000). Upon TLR9-CpG ODN interaction, CpG ODN stimulates monocytes to express proinflammatory cytokines which in turn promotes release of IFN-γ from natural killer cells (Bauer et al., 1999; Brown et al., 1998; Cowdery et al., 1996; Hartmann and Krieg, 1999; Jakob et al., 1998; Krieg et al., 1995; Krug et al., 2001; Messina et al., 1991; Qiao et al., 2005; Shoda et al., 2001; Sparwasser et al., 1997; Stacey et al., 1996; Takeshita and Klinman, 2000). IFN-γ triggers myeloid cell-derived B-cell Activating Factor (BAFF) expression which in turn promotes B-cell survival, proliferation, and Ig class switching (Bergamin et al., 2007; Craxton et al., 2003; Jego et al., 2005; Litinskiy et al., 2002). CpG ODN has also been shown to promote expression of BAFF or a BAFF family member in murine B cells (Chu et al., 2007; Goto et al., 2008) suggesting that B cell survival, proliferation, and differentiation are supported by paracrine and autocrine pathways.

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Whether naïve murine or human B cells respond to CpG ODN remains controversial and may be highly dependent on the B cell subtype under investigation and their state of maturation. For instance, Krieg et al. (1995) reported that murine splenic B cells were directly activated by CpG motifs in bacterial DNA, however, the cell population used was depleted of T cells but they were not specifically depleted of myeloid cells, such as monocytes and DCs, or NK cells (Krieg et al., 1995). Bernasconi et al. (2003) showed that highly purified human naive B cells proliferate, undergo isotypic switch to IgG and IgA, and differentiate into antibody secreting cells in response to TLR9 agonists only when the BCR is engaged and in the presence of cognate T-cell help (Bernasconi et al., 2003). Ruprecht and Lanzavecchia (2006) showed that antigen-specific proliferation and differentiation of highly purified human naïve B cells requires a third stimulus such as that provided by triggering of TLRs on B cells or by cytokines produced by TLR-activated DCs (Ruprecht and Lanzavecchia, 2006). In contrast, Jiang et al. (2007) reported that independent from other cell types and in the absence of B cell receptor stimulation, CpG ODN efficiently induced human, blood-derived, naïve B cell proliferation, differentiation into IgM-secreting cells and increased surface expression of HLA-DR, CD40 and CD80 (Jiang et al., 2007). These expanded naïve B cells failed, however, to mature into $CD27^+$ or $IgG^+$ memory B cells. We reported that although bovine blood-derived $CD21^+$ B cells express TLR9 and proliferate in response to CpG in mixed-cell populations, purified bovine B cells do not proliferate significantly in response to CpG ODN, even when the B cell receptor is engaged (Buchanan et al., 2011). When co-cultured with $CD14^+$ myeloid cells and/or B-cell Activating Factor (BAFF), a cytokine produced by activated myeloid cells, there was a significant increase in CpG-specific B cell proliferation and an increased number of large B cells expressing the activation marker, CD25. These data suggest that activated myeloid cells and BAFF prime B cells for significant CpG-specific activation.

Observations by Pontarollo et al. (2002) and Buchanan et al. (2011) indicated that myeloid cells depleted from bovine PBMCs reduced CpG-induced B cell proliferation (Buchanan et al., 2011; Pontarollo et al., 2002). These observations suggest two scenarios for B cell activation: (1) Myeloid cells are the primary cells responsive to CpG ODN which, upon activation, produce one or more factors which augment B cell activation, or (2) CpG ODN directly acts on B cells but myeloid cells produce one or more factors which augment CpG-induced B cell activation and proliferation. In the current report, we seek to clarify which of these mechanisms may be correct. A better understanding of the role of TLR agonists and co-signaling cytokines in stimulating antigen-independent and T cell-independent B cell activation will further define how innate and adaptive immune responses are integrated.

## 2. Materials and methods

### 2.1. Animals

Cattle used throughout these studies were castrated males of mixed breed between 6 and 12 months of age. All animals were housed at the Vaccine and Infectious Disease Organization animal facility and all experimental procedures were conducted in accordance with the Guide to the Care and Use of Experimental Animals, provided by the Canadian Council on Animal Care.

### 2.2. Oligodeoxynucleotides

Class C CpG 2429 (5′ TCGTCGTTTTCGGCGGCCGCCG 3′) was purchased from Qiagen Operon (Alameda, CA, USA).

### 2.3. Cytokines

Recombinant bovine IFNγ was supplied by Ciba Geigy (Basel, Switzerland) and recombinant human BAFF (2149-BF) was obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Unless otherwise indicated, cytokines were used at a final concentration of 10 ng/ml.

### 2.4. PBMC isolation

PBMCs were isolated following the protocol described in Buchanan et al. (2011).

### 2.5. MACS isolation of $CD14^+$ monocytes, $CD21^+$ B lymphocytes and PBMCs MACS-depleted of $CD14^+$ monocytes

Magnetic labeling was performed according to the protocol provided by the manufacturer (Miltenyi Biotec Inc., Auburn, CA, USA). To prevent myeloid contamination of the B cell population, PBMCs were subjected to $CD14^+$ MACS-depletion prior to $CD21^+$ B cell isolation as detailed in Buchanan et al. (2011). MACS-purified $CD14^+$ monocytes and $CD21^+$ B cells, and the PBMC-$CD14^+$ cell populations were characterized as by subsequent flow cytometric analysis.

### 2.6. Flow cytometry analysis of cell phenotypes and cell surface marker expression

For FACS analysis was performed as detailed in Buchanan et al. (2011). Results indicate that $CD21^+$ B cells constituted $26.6\% \pm 9.0$ of the PBMC population. MACS-purification resulted in a highly enriched $CD21^+$ B cell population (purity = $98.04\% \pm 0.85$ $CD21^+$ B cells; $n = 6$). Bovine PBMCs contained $14.6\% \pm 4.6$ $CD14^+$ cells, and the purity of MACS-selected $CD14^+$ myeloid cell population was $97.8\% \pm 1.6$ ($n = 5$ animals) (data not shown).

For Fig. 5, FACS analysis is presented as a scatter plot of side-scatter ($y$-axis; SCC-Height) versus $\log_{10}$ forward angle light scatter ($x$-axis; FSC-Height) for each stimuli and control cell population. All viable cells in gate R2 were quantified. For each biological replicate ($n = 4$), the number of cells in R2 from each stimulated population was divided by the number of cells in R2 from the relevant control (media) population to generate the fold change in Table 1.