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Blood stage *Plasmodium falciparum* antigens induce T cell independent immunoglobulin production via B cell activation factor of the TNF family (BAFF) pathway

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ABSTRACT

T independent (TI) antigens (Ags) activate monocytes to produce a cytokine, termed B cell activation factor (BAFF), involved in immunoglobulin (Ig) production. This study aimed to investigate whether the soluble schizont fraction of *Plasmodium falciparum* antigen (sPfAg) and hemozoin (HZ) could act as TI Ag to induce *P. falciparum* (Pf) specific Ig production via BAFF pathway. Co-cultures of monocytes and naïve B cells from 6 healthy donors were stimulated with sPfAg (10 mg/ml) or HZ (10 μM). At interval times, the expressions of BAFF on activated monocytes, BAFF receptor (BAFF-R) and proliferation nuclear Ag in activated B cells were determined by flow cytometry. The soluble BAFF (sBAFF), total and specific IgG levels in the supernatants were assessed by enzyme-linked immunosorbent assay (ELISA). The finding revealed both sPfAg and HZ could activate monocytes to express BAFF on surface and release sBAFF in the supernatant within 72 h of stimulation. The B cells responded to specific activation, indicated by BAFF-R expression on the surface within 72 h, marked proliferation on day 7, and final production of total and specific IgG during days 7–12. Comparing to sPfAg, HZ stimulated monocyte and B cell co-culture to express higher levels of BAFF and sBAFF during 24–48 h, more BAFF-R on HZ activated B cells within 24 h and induced marked proliferation of B cells with higher Pf specific IgG level. However, stimulation with sPfAg showed a more significant correlation between BAFF expression on the activated monocytes at 72 h and the Pf specific IgG level on day 12 ($r = 0.961$, $p = 0.039$, Pearson Correlation). In conclusion, it is possible that both sPfAg and HZ stimulated B cells to produce specific IgG with BAFF involvement.

1. Introduction

When antigen (Ag) enters the body, antibody diversity is essential for B cells to mount protective responses against invading pathogens. Naïve B cells undergo Ag-dependent antibody diversification through immunoglobulin (Ig) V(D)J gene somatic hypermutation and immunoglobulin heavy chain (IgH) class switching. During a T dependent (TD) immune response, IgH class switching occurs through class switch recombination (CSR) which requires two signals, one provided by CD40 ligand (CD40L), a tumor necrosis factor (TNF) family member expressed by Ag-activated $CD4^+$ T helper cells (Th), and the other provided by a Th-derived cytokines, including interleukin (IL)-4, IL-10 or transforming growth factor (TGF)-β (Storb and Stavnezer, 2002).

In T independent (TI) immune response, Ig class switching can occur though B cell activating factor (BAFF), a novel member of the TNF family protein (Schneider et al., 1999), and/or a proliferation-inducing ligand (APRIL), another member of the TNF family (Stein et al., 2002). BAFF is produced predominantly by myeloid cells (monocytes, macrophages, dendritic cells), neutrophils (Scapini et al., 2005), and also B lymphocytes (Chu et al., 2007). In addition, BAFF mRNA expression can be detected in resting and stimulated T cells, mast cells (Ng et al., 2004), and stromal cells (Gorelik et al., 2003). BAFF plays a fundamental role in B cell physiology including differentiation, proliferation and Ig production (Litinskiy et al., 2002; Craxton et al., 2003).

In the process of the TI immune response, when viral or bacterial antigens (Ags) are taken up, dendritic cells or macrophages or antigen presenting cells (APCs) trigger the production of interferon

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(IFN)-α/β and TGF-β that could up-regulate expression of BAFF and APRIL. IL-10, IFN-γ, and bacterial components such as lipopolysaccharide (LPS) and peptidoglycan also enhance BAFF expression (Litinskiy et al., 2002; Craxton et al., 2003). Both BAFF and APRIL bind to three receptors expressed on B cells including B cell activating factor-receptor (BAFF-R), B cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). Then, BAFF and APRIL interactions with their respective receptors induce CD40-independent CSR through the action of nuclear factor kappa B (NF-κB). Soluble BAFF (sBAFF) released from activated monocytes or APCs binds to their receptors on the B cell surface, leading to B cell activation, proliferation and isotype switching to IgA and IgG (Litinskiy et al., 2002). Although not required to initiate CSR, B cell receptor (BCR) cross-linking by Ag is needed to promote plasma cell differentiation and antibody secretion. These effects were dependent on BAFF but not CD40L (Litinskiy et al., 2002).

Recently, BAFF protein has been investigated in peripheral blood mononuclear cells (PBMCs), monocytes and T cells from patients with primary Sjögren's syndrome (SjS) both *ex vivo* and *in vitro* after IFN-α, IFN-γ and IL-10 stimulation (Lavie et al., 2008). Serum BAFF levels were increased in some infection related to autoimmune diseases, e.g. systemic lupus erythematosus, chronic hepatitis C virus, idiopathic inflammatory myopathies (Toubi et al., 2006; Sene et al., 2007; Krystufkova et al., 2009), and chronic lymphocytic leukemia (Molica et al., 2009).

Presently, falciparum malaria remains an important parasitic public health problem causing significant morbidity and mortality in both children and adults (WHO, 2009). Immunity to malaria mostly involves TD immunity, including both cell-mediated and humoral immune mechanisms (Ndungu et al., 2005; Beeson et al., 2008; Nebie et al., 2008; Troye-Blomberg and Berzins, 2008; Iriemenam et al., 2009; Balogun et al., 2009).

Previous studies have revealed that the activated immune-regulatory cells in response to various parts of the blood stage *Plasmodium falciparum* released numerous cytokines which play roles in protective immune response and pathogenesis of the disease (Wahlgren et al., 1995; Akanmori et al., 2000; Walther et al., 2006; Robinson et al., 2009; Clark, 2009; McCall et al., 2010). Particularly, the significant increase of IFN-γ and TGF-β levels observed in falciparum malaria (Walther et al., 2006; Robinson et al., 2009; McCall et al., 2010) are suggested to be the important cytokines regulating BAFF expression (Litinskiy et al., 2002; Craxton et al., 2003).

Presently, there have been very few studies focusing on human malaria parasite components acted as TI antigens. In an earlier study, mice depleted of $CD4^+$ T cells infected with *Plasmodium yoelii* could induce IgG and IgM production (Bate et al., 1990). Furthermore, synthetic peptide circumsporozoite (CS) protein did not require the presence of Ag-specific T helper cell in mice (Schofield and Uadia, 1990). In a recent study, cysteine-rich interdomain region 1-alpha (CIDR1-α) of *P. falciparum* erythrocyte membrane protein-1 was tended to play role in TI polyclonal B cell activator that activated B cells and increased APRIL expression (Donati et al., 2006).

However, only few studies have addressed whether BAFF regulates antibody production in falciparum malaria, although the blood stage *P. falciparum* parasites could induce expression of BAFF regulated cytokine, IFN-γ and TGF-β (Wahlgren et al., 1995; Walther et al., 2006; Robinson et al., 2009; McCall et al., 2010). The components of the *P. falciparum* parasite, particularly the soluble schizont fraction of *P. falciparum* and hemozoin (HZ), with properties of TI Ag, may be able to stimulate IFN-γ involving in BAFF expression on APC leading to bind on BCR and further induce CSR without CD40L from T cells.

The present study was the first to demonstrate whether *P. falciparum* Ag, including sPfAg and HZ representing TI Ags could induce Ig production via the BAFF pathway. Purified human monocytes and naïve B cells from healthy buffy coats were stimulated with sPfAg or HZ *in vitro*. The expressions of BAFF on activated monocytes and BAFF-R on B cells were determined by flow cytometry and enzyme-linked immunosorbent assay (ELISA). The final production of Ig by activated B cells was determined by ELISA. Our findings showed the incidence of *P. falciparum* Ag-activated B cells via BAFF and specific Ab production, despite a limitation of contaminated T and memory cells. Taken together, it is possible that sPfAg and HZ from the blood stage parasites induce TI immunity with BAFF involvement. This study is worthy for further study to clarify the T independent immune response to falciparum malaria.

2. Materials and methods

2.1. Subjects

Buffy coats were obtained from healthy donors who provided informed consent to the blood donation from The Thai Red Cross Society and Rajvithi Hospital, Bangkok, Thailand. The donors had no clinical history of falciparum malaria and other infectious diseases. This study was approved by the Ethical Committees of Mahidol University and Rajvithi Hospital.

2.2. Soluble schizont fraction parasite antigen preparation

The parasites from a laboratory isolate of *P. falciparum* TM267 were cultured in complete RPMI 1640 and 5% hematocrit of fresh-washed healthy human type O erythrocyte suspension under an atmosphere of 90% N_2, 5% CO_2 at 37 °C by the conventional *in vitro* culture method as described previously (Trager and Jensen, 1976). When the parasite suspension cultures contained more than 10% of 24–36 h late stage schizonts, 50 ml of the cultures were enriched by magnetic separation using MACS® (LD column, Miltenyi Biotec, Germany) (Ribaut et al., 2008). The yield of schizont stage parasitized infected red blood cells (PRBCs) was approximately 95%. Then, the pack of PRBCs was used to prepare soluble schizont fraction of *P. falciparum* antigen (sPfAg) as described previously (Pichyangkul et al., 2004). Briefly, schizonts were suspended in RPMI 1640 and subjected to two or three freeze-thaw cycles to obtain the lysate. The schizont-soluble fraction was obtained by centrifugation of the lysate at 14,000 × *g* for 5 min using a microcentrifuge (Hitashi; Brinkmann Instruments, Westbury, NY). This centrifugation process was repeated three times until a clarified fraction was obtained, then filtered through 0.2 μm membrane filter (PALL Life Science, MI, USA). The soluble fraction of infected red cells was used as sPfAg. The concentration of protein in sPfAg was determined by the Coomassie® Plus Protein Assay Reagent Kit (Pierce, IL, USA).

2.3. Preparation of HZ

The laboratory isolate of *P. falciparum* (TM267) was also used to prepare purified HZ as previously described (Coban et al., 2002). Briefly, 10 ml of the pack of enriched late stage PRBC, as mentioned above, were lysed by saponin lysis, washed five times with 0.01 M phosphate-buffered saline (PBS) and sonicated in 2% sodium dodecyl sulfate (SDS). Following eight washes in 2% SDS, the pellet was resuspended in a solution of 10 mM Tris–HCl (pH 8.0), 0.5% SDS, and 1 mM $CaCl_2$ containing 2 mg/ml of proteinase K, then incubated at 37 °C overnight. The pellet was then washed three times in 2% SDS and incubated in 6 M urea for 3 h at room temperature on a shaker. Following five washes in 2% SDS, the HZ pellet was suspended in distilled water and kept at 4 °C. The HZ was sonicated again prior