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Rescue of the mature B cell compartment in BAFF-deficient mice by treatment with recombinant Fc-BAFF

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ABSTRACT

BAFF deficiency in mice impairs B cell development beyond the transitional stage 1 in the spleen and thus severely reduces the size of follicular and marginal zone B cell compartments. Moreover, humoral immune responses in these mice are dramatically impaired. We now addressed the question whether the decrease in mature B cell numbers and the reduced humoral immune responses in BAFF-deficient mice could be overcome by the injection of recombinant BAFF. We therefore engineered a recombinant protein containing the human IgG1 Fc moiety fused to receptor-binding domain of human BAFF (Fc-BAFF). At 1 week after the second injection of this fusion protein a complete rescue of the marginal zone B cell compartment and a 50% rescue of the follicular B cell compartment was observed. Moreover these mice mounted a T cell-dependent humoral immune response indistinguishable from wild-type mice. By day 14 upon arrest of Fc-BAFF treatment mature B cell numbers in the blood dropped by 50%, indicating that the life span of mature B cells in the absence of BAFF is 14 days or less.

Collectively these findings demonstrate that injection of Fc-BAFF in BAFF-deficient mice results in a temporary rescue of a functional mature B cell compartment.

1. Introduction

The first steps of B cell development take place in the bone marrow, where cells are selected for productive rearrangement of the immunoglobulin heavy and light chain genes and subsequently for the surface expression of a proper non-autoreactive B cell receptor (BCR) [1–4]. These immature B cells then leave the bone marrow and differentiate further in the spleen through transitional phases 1, 2 and 3 (T1, T2 and T3). These stages can be distinguished by the expression of CD21, CD23, IgM and IgD, T1 being defined as $CD21^{-}CD23^{-}IgM^{high}IgD^{low}$, T2 $CD21^{+}CD23^{+}IgM^{high}IgD^{high}$ and T3 $CD21^{+}CD23^{+}IgM^{low}IgD^{high}$ [5–9]. Transitional B cells then differentiate into follicular or marginal zone mature B cells (FO B and MZB). The hallmark of the transition from immature to mature B cells is the loss of CD93 cell surface marker [6].

B cell activator factor of the TNF family (BAFF) [10], a member of the Tumor Necrosis Factor (TNF) family, has been identified as the key factor in the survival of immature B cells as they differentiate to mature B cells. BAFF-deficient mice were initially described as having a major block at the T1 stage of B cell differentiation and very low numbers of mature B cells [11]. It was shown later that B cells beyond the T1 stage do exist in BAFF-deficient mice, but that they are present in reduced number and fail to express CD21 and CD23 [12]. BAFF binds to three different receptors, BAFF-R, TACI and BCMA [13–15]. Among these receptors, only BAFF-R deficiency recapitulates the B cell lymphopenia observed in BAFF-deficient mice, demonstrating that BAFF binding to BAFF-R is the critical event in mature B cell differentiation [16–19]. Upon binding, BAFF-R induces the activation of the NF-κB pathway, which then induces the expression of anti-apoptotic members of the Bcl-2 family [20,21]. Transgenic expression of Bcl-2 in B cells of BAFF-deficient mice rescue mature FO B cells development but not MZB [22]. This suggests that at transitional stage survival signals are necessary and sufficient to allow differentiation into the FO B cell lineage, whereas it is not sufficient to induce MZB differentiation.

BAFF-mediated survival signals are not only required during the transition from immature to mature B cells but also during the entire life of naïve B cells. Inhibition of BAFF-R signalling by injection of TACI-Ig, BAFF-R-Ig or BAFF-R-specific antagonist antibodies in wild-type mice results in the rapid loss of mature B cells [23–26].

We demonstrate here that injection of BAFF-deficient mice with a recombinant Fc-BAFF protein can rescue mature B cell develop-

Abbreviations: BAFF, B cell activator factor belonging to the TNF family; BAFF-R, BAFF receptor; BCMA, B cell maturation antigen; FO B, follicular B cells; MZB, marginal zone B cells; TACI, transmembrane activator and CAML interactor.

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ment and leads to the formation of a normal splenic B cell zone architecture. Moreover, treatment completely restored the ability of BAFF-deficient mice to mount an antibody response against T-dependent antigen. When Fc-BAFF treatment was interrupted, mature B cell population decreased with an estimated half-life time of $\leq$14 days.

Altogether, these findings demonstrate that BAFF-deficiency can be almost completely corrected by exogenous administration of recombinant Fc-BAFF.

2. Materials and methods

2.1. Mice

C56BL/6 mice were obtained from RCC (Füllinsdorf, Switzerland) or Harlan Netherlands (Horst, The Netherlands) or bred under pathogen-free conditions at the Center for Biomedicine at the University of Basel or at the Biochemistry Department of the University of Lausanne. BAFF-deficient mice [11] were bred at the Biochemistry Department of the University of Lausanne. All animal experiments were carried out within institutional guidelines with the permission of national or local authorities (authorization numbers 1370, 1886, 1887 and 1888).

2.2. Recombinant Fc-BAFF

The Fc-BAFF expression plasmid encodes the hemagglutinin signal peptide, the Fc portion of human IgG1 (amino acids 108–338 of GenBank accession number AAC82527, excluding the stop codon), a linker sequence (RSPQPQPKPQPKPEPEGSLQVD) and the receptor-binding domain of human BAFF (amino acids 136–285) [27]. An IRES-GFP EcoRI/SalI fragment of pMIG-IRES-GFP was cloned in the EcoRI/XbaI sites 3′ of the Fc-BAFF construct. Chinese hamster ovary cells were transfected using Lipofectamine (Invitrogen) according to the manufacturer's protocol and selected 24 h later with 0.5 mg/ml of G418 (Invitrogen). After 14 days of selection, GFP-bright cells were sorted using a FACS aria (BD Biosciences), amplified and grown in 2-liter roller bottles for 14 days at 37 °C in IMDM supplemented with 5×10^{-5} M β-mercaptoethanol, 1 mM glutamine, 0.03% (w/v) Primatone (Quest, Naarden, The Netherlands), 100 U/mL penicillin, 100 μg/mL streptomycin and 2% heat-inactivated fetal bovine serum. Fc-BAFF was purified on protein A-Sepharose, eluted with 100 mM citrate-NaOH (pH 2.8) and dialysed against PBS.

SDS-PAGE: Fc-BAFF was resolved by 12% SDS-PAGE in presence or absence of 50 mM DTT. Proteins were stained with Coomassie Blue.

Gel permeation chromatography: 180 μg of Fc-BAFF was applied onto a Superdex 200 column (GE Healthcare) eluted in PBS at 0.5 ml/min. Fractions of 1 ml were collected of which 5 μl were analyzed by ELISA against human IgG1.

2.3. ELISA

Unlabeled goat anti-human IgG1 and alkaline phosphatase-coupled goat anti-human IgG1 were purchased form Southern-Biotech. An anti-human BAFF sandwich ELISA was developed in our laboratory. In brief monoclonal antibodies were raised by immunization of Lewis Rats with recombinant hBAFF. Reagents are thoroughly described in manuscript under preparation. Unlabelled anti-human BAFF mAb 2.81.5 was used for capture. Human BAFF was subsequently revealed with biotinylated-anti-human BAFF mAb 4.62, followed by alkaline phosphatase-coupled streptavidin (GE Healthcare).

2.4. Fc-BAFF treatment and immunization of mice

Unless specified otherwise in the figure legend, mice were injected i.v. at day 0 with 100 μg of Fc-BAFF in PBS, and then injected i.p. at day 14 with 50 μg of Fc-BAFF in PBS. In the immunization experiment, treated mice were immunized i.p. with 100 μg of NP-CGG in alum at day 15, and administered with an additional 50 μg of Fc-BAFF i.p at day 21. Anti-NP IgG titers were measured by ELISA with coated NP-BSA 2 weeks post-immunization (day 29).

2.5. Antibody and flow cytometry

Anti-CD19-PE-Cy7 (1D3) and anti-CD19-PECy5 (1D3) were purchased from BD Bioscience (BD Pharmingen). Anti-CD93 (PB493), anti-CD19 (1D3), anti-CD21 (7G6), anti-CD23 (B3B4), anti-CD90 (T24), anti-IgM (M41) were purified and labelled with biotin, Alexa (488) or Alexa (647) using standards procedures. Biotin-labelled antibodies were revealed by streptavidin-PE (SouthernBiotech),-PECy7 or -APC (BD Pharmingen). Anti-hIgG1 Fc monoclonal antibody was developed in our laboratory using a standard protocol. Staining of cells was performed as described previously [28]. Propidium iodide was used at 0.5 μg/ml. Flow cytometry was performed using a FACS Calibur (BD Biosciences) and data were analyzed using the FlowJo software.

2.6. Immunofluorescence

Spleens were snap frozen and embedded into OCT (Sakura, Zoetermeer, NL). Cryostat sections of 5 μm were prepared and fixed in acetone for 10 min. Sections were stained with anti-IgM^{biot} (M41) and anti-CD90^{fitc} (T24) or anti-IgM^{fitc} (M41) and MOMA^{biot}. Biotin-labelled antibodies revealed using Streptavidin-Texas Red (SouthernBiotech). Antibodies were visualized using an Axioskop Immunofluorescence (Zeis, Feldbach, Switzerland) equipped with a Nikon digital camera.

2.7. Binding assay

40E1 cells were mock- or BAFF-R-tranduced using a previously described protocol [26]. Cells were incubated in presence of 100 ng/ml Fc-BAFF. Binding was demonstrated using a biotin-labelled monoclonal anti-hIgG1 revealed by Streptavidin-PE.

2.8. In vitro B cell survival assay

CD19+ cells were sorted form the spleen of C57BL/6 WT mice using a FACS aria (BD Biosciences) and seeded at 2×10^5 cells/wells in 96 wells plates in 200 μl of IMDM supplemented with 5×10^{-5} M β-mercaptoethanol, 1 mM glutamine, 0.03% (w/v) Primatone (Quest, Naarden, The Netherlands), 100 U/mL penicillin, 100 μg/mL streptomycin and 2% heat-inactivated fetal bovine serum in the presence or absence of the indicated concentrations of Fc-BAFF. After 5 days of culture, percentages of living cells were estimated by flow cytometry using propidium iodide and anti-CD19 stainings.

3. Results

3.1. Fc-BAFF recombinant protein forms oligomeric complexes, binds to mouse BAFF receptors and induces survival of sorted mouse mature B cells

We generated a recombinant fusion protein consisting of the TNF homology domain of the human BAFF and the Fc part of human IgG1 (Fc-BAFF) (Fig. 1a). This protein was produced in Chinese hamster ovary cells and purified from supernatants by affinity chromatography on Protein A. Fc-BAFF had apparent molecular