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The transcription factor Bright plays a role in marginal zone B lymphocyte development and autoantibody production

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ABSTRACT

Previous data suggested that constitutive expression of the transcription factor Bright (B cell regulator of immunoglobulin heavy chain transcription), normally tightly regulated during B cell differentiation, was associated with autoantibody production. Here we show that constitutive Bright expression results in skewing of mature B lineage subpopulations toward marginal zone cells at the expense of the follicular subpopulation. C57Bl/6 transgenic mice constitutively expressing Bright in B lineage cells generated autoantibodies that were not the result of global increases in immunoglobulin or of breaches in key tolerance checkpoints typically defective in other autoimmune mouse models. Rather, autoimmunity correlated with increased numbers of marginal zone B cells and alterations in the phenotype and gene expression profiles of lymphocytes within the follicular B cell compartment. These data suggest a novel role for Bright in the normal development of mature B cell subsets and in autoantibody production.

1. Introduction

Autoimmunity results from a loss of tolerance to self-antigen. Due to the complexity of this disease, its cause is still largely unknown and treatment options are limited (Fairhurst et al., 2006). Mouse models have been instrumental in identifying normal B cell tolerance checkpoints (Gay et al., 1993; Goodnow et al., 1988; Hartley et al., 1993; Nemazee and Burki, 1989; Okamoto et al., 1992). Receptor editing is the process of continued light chain rearrangement, which utilizes other gene segments within the kappa (κ) light chain loci and resorts to the lambda (λ) light chain loci if necessary to change the antigen receptor specificity (Gay et al., 1993; Nemazee and Weigert, 2000). Anergy is a property of low-affinity autoreactive cells that persist in the periphery and demonstrate a reduced capacity to participate in immune responses (Merrell et al., 2006). At the cellular level they do not mobilize Ca^{2+}, initiate tyrosine phosphorylation or proliferate (Merrell et al., 2006; Teague et al., 2007). These anergic cells are commonly referred to as transitional type 3 (T3) immature B cells, and this subset is selected away from the T1 → T2 → mature B cell developmental pathway (Teague et al., 2007). In multiple lupus-prone strains, this subset is decreased (Teague et al., 2007). BCR stimulation can also lead to clonal deletion and occurs predominantly at the immature T1 B cell stage (Carsetti et al., 1995; Petro et al., 2002; Su and Rawlings, 2002). Furthermore, others have shown that intrinsic hyperactivity and expansions in mature marginal zone (MZ) B cells, which are enriched for autoreactive clones (Martin and Kearney, 2002), are associated with breaches in tolerance. Therefore, defects in multiple pathways and at multiple stages of B cell development contribute to autoimmunity.

Bright (for B cell regulator of Ig heavy chain transcription) is a transcription factor that belongs to the ARID (AT-rich interaction domain) family (Herrscher et al., 1995), which consists of 15 proteins divided into seven subfamilies. Bright and two ARID orthologs in the human and yeast have been associated with chromatin remodeling (Ayoub et al., 2003; Lai et al., 2001; Lin et al., 2007), suggesting a possible function in epigenetic regulation. However, the first Bright binding sites discovered were in the Ig loci and the best characterized function of Bright is its ability to increase immunoglobulin (Ig) production (Webb et al., 1989; Webb, 2001). Predicted Bright-binding sites are present upstream of over half of the variable region genes and within the intronic Eμ enhancer necessary for expression of all Igs (Herrscher et al., 1995; Johnston et al., 2006). Bright Ig enhancing

Abbreviations: Bright, B cell regulator of immunoglobulin heavy chain transcription; MZ, marginal zone; ARID, AT-rich interaction domain; Ig, immunoglobulin; Btk, Bruton's tyrosine kinase; FO, follicular; BrTg, Bright transgenic; ANA, antinuclear antibody; BCR, B cell receptor.

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function requires a multi-protein complex containing Bright dimers (Nixon et al., 2004a) and both Bruton's tyrosine kinase (Btk) and its substrate, TFII-I (Rajaiya et al., 2006, 2005). More recent data suggest that Bright may also affect genes outside of the Ig locus.

Bright is expressed in multiple embryonic tissues where its function is unlikely to be associated with Ig expression. Although transgenic mice that expressed a dominant negative form of Bright specifically in the B cell lineage showed defects in B1 B cell Ig secretion and decreased levels of serum IgM (Nixon et al., 2008), Bright knockout mice died between days 12 and 14 of gestation due to defects in their ability to generate sufficient red blood cells (Webb et al., 2011). In addition, Bright deficient cells from multiple tissues including spleen, bone marrow, and lymph node became developmentally plastic with the ability to differentiate into cells expressing markers from all three germ lines (An et al., 2010). These data demonstrate that Bright plays a role in non-Ig related functions and suggest that it regulates genes other than the immunoglobulin loci.

Bright expression in the adult is restricted to discrete stages during B cell development in both the mouse and human. Bright mRNA expression occurs in large pro-B, pre-B and recirculating B cells in the bone marrow and in germinal center B cells in the spleen, but it is actively down regulated in the majority of peripheral B cells in the spleen (Nixon et al., 2004b; Webb et al., 1998). Normal follicular B cells do not express Bright mRNA transcripts, while marginal zone B cells maintain low levels of expression. These two phenotypically and functionally distinct mature B cell subsets can terminally differentiate into memory or plasma B cells once they have initiated an antibody response. Follicular (FO) B cells represent the largest B cell subset in the periphery, are defined as $B220^{+}CD93^{-}CD23^{+}IgM^{+}$ (Allman et al., 2001; Allman and Pillai, 2008), and re-circulate between various lymphoid sites where they come into contact with activated T cells and undergo germinal center reactions. In contrast, MZ B cells are a minor mature subset accounting for roughly 5% of the total B cell population, are defined as $B220^{+}CD93^{-}CD23^{-}IgM^{hi}$ (Allman et al., 2001; Allman and Pillai, 2008), and produce natural IgM antibody rapidly to blood-borne antigens. Relative to FO B cells, MZ B cells are partially activated, expressing higher basal levels of co-stimulatory molecules and developing into plasma cells more rapidly following activation (Oliver et al., 1999, 1997). MZ B cells have a relatively restricted B cell repertoire biased toward common bacterial and self-antigen that tends to be polyreactive, and thus capable of being autoreactive (reviewed in (Loder et al., 1999)). Generally, autoreactive cells are excluded from the FO compartment, whereas the MZ compartment is enriched for autoreactive clones (reviewed in (Martin and Kearney, 2002)).

Previously, we demonstrated that over-expression of the transcription factor Bright throughout B cell development was associated with ANA production, proteinuria and kidney glomerulonephritis as transgenic FVB/N mice aged (Shankar et al., 2007). However, other studies suggest that normal FVB/N mice exhibit some traits commonly associated with autoimmunity (Maier et al., 2007), suggesting that the genetic background could have influenced the phenotype of the Bright transgenic mice. Here, we sought to analyze how Bright over-expression resulted in autoantibody production. Therefore, it was important to backcross the FVB/N Bright transgenic lines onto the non-autoimmune strain C57Bl/6 (Bygrave et al., 2004; Vidal et al., 1998) for those analyses. Bright expression was also sufficient to generate autoantibodies on this background in mice only 4 weeks old, and these mice were analyzed to determine if losses in tolerance were consistent with mechanisms observed in other autoimmune models. Intriguingly, the data presented here suggest new roles for Bright in both B cell development and autoimmunity.

2. Material and methods

2.1. Mice

FVB/N mice that express the Bright transgene driven by the B lineage restricted CD19 promoter were described previously (Shankar et al., 2007). Two independent transgenic lines were backcrossed onto the C57Bl/6 background for more than 10 generations and were genotyped as described (Shankar et al., 2007). All BrTg mice were maintained as heterozygotes. C57Bl/6 and Bcl2 Eμ transgenic mice on the C57Bl/6 background were purchased from Jackson ImmunResearch Laboratories. $Btk^{-/-}$ mice, also on the C57Bl/6 background, were a gift from Wasif Khan (Vanderbilt University School of Medicine, TN). C57Bl/6 (SJL Ly5.1) congenic mice were a gift from Paul W. Kincade (Oklahoma Medical Research Foundation) and were lethally irradiated (1300 rad) before retro-orbital injection with 2×10^6 whole bone marrow cells from Bright transgenic and/or C57Bl/6 donors (both Ly5.2). 5-weeks post injections, recipients were sacrificed and analyzed. All mice were bred and maintained in the OMRF animal care facility according to Institutional Animal Care and Use Committee approved protocols. Male and female mice were analyzed between 4 and 12 weeks of age.

2.2. ANA

Mouse sera were tested at multiple dilutions for the presence of anti-nuclear antibodies using the NOVA Lite HEp-2 ANA Kit (INOVA diagnostics) as previously described (Shankar et al., 2007). ANA positive control sera from adult mice typically showed strong staining compared to sera from negative littermate controls even at dilutions of 1:360 (Maier et al., 2007). However, for younger mice and mice with low serum Ig levels, higher dilutions (1:45) were also assayed. Antibodies to nuclear antigens were detected using a secondary FITC-conjugated anti-mouse IgG antibody (Jackson Immunoresearch Laboratories) and visualized using a Zeiss Axioplan 2i inverted microscope with an AxioCam HRm camera (Carl Zeiss).

2.3. ELISA

Serum Ig levels were determined using the clonotyping system-AP kit (Southern biotech) and a Biotech EL800 plate reader with GEN5 Biotech software as previously described (Shankar et al., 2007). Briefly, duplicate serum samples were serially diluted from 1:400 to 1:51,200 and concentrations were determined using standard curves generated with appropriate Ig isotype controls of known concentration. Ig secretion from purified B cells plated at 1×10^6 cells/ml and stimulated with 10 μg/ml goat $F(ab')_2$ anti-mouse IgM or LPS for 5 days was measured similarly from supernatants by ELISA using anti-kappa light chain reagents as previously described (Nixon et al., 2008).

2.4. Flow cytometry

Cells from spleens, bone marrow, peritoneal cavity and thymuses were isolated and prepared, and B cell staining was performed as described (Shankar et al., 2007). Data were collected using an LSRII (BD Biogenics) and DIVA software ver. 4.1. A minimum of 100,000 events were recorded. Data were analyzed using Flojo (Treestar) software ver. 8.3. Fluorescence conjugated antibodies phycoerythrin-cyanin 5 (PE-Cy5)-B220 (Ra3-6B2), peridinin chlorophyll-a protein (PerCP)-B220 (RA3-6B2), phycoerythrin (PE)-CD23 (B3B4), and -CD43 (S7) were purchased from BD Biosciences; allophycocyanin (APC)-CD93/C1qRp (AA4.1), biotin-CD86/B7.2 (GL1), -TLR4/MD2 (MTS510), and -RP105 (RP/14), fluorescein isothiocyanate (FITC)-CD21/35 (7G6), -CD19 (MB19-1), -IgM (II/41),