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Distinct and synergistic roles of FcγRIIB deficiency and 129 strain-derived SLAM family proteins in the development of spontaneous germinal centers and autoimmunity

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ABSTRACT

The inhibitory IgG Fc receptor (FcγRIIB) deficiency and 129 strain-derived signaling lymphocyte activation molecules (129-SLAMs) are proposed to contribute to the lupus phenotype in FcγRIIB-deficient mice generated using 129 ES cells and backcrossed to C57BL/6 mice (B6.129.RIIBKO). In this study, we examine the individual contributions and the cellular mechanisms by which FcγRIIB deficiency and 129-derived SLAM family genes promote dysregulated spontaneous germinal center (Spt-GC) B cell and follicular helper T cell (Tfh) responses in B6.129.RIIBKO mice. We find that B6 mice congenic for the 129-derived SLAM locus (B6.129-SLAM) and B6 mice deficient in FcγRIIB (B6.RIIBKO) have increased Spt-GC B cell responses compared to B6 controls but significantly lower than B6.129.RIIBKO mice. These data indicate that both FcγRIIB deficiency and 129-SLAMs contribute to elevated Spt-GC B cell responses in B6.129.RIIBKO mice. However, only 129-SLAMs contribute significantly to augmented Tfh responses in B6.129.RIIBKO mice, and do so by a combination of T cell-dependent effects and enhanced B cell and DC-dependent antigen presentation to T cells. Elevated Spt-GC B cell responses in mice with FcγRIIB deficiency and polymorphic 129-SLAMs were associated with elevated metabolic activity, improved GC B cell survival and increased differentiation of naïve B cells into GC B cell phenotype. Our data suggest that the interplay between 129-SLAM expression on B cells, T cells and DCs is central to the alteration of the GC tolerance checkpoint, and that deficiency of FcγRIIB on B cells is necessary to augment Spt-GC responses, pathogenic autoantibodies, and lupus disease.

1. Introduction

Production of autoantibodies (autoAbs) is a serological hallmark of systemic lupus erythematosus (SLE), a multigenic disorder with genetic predisposition contributing to disease susceptibility [1,2]. Several mouse models that develop spontaneous (Spt) human SLE-like autoimmunity have been used to delineate the genetic factors involved in the pathogenesis of the disease [2]. Genetic linkage studies in several lupus mouse models (NZB, NZW, NZM2410, BXSB and MRL/*lpr*) have identified at least 31 loci on different chromosomes that contribute to the disease phenotype [3]. The most well studied are the overlapping genetic loci *Sle1* and *Nba-2*, derived from New Zealand White (NZW) and Black (NZB) mice, respectively; *Sle1* and *Nba-2* loci are located on the telomeric region of chromosome 1 [3–7]. B6 mice carrying a similar chromosome 1 region (named *Sle16*) derived from 129/Sv mice also develop lupus-like autoimmunity [8]. This region is syntenic to the human

Abbreviations: GC, germinal center; Spt-GC, spontaneous germinal center; PNA, peanut agglutinin; ANA, anti-nuclear antibody; SRBC, sheep red blood cells; Tfh, follicular helper T cells; SLE, systemic lupus erythematosus; CFSE, carboxyfluorescein succinimidyl ester; SLAM, signaling lymphocyte activation molecule.

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chromosome 1 region 1q22-25, which is also associated with human SLE [9,10]. Extensive studies on lupus susceptible and non-susceptible mouse strains have identified several polymorphic variations between the two groups in the *FcγR* interval and SLAM family genes, located in the *Sle1*/*Nba2*/*Sle16* region [8,11–13].

Within the *FcγR* locus, *Fcγr2b* encodes the low affinity inhibitory receptor for IgG, FcγRIIB, which signals through an immunoreceptor tyrosine based inhibitory motif (ITIM) [14]. FcγRIIB is the only Fc receptor expressed on B cells, functioning to negatively regulate BCR-mediated activation signals [15]. In DCs and other myeloid cells, FcγRIIB opposes the activation signals transduced by the immunoreceptor tyrosine based activation motif (ITAM)-bearing Fc receptors, FcγRI and FcγRIII [16]. FcγRIIB-deficient mice generated using 129/Sv-derived ES cells and backcrossed to C57BL/6 (B6) mice (designated B6.129.RIIBKO) have served as a model for human lupus, exhibiting the accumulation of autoAbs and progression to fatal glomerulonephritis [17,18].

The close proximity of the FcγRIIB and SLAM family genes located in the *Sle1*/*Nba2*/*Sle16* locus and their involvement in autoimmune susceptibility underlie studies to define the contribution of the FcγRIIB and SLAM family genes to autoimmunity [19–22]. Analysis of B6.129.RIIBKO mice and FcγRIIB-deficient mice generated using B6 ES cells (named B6.RIIBKO) show that the SLAM family genes derived from 129 mice (designated 129-SLAMs) are the major autoimmune susceptibility conferring genes in the *Sle1*/*Nba2*/*Sle16* interval, whereas FcγRIIB acts as a modifier of autoimmune susceptibility in B6.129.RIIBKO mice [23]. Verbeek and co-workers (23) found that FcγRIIB deficiency did not contribute to spontaneous autoimmunity on a B6 background. By generating FcγRIIB germline and conditional knockout mice from B6 ES cells, Li et al. recently showed that FcγRIIB is required for maintaining tolerance [24]. However, the cellular mechanisms regulated by 129-SLAMs and FcγRIIB deficiency, which contribute to lupus pathogenesis in B6.129.RIIBKO mice remain to be defined.

Spontaneously developed germinal centers (Spt-GCs) play a significant role in generating somatically hypermutated and class-switched pathogenic autoAbs, which cause lupus autoimmunity [25–30]. Somatically mutated and pathogenic autoAbs in B6.129.RIIBKO mice have recently been shown to develop through the GC pathway [19], indicating dysregulation at the level of the GC checkpoint. However, the mechanisms by which FcγRIIB deficiency and/or 129-SLAMs contribute to the perturbation of the Spt-GC responses in B6.129.RIIBKO mice are not clear. To determine the cellular functions of various immune cells that may contribute to Spt-GC formation, we compared B6, B6.RIIBKO (FcγRIIB deficient mice generated using B6 ES cells), B6.129-SLAM (B6 mice congenic for the 129-derived SLAM locus) and B6.129.RIIBKO mice (FcγRIIB deficiency on a B6/129 mixed background).

In this study, we provide evidence that both 129-SLAMs and FcγRIIB deficiency independently contribute to the heightened Spt-GC B cell responses in B6.129.RIIBKO mice. 129-SLAMs were found to play a central role in regulating GC Tfh (follicular helper-T cell) responses, which were mostly unaffected by FcγRIIB deficiency. B cells and DCs expressing 129-SLAMs had increased antigen presentation capability, and augmented T cell proliferation and functionality. B cells from both B6.129-SLAM and B6.RIIBKO mice showed increased differentiation into GC B cells and improved survival in the GC microenvironment, which was associated with higher energy metabolism than B6 B cells. Our data suggest that the expression of 129-SLAMs on B cells, T cells and DCs was cumulatively important for the observed increase in the Spt-GC and Tfh responses in B6.129-SLAM and B6.129.RIIBKO mice. However, the expression of 129-SLAMs on B cells was particularly important for the selection of autoimmune B cells within GCs. Altogether, this study demonstrates the co-operative yet mutually exclusive roles of FcγRIIB deficiency and 129-SLAMs in altering the GC tolerance checkpoint, leading to the production of autoAbs and lupus pathology.

2. Materials and methods

2.1. Mice

Breeding pairs for C57BL/6J (B6, stock number 000664), B6.μMT (B6.129S2-*Ighm*^{tm1Cgn}/J, stock number 002288), and OT-II transgenic (B6.Cg-Tg (TcraTcrb)425Cbn/J, stock number 004194) mice were originally purchased from the Jackson Laboratory (Bar Harbor, Maine) and bred in-house. FcγRIIBKO mice (Fcgr2b < tm1.2Rav) were generated on a pure B6 background (B6.RIIBKO) as described [31,32]. The Ig V_H knock-in mouse line, HKIR (*Igh*^{tm1Tim}), was described previously [33,34]. B6.129.RIIBKO (B6.129S4-*Fcgr2b*^{tm1TtK} N12, model 580) mice were purchased from the Taconic Farms (Hudson, NY). B6.129-SLAM congenic mice were generated through the transfer of the SLAM locus from 129 to C57BL/6 mice by the marker-assisted speed congenics approach as described [8]. All animals were housed in the barrier mouse facility at Penn State Hershey Medical Center. All experimental procedures were performed in accordance with the guidelines and approved by the Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee.

2.2. Genetic background analysis

Genetic background analysis of B6.129-SLAM and B6.129.RIIBKO mice was performed using tail DNA samples, on a 1449 SNP Illumina Bead Chip, and analyzed on SNAP-Map software at DartMouse speed congenic core facility, the Geisel School of Medicine, Dartmouth College.

2.3. Flow cytometry reagents

The following antibodies were utilized for flow cytometric analysis of mouse splenocytes: Pacific Blue-anti-B220 (RA3-6B2); Alexa Fluor-700-anti-CD4 (RM4-5); PE-anti-PD-1 (29F.1A12); BV605-anti-CD69 (H1.2F3); Allophycocyanin-anti-TCR-Vα2 (B20.1); Allophycocyanin-Cy7-anti-CD25 (PC61); PeCy7-anti-CD95 (FAS, Jo2); PeCy7-anti-MHC-II (M5/114.15.2); APC-anti-2B4 (m2B4(B6) 458.1); APC-anti-CD48 (HM48-1); PerCP-Cy5.5-anti-CD150 (TC15-12F12.2); Biotin-anti-Ly9 (Ly9ab3); Biotin-anti-CD84 (mCD84.7); Pacific Blue-anti-Ly108 (330-AJ) and PE-Cy5-streptavidin (SA) were purchased from BioLegend, San Diego, CA. Biotin-anti-CXCR5 (2G8); FITC-GL7 and FITC-anti-CD11c (HL3) were from BD Pharmingen, San Diego, CA. FITC-peanut-agglutinin (PNA) from Vector Labs, Burlingame, CA. CD16/32 (clone 2.4G2) was from eBiosciences, San Diego, CA. SA-PE conjugate was purchased from Sigma Aldrich, St. Louis, MO. Anti-idiotypic mAb E4, recognizing DNA/Ars dual reactive HKIR B cells [35] and mAb K9.361 (kind gift from Dr. U Hammerling, Sloan-Kettering Memorial Hospital, New York, NY), recognizing FcγRIIB from the B6 background were purified from the respective hybridoma and biotinylated in-house. Stained cells were analyzed using the BD LSR II flow cytometer (BD Biosciences, Franklin lakes, NJ). Data were analyzed using FlowJo software (Tree Star, San Carlos, CA). Dead cells were excluded from analysis by flow cytometry using 4′, 6-diamidino-2-phenylindole exclusion (DAPI) (Sigma–Aldrich, St. Louis, MO). The SR-FLICA *in vitro* caspase detection kit (Poly-caspase SR-VAD-FMK) was purchased from AbD Serotec (Kidlington, U.K.).