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Excessive interferon-α signaling in autoimmunity alters glycosphingolipid processing in B cells

Andy Hee-Meng Tan [a, **], Arleen Sanny [a], Sze-Wai Ng [a], Ying-Swan Ho [a], Nurhidayah Basri [a], Alison Ping Lee [a], Kong-Peng Lam [a, b, c, d, *]

[a] Bioprocessing Technology Institute, Agency for Science, Technology and Research, Singapore 138668, Singapore
[b] Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 11799, Singapore
[c] Department of Microbiology, and Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117599, Singapore
[d] Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117599, Singapore

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ABSTRACT

Excessive interferon-α (IFN-α) production by innate immune cells is a hallmark of autoimmune diseases. What other cell type secretes IFN-α and how IFN-α affects immune cell metabolism and homeostasis in autoimmunity are largely unclear. Here, we report that autoimmune B cells, arising from two different B cell-specific genetic lesions in mice, secrete IFN-α. In addition, IFN-α, found in abundance in autoimmunity, elicited profound changes in the B cell lipidome, increasing their expression of glycosphingolipids (GSLs) and leading to their CD1d-mediated depletion of iNKT cells *in vitro* and *in vivo*. IFN-α receptor blockade could reverse the loss of iNKT cells. Excessive stimulation of B cells with IFN-α altered the expression of enzymes that catalyze critical steps in GSL processing, increasing the expressions of glucosylceramide synthase (GCS) and globotrihexosylceramide synthase (Gb3S) but decreasing that of α-galactosidase A (α-galA). Inhibiting GCS or restoring α-galA expression prevented iNKT depletion by IFN-α-activated B cells. Taken together, our work indicated that excessive IFN-α perturbs GSL metabolism in B cells which in turn adversely affects iNKT homeostasis.

1. Introduction

Autoimmune patients suffering from systemic lupus erythematosus (SLE) have high serum levels of cytokines such as interferon-α (IFN-α) [1], interleukin-6 (IL-6) [2,3], tumour necrosis factor-α (TNF-α) [4,5] and IL-10 [6–8]. In particular, IFN-α is produced by plasmacytoid dendritic cells (pDCs) that are activated by opsonized apoptotic material or circulating IgG-nucleic acid complexes (ICs) [9]. In these cells, ICs are internalized via surface FcγRIIa (CD32) and trafficked to endosomes where their nucleic acid components stimulate Toll-like receptor (TLR)-7 or −9 to induce *ifna* gene transcription. Elevated IFN-α drives the maturation of conventional DCs (cDCs) that subsequently activate autoreactive T and B cells to amplify the autoimmune syndrome [10].

B cell-specific loss of the death receptor FAS (CD95, Apo-1) or protein tyrosine phosphatase SHP-1 in mice results in some features recapitulating human SLE such as autoantibody production by B cells and accompanying IC-mediated renal damage [11,12]. That B cells produce cytokines during immunity against infection is well documented [13,14] but less is known about what cytokines they produce in autoimmunity; in particular, whether they produce IFN-α in similar fashion to pDCs.

Type I IFNs have been shown to induce Warburg-like glycolytic re-programming in macrophages and DCs that is required for the maturation of their effector functions [15]. IFN-γ or β but not IL-6 or TNF-α reduces sterol biosynthesis in macrophages which compromises host protection against viral infection [16]. In comparison, it is not known what effects IFN-α has on B cells, particularly in the context of autoimmunity. SLE T cells had been found to have

Abbreviations used: IFN, interferon; TNF, tumour necrosis factor; IL, interleukin; iNKT, invariant natural killer T; GSL, glycosphingolipid; Cer, ceramide; LacCer, lactosylceramide; GCS, glucosylceramide synthase; Gb3S, globotrihexosylceramide synthase; α-galA, α-galactosidase A; NB-DNJ, N-butyldeoxynojirimycin; PMA, phorbol-12-myristate-13-acetate.

* Corresponding author. Bioprocessing Technology Institute, 20 Biopolis Way, #06-01 Centros, Singapore, 138668 Singapore.
** Corresponding author. Bioprocessing Technology Institute, 20 Biopolis Way, #06-01 Centros, Singapore 138668, Singapore.
E-mail addresses: andy_tan@bti.a-star.edu.sg (A.H.-M. Tan), lam_kong_peng@bti.a-star.edu.sg (K.-P. Lam).

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increased cholesterol-sphingolipid enriched lipid rafts, resulting in attenuated T cell receptor (TCR) signaling and other functional defects [17,18]. However, it was not clear from these studies whether and what cytokines led to the lipid raft defect in SLE T cells.

In this study, we set out to investigate what cytokines are produced by autoimmune B cells and if these cytokines could alter lipid metabolism in B cells. Previously, we documented that autoimmune B cells could deplete peripheral iNKT cells via CD1d-dependent presentation of self-lipids, suggesting that these B cells likely harboured alterations in their lipid composition [19]. Here, we found autoimmune FAS- and SHP-1-deficient B cells to produce aberrant levels of IFN-α, IL-6, IL-10 and TNF-α. Moreover, we found IFN-α to elicit profound changes in the intracellular lipid composition, including selected GSLs, of B cells. B cells stimulated with IFN-α depleted co-cultured iNKT cells *in vitro* and blocking IFN-α signaling could significantly rescue iNKT cells in autoimmune mice *in vivo*. Finally, we showed that IFN-α altered the expression of enzymes critical for GSL processing in B cells.

2. Materials and methods

2.1. Mice

C57BL/6, $Fas^{f/f}$ [20], $Ptpn6^{f/f}$ [12], $Cd1d1^{f/f}$ [21] and $Cd19^{Cre/+}$ [22] mice were purchased from The Jackson Laboratory. All mice were bred in our animal facilities and maintained under specific pathogen-free conditions. $Fas^{f/f}$, $Ptpn6^{f/f}$ and $Cd1d1^{f/f}$ mice were bred with $Cd19^{Cre/+}$ mice to generate $Fas^{f/f}Cd19^{Cre/+}$, $Ptpn6^{f/f}Cd19^{Cre/+}$ and $Cd1d1^{f/f}Cd19^{Cre/+}$ mice, respectively. Mice used were female and between 12 and 20 weeks of age. Experiments with mice were conducted according to guidelines issued by A*STAR Biological Resource Centre Institutional Animal Care and Use Committee.

2.2. Cell suspensions and flow cytometry

Single cell suspensions of spleens and thymi were prepared by standard methods. Liver mononuclear cells were obtained by perfusion, lysis of red blood cells and overlaying of cells with Ficoll-Paque (GE Healthcare). Cells were centrifuged for 20 min at 700 g without brakes and live lymphocytes were collected at the interface. Bone marrow cells were isolated by gentle flushing of femurs and tibia. Before labeling with relevant fluorochome-conjugated antibodies (Abs), cells were treated with F_c block (Ab against CD16/32). iNKT cells were stained with allophycocyanin (APC)-conjugated CD1d-PBS57 tetramer (provided by the US National Institutes of Health Tetramer Core Facility) and other relevant cell surface markers. Abs against TCRβ (H57-597) and B220 (RA3-6B2) were from BioLegend. Ab against CD317 (BST2, PDCA-1; eBio927) was from eBioscience and Ab against CD16/32 (2.4G2) was from BD Pharmingen. Samples were acquired on LSRII cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar).

2.3. Lipid Extraction and lipid profiling with LC-MS

B cells were enriched from spleens of C57BL/6 mice using anti-CD19-conjugated microbeads (Milenyi Biotec) and cultured in complete media alone or in the presence of 10^3 U/ml IFN-α (Miltenyi Biotec), 20 ng/ml IL-6, 10 ng/ml IL-10 or 25 ng/ml TNF-α (all from Peprotech) for 3 days. Lipids were extracted from B cells and LC-MS analysis was performed as previously described [19]. Raw LC-MS data were pre-processed using an in-house bioinformatics software incorporating the XCMS algorithm [23]. Masses of shortlisted peaks of interest were first compared against entries in the Kyoto Encyclopedia of Genes and Genome and Human Metabolome Database. Mass peaks with matches within a 5 ppm mass accuracy window were assigned putative identities. Metabolite identities were verified by MS^2 spectral comparison with commercially available standards where possible, by comparison to mass spectral databases available online or by theoretical fragments generated by Mass Frontier 5.1 software (HighChem).

2.4. Intracellular cytokine production

5×10^6 splenocytes or B cells enriched from spleens of $Cd19^{Cre/+}$, $Fas^{f/f}Cd19^{Cre/+}$ and $Ptpn6^{f/f}Cd19^{Cre/+}$ mice were stimulated with 50 ng/ml PMA and 750 ng/ml ionomycin (Sigma-Aldrich) in the presence of Brefeldin A at 37 °C for 5 h. B cells and pDCs were visualized with Abs against B220 and BST2, fixed and permeabilized as above before staining with Abs against IFN-α (RMMA-1; PBL Interferon Source) and IL-10 (JES5-16E3; eBioscience) or IL-6 (MP5-20F3; eBioscience) and TNF-α (MP6-XT22; eBioscience).

2.5. B and iNKT cell co-culture

To assess how IFN-α-activated B cells affect iNKT cells in co-culture, 5×10^6 B cells enriched from spleens of C57BL/6, $Cd1d1^{+/+}Cd19^{Cre/+}$ or $Cd1d1^{f/f}Cd19^{Cre/+}$ mice were incubated in the presence of 10^3 U/ml IFN-α or no cytokine for 3 days. In some experiments, IFN-α-activated C57BL/6 B cells were pulsed concurrently with 100 ng/ml heated and sonicated α-GalCer (BioVision). In others, cells were treated concurrently with 10 μM NB-DNJ. Cells were then collected, washed twice with complete media, counted and 2×10^6 B cells seeded with 2×10^5 iNKT cells enriched from C57BL/6 thymi using APC-conjugated CD1d-PBS57 tetramer and anti-APC microbeads for a further 3 days in media alone. In some experiments, B and iNKT cells were co-cultured in the presence of 20 μg/ml anti-mouse CD1d Ab (1B1; Biolegend) or rat IgG2b, κ isotype control Ab (RTK4530; BioLegend). Cells were then enumerated, stained with Abs against cell surface molecules and visualized by flow cytometry.

2.6. Retroviral constructs and retroviral infection of B cells

Full-length murine *Gla* (NM_013463.2) was amplified from cDNA of enriched B cells by PCR with primers 5′-CGC CGG AAT TAG ATC GGG AAA CTG AAC CAA AGG ATT-3′ and 5′-TAA CCT CGA GAG ATC CGG GAC ATA GAA CTC TGC CTA-3'. The PCR fragment was cloned into the bicistronic vector GFP-RV (MIGR1 [24]) via *Bgl*II sites using In-Fusion HD Cloning kit (Clontech) to achieve directional ligation. Both EV and *Gla* retroviral vectors were transfected into the Phoenix-Eco packaging cell line obtained from Dr. Gary P. Nolan (Stanford University) using Fugene 6 (Promega) and viral supernatants were collected 24 h later. Enriched C57BL/6 B cells activated with 10 μg/ml anti-IgM Ab (Jackson ImmunoResearch) and 2 μg/ml anti-CD40 Ab (FGK45; Adipogen) were infected with viral supernatant in the presence of 1:100 dilution of DOTAP liposomal transfection reagent (Roche), centrifuged at 2800 rpm for 90 min at room temperature, and replaced after overnight incubation with fresh media containing anti-CD40 Ab for an additional 48 h. In some experiments, IFN-α was added together with anti-CD40 Ab. Infected (GFP^+) B cells were then sorted on FACSAria II sorter (BD Biosciences). 2×10^5 GFP^+ B cells were co-cultured with 2×10^4 iNKT cells for 3 days and iNKT cells remaining in culture were enumerated.

2.7. RNA isolation and quantitative real-time PCR

Total RNA was isolated using TRIzol (Invitrogen) and precipitated with isopropanol. cDNA was prepared using iScript Reverse

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