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Impact of coding risk variant *IFNGR2* on the B cell-intrinsic IFN-γ signaling pathway in multiple sclerosis

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ABSTRACT

B cells of people with multiple sclerosis (MS) are more responsive to IFN-γ, corresponding to their brain-homing potential. We studied how a coding single nucleotide polymorphism (SNP) in *IFNGR2* (rs9808753) co-operates with Epstein-Barr virus (EBV) infection as MS risk factors to affect the IFN-γ signaling pathway in human B cells. In both cell lines and primary cells, EBV infection positively associated with IFN-γ receptor expression and STAT1 phosphorylation. The *IFNGR2* risk SNP selectively promoted downstream signaling via STAT1, particularly in transitional B cells. Altogether, EBV and the *IFNGR2* risk SNP independently amplify IFN-γ signaling, potentially driving B cells to enter the MS brain.

1. Introduction

In many autoimmune diseases, both central and peripheral B-cell tolerance checkpoints are impaired [1], resulting in the escape of autoreactive, naive B cells that enter the circulation. Strikingly, in people with multiple sclerosis (MS), an inflammatory disease of the central nervous system (CNS), only defects in peripheral B-cell selection have been found [1,2]. In addition, B cells of people with MS are highly sensitive to IFN-γ, a trigger that mediates escape from peripheral tolerance [3,4] and promotes differentiation into memory subsets expressing T-box transcription factor T-bet [5]. Probably as a result, these types of B cells infiltrate the CNS to mature into antibody-secreting cells (mostly IgG) and contribute to MS pathology [5–8]. IFN-γ induces T-bet transcription through dimerization of its receptor subunits IFNGR1 (α-chain) and IFNGR2 (β-chain), which is followed by phosphorylation of downstream signaling molecule STAT1 [9–12]. Importantly, while IFNGR1 is required for ligand binding, IFNGR2 is crucial for initiating downstream activity [9,10]. It is currently unclear how the IFN-γ signaling pathway in B cells is affected and contributes to enhanced IFN-γ responsiveness in MS.

For the development of MS, B cells are likely affected by a complex interaction between genetic and environmental risk factors [13–16]. Results from large genome-wide association studies (GWAS) indicate that one of the few coding risk single nucleotide polymorphisms (SNPs) for MS is located in *IFNGR2* (rs9808753; A > G) [17,18]. It causes a change in amino acid, although its functional implications are not known. Furthermore, there is a 32-fold increased risk to develop MS after infection with the Epstein-Barr virus (EBV) [14], a B-lymphotropic human herpesvirus that can interact with risk variants either in a direct or indirect manner [16,19]. Notably, the presence of rs9808753 is associated with a lower abundance of EBV in human B-cell lines [20]. Here, we elaborated on the enhanced IFN-γ responsiveness of B cells in MS by exploring the impact of the *IFNGR2* risk SNP and EBV on the IFN-γ signaling pathway, both in human B-cell lines and blood B cells from healthy and MS donors.

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Abbreviations

B-LCL	B lymphoblastoid cell line
CNS	central nervous system
EBV	Epstein-Barr virus
eQTL	expression quantitative trait loci
FACS	fluorescence activated cell sorting
IFNGR	interferon-gamma receptor
MACS	magnetic activated cell sorting
MS	multiple sclerosis
PBMC	peripheral blood mononuclear cell
qPCR	quantitative polymerase chain reaction
RQ-PCR	real-time quantitative reverse transcriptase polymerase chain reaction
SNP	single nucleotide polymorphism
STAT1	signal transducer and activator of transcription 1
T-bet	T-box transcription factor

2. Materials and methods

2.1. Human B-cell lines and patients

To uncover the effect of EBV infection and *IFNGR2* risk SNP rs9808753 on IFN-γ signaling in B cells, we analyzed IFN-γ receptor, (p-) STAT1 and T-bet expression profiles in human B-cell lines and primary B cells. 19 different human B lymphoblastoid cell lines (B-LCLs), authenticated by cytogenetics and tested for mycoplasma contamination, were analyzed for EBV load and the presence of rs9808753 (Table S1). We selected 4 B-LCLs (Karpas-422, MC116, EHEB and JVM13) based on the presence (homozygote risk: GG) or absence (non-risk: AA) of rs9808753 and high or low EBV load. Besides, we selected natalizumab-treated MS patients based on the presence of rs9808753 (risk: AG or GG) (n = 8, 100 % female, average age = 40.4) or its absence (non-risk: AA) (n = 8, 100 % female, average age = 42.8) and matched them with healthy controls (n = 8, 100 % female, average age = 43.9), for whom rs9808753 genotyping could not be performed. An additional cohort of untreated early MS patients was selected based on the homozygote presence (homozygote risk: GG) (n = 4, 75 % female, average age = 35.8), heterozygote presence (heterozygote risk: AG) (n = 4, 75 % female, average age = 35.8) or absence (non-risk: AA) (n = 4, 75 % female, average age = 28.8) of rs9808753. All patients provided written informed consent. Study protocols were approved by the medical ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands).

2.2. Isolation of mononuclear cells from peripheral blood

As described previously [5], peripheral blood from healthy controls and MS patients was collected via venipuncture into BD vacutainer® CPT™ mononuclear cell preparation tubes containing sodium heparin (BD Biosciences). Peripheral blood mononuclear cells (PBMCs) were isolated according to the manufacturer's protocol and frozen in liquid nitrogen for further use.

2.3. IFNGR2 risk SNP (rs9808753) genotyping

DNA was extracted from B-LCLs and whole blood samples of patients as reported previously [21]. For genotyping of B-LCLs, the following primer sequences were used: forward primer – TTTGGGCGGCGACGT-GAG, reverse primers – GACTGGCGGCAGTGAAGTCAC & GTTTTCTGGAGGCCCGACAGT. A polymerase chain reaction (PCR) was performed using AmpliTaq Gold™ DNA Polymerase with Buffer II and $MgCl_2$ (Life Technologies) according to manufacturer's protocol in a 9800 Fast Thermal Cycler (Applied Biosystems). Afterwards, sequencing was done using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Life Technologies) according to the manufacturer's protocol and measured with a 3500 Genetic Analyzer (Applied Biosystems). Data were analyzed with CLC Genomics Workbench (Qiagen). For genotyping of natalizumab-treated MS patients, the following primer sequences were used: forward primer – AAGATTCGCCTGTACAAC, reverse primer – TACAAGCTCCAAGAACGAT. A PCR was performed using the Q5® High-Fidelity PCR Kit (New England Biolabs) according to the manufacturer's protocol in a Mastercycler™ Nexus Gradient (Eppendorf). The PCR product was purified by performing electrophoresis on a 1 % agarose gel at 100 V and cutting out the bands from the gel. Isolation of the PCR product was done using the MinElute Gel Extraction Kit (Qiagen) according to the manufacturer's protocol. Subsequently, sequencing was done using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Life Technologies) according to the manufacturer's protocol and measured with a 3500 Genetic Analyzer (Applied Biosystems). Data were analyzed with CLC Genomics Workbench (Qiagen). For genotyping of untreated early MS patients, samples were genotyped on the Immuno-Chip (Illumina) as described previously [18].

2.4. EBV load determination

As reported previously [15], DNA was isolated from B-LCLs and used for a multiplex real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR). EBV load was measured with a QuantStudio™ 5 Real-Time PCR Systems (Thermo Fisher Scientific). Data were analyzed with QuantStudio (Thermo Fisher Scientific) using the B-LCL Namalwa as the standard curve.

2.5. Fluorescence activated cell sorting (FACS) and Epstein-Barr virus (EBV) infection

Naive mature B cells ($CD3^-CD19^+CD38^{dim/-}CD27^-IgD^+$) were sorted from PBMCs of healthy controls with a FACSAria™ Fusion cell sorter (BD Biosciences) using fluorochrome-labeled monoclonal anti-human antibodies (Table S2). In order to infect with EBV, naive mature B cells were incubated with EBV (strain B95.8) and 2.5 μg/mL CpG-ODN 2006-G5 (InvivoGen via Bio-Connect) for 3 h at 37 °C and 5 % CO_2 [22]. Afterwards, the cells were cultured for 6 weeks in the presence of 2.5 μg/mL CpG-ODN 2006-G5 in RPMI supplemented with 10 % FCS.

2.6. Phosphoflow

B-LCLs and primary B cells, risk and non-risk evenly distributed across experiments, were stimulated and stained in 96-wells round-bottom plates. We seeded $1–5*10^5$ cells in 50 μL RPMI with 2 % FCS in each well on ice. For stimulation, the plate was placed in a water bath at 37°. 100 ng/mL human recombinant IFN-γ (PeproTech via Tebu-Bio) was added for 10 min and the cells were directly placed on ice afterwards. Subsequently, cells ± IFN-γ stimulation were washed and stained extracellularly for 30 min at 4 °C in the dark using fluorochrome-labeled monoclonal anti-human antibodies (Table S3). Then, the cells were washed and stained with fixable viability dye (Zombie-NIR, Biolegend) for 10 min at 4 °C in the dark. Afterwards, fixation and permeabilization were done using the Transcription Factor Phospho Buffer Set (BD Biosciences) according to the manufacturer's protocol. The cells were stained intracellularly for p-STAT1 (Tyr701, AF647, Cell Signaling Technologies) for 30 min at 4 °C in the dark (Table S3). Stained cells were measured with a 5-laser Cytek Aurora flow cytometer (Cytek Biosciences). Data analysis was done using the OMIQ software from Dotmatics (www.omiq.ai, www.dotmatics.com).

2.7. Multicolor flow cytometry

B-LCLs and primary B cells, risk and non-risk evenly distributed

across experiments, were stained in 96-wells round-bottom plates. We seeded 0.5–2*10^6 cells in each well and stained them with fixable viability dye (eFluor520, Invitrogen) for 15 min at 4 °C in the dark. Then, the cells were washed and stained extracellularly using fluorochrome-labeled monoclonal anti-human antibodies (Table S4). Afterwards, fixation and permeabilization were done using the eBioscience™ Foxp3 Transcription Factor Staining Buffer Set (Invitrogen) according to the manufacturer's protocol. The cells were stained intracellularly for IFNGR1 (PE, Biolegend) and IFNGR2 (PE, Biolegend) for 45 min at 4 °C in the dark (Table S4). Stained cells were measured with a LSRII-Fortessa flow cytometer (BD Biosciences). Data analysis was done using the OMIQ software from Dotmatics (www.omiq.ai, www.dotmatics.com).

2.8. Immunoblotting

B-LCLs were stimulated in 5 mL tubes. We added 1*10^6 cells in 1 mL RPMI with 20 % FCS in each tube on ice. For stimulation, 100 ng/mL human recombinant IFN-γ (PeproTech via Tebu-Bio) was added for 10 min at 37 °C. Afterwards, cells ± IFN-γ stimulation were lysed and used for immunoblotting as reported previously [21]. For IFN-γRβ immunoblotting, cell lysates were loaded on a 4–15 % precast polyacrylamide gel and blotting was done on a *trans*-blot turbo mini PVDF membrane (Bio-Rad), which was blocked in 4 % BSA. Primary antibodies used were purified mouse anti-total Stat1 N-terminus (1/STAT1, BD Biosciences), purified mouse anti-Stat1 pY701 (4a, BD Biosciences), Armenian hamster anti-human IFN-γRβ (2HUB159, Santa Cruz), and mouse anti-human β-actin (AC-15, Abcam). Horseradish peroxidase (HRP)-conjugated goat-anti-mouse Ig (Dako) and goat anti-hamster Ig (Jackson ImmunoResearch) were used as secondary antibodies. Protein bands were visualized using Western Lightning Plus-ECL (PerkinElmer Inc.).

2.9. Quantitative polymerase chain reaction (qPCR)

For primary cells, $CD19^+$ B cells were isolated untouched from blood of untreated early MS patients using the B-cell isolation kit II (Miltenyi Biotec) according to protocol. For B cell lines, total fractions were used. RNA was extracted from the cells and converted to cDNA, followed by qPCR as reported previously [21]. *TBX21*, *IFNGR1*, *IFNGR2* and *STAT1* gene expression profiles were measured using forward en reverse primers (Table S5).

2.10. Statistics

GraphPad Prism 9 (GraphPad Software) and Rstudio (version 4.3.2., Posit) were used for interpreting all datasets and performing statistical analysis. For comparing two groups, non-parametric Wilcoxon signed-rank tests or Mann-Whitney U tests were performed. For comparing more than two groups, non-parametric Kruskal-Wallis or Friedman tests were performed. Post-hoc comparisons were performed using the Dunn's post-hoc test. Furthermore, correlations were analyzed by calculating the Spearman correlation coefficient. Comparisons were considered significant with a p-value <0.05 (two-tailed). Statistical significance is represented in all graphs using asterisks and all data are shown using the median.

3. Results and discussion

From 19 different human B lymphoblastoid cell lines (B-LCLs) analyzed, we selected 4 B-LCLs for exploration of the IFN-γ signaling pathway based on the amount of EBV DNA and the presence of *IFNGR2* risk SNP rs9808753: Karpas-422 (EBV^{low}, non-risk), MC-116 (EBV^{low}, risk), EHEB (EBV^{high}, non-risk), and JVM-13 (EBV^{high}, risk) (Table S1). In line with a previous expression quantitative trait loci (eQTL) analysis [20], qPCR showed that the risk SNP was associated with a higher *IFNGR2* gene expression in these B-LCLs (Fig. S1A). This was not seen for *IFNGR1* gene expression (Fig. S1B) and validated in primary B cells from untreated MS patients with and without this SNP (Fig. S2A). Flow cytometry analysis revealed that, in contrast to IFNGR1, both total and surface IFNGR2 protein was highly expressed in EBV^{high} versus EBV^{low} B-LCLs. However, this was not affected by the presence of rs9808753 (Fig. S3A). These findings were supported by immunoblotting (Fig. S3B). In patients from our natalizumab-treated MS cohort (Figs. S4A–B), whose blood samples are enriched for B cells with the tendency to infiltrate the CNS [5,23], IFNGR1 and IFNGR2 protein levels were not different between risk and non-risk SNP carriers (Fig. S4C). Despite the increased expression of both IFNGR1 and IFNGR2 for memory subsets (Fig. S4D), these levels also did not associate with rs9808753 at the B-cell subset level (Fig. S4E). We did find a strong, but risk SNP-unrelated increase in IFNGR1 and IFNGR2 levels in B cells after *in vitro* EBV infection (Fig. S4F).

The observed association of rs9808753 with increased IFNGR2 gene but not protein expression could imply that *IFNGR2* is more translated and directed to the B-cell surface at the moment of interaction with an IFN-γ-producing T cell [9,24], resulting in increased signaling and transcription of its target gene, *TBX21* (T-bet). Indeed, after IFN-γ stimulation, *TBX21* expression was more induced in B cells from untreated MS patients carrying rs9808753 (Fig. S2B).

To further assess this in conjunction with EBV infection, we next analyzed the expression level and activity of downstream molecule STAT1 in our 4 selected B-LCLs. First, *STAT1* gene expression was increased in the EBV^{high} B-LCL carrying the risk SNP (Fig. S1C). Second, basal p-STAT1 levels were higher in EBV^{high} compared to EBV^{low} B-LCLs, as determined by phosphoflow (Fig. 1A). Third, STAT1 phosphorylation was triggered by IFN-γ, especially in EBV^{low} B-LCLs, in which levels were approximately two times more induced in the presence of rs9808753 ($p = 0.0003$). This effect was less, but still significant in EBV^{high} B-LCLs (Fig. 1A). Thus, not only STAT1, but also p-STAT1 seems to be more constitutively expressed in the presence of EBV, thereby dampening the risk SNP-related IFN-γ responsiveness. To validate these results and analyze the dependency of the risk SNP effect on total STAT1 protein, we measured both STAT1 and p-STAT1 using immunoblotting (Fig. 1B). In line with the flow cytometry data, EBV^{low} B-LCLs carrying the risk SNP showed the most pronounced induction of p-STAT1/STAT1 ratios upon IFN-γ stimulation (Fig. 1B). Interestingly, p-STAT1/STAT1 ratios showed a relative increase already at baseline in B-LCLs carrying rs9808753 (Fig. 1B).

In primary B cells (Fig. 2A–B), *in vitro* EBV infection induced STAT1 phosphorylation (Fig. 2C), which was not influenced by the presence of rs9808753 or different between patients and controls (data not shown). In line with our B-LCL data, p-STAT1 was less induced by IFN-γ in EBV-infected B cells (Fig. 2C). At baseline, B cells showed a trend increase in p-STAT1 levels in risk versus non-risk SNP carriers (Fig. 2D). To further specify the latter difference, we subsequently analyzed the phosphorylation of STAT1 in blood B-cell subpopulations, which showed no differences in frequencies between non-risk and risk SNP carriers (Fig. S5). Interestingly, of all subpopulations analyzed, we found the highest p-STAT1 levels in transitional (early emigrant) B cells ($CD38^{high}CD27^-$), which, to our knowledge, has not been described before (Fig. 2E). These levels were even further increased in transitional B cells from MS patients carrying rs9808753, both at baseline and after IFN-γ stimulation (Fig. 2F). When discriminating between two successive stages of transitional B-cell development, we found that transitional type 1 (T1; $IgM^{high}IgD^{dim}$) cells expressed higher levels of p-STAT1 than transitional type 2 (T2; $IgM^{dim}IgD^+$) cells, irrespective of stimulation with IFN-γ (Fig. 2G) [25,26]. Accordingly, T1 versus T2 transitional B-cell ratios were associated with the increased p-STAT1 levels in risk versus non-risk SNP carriers (Fig. 2H). Counterintuitively, independent of the risk SNP, T1 transitional B cells showed the lowest IFNGR1 and IFNGR2 protein levels (Figs. S6A–D), which could possibly be explained by a more efficient internalization and recycling of the receptor complex [27].

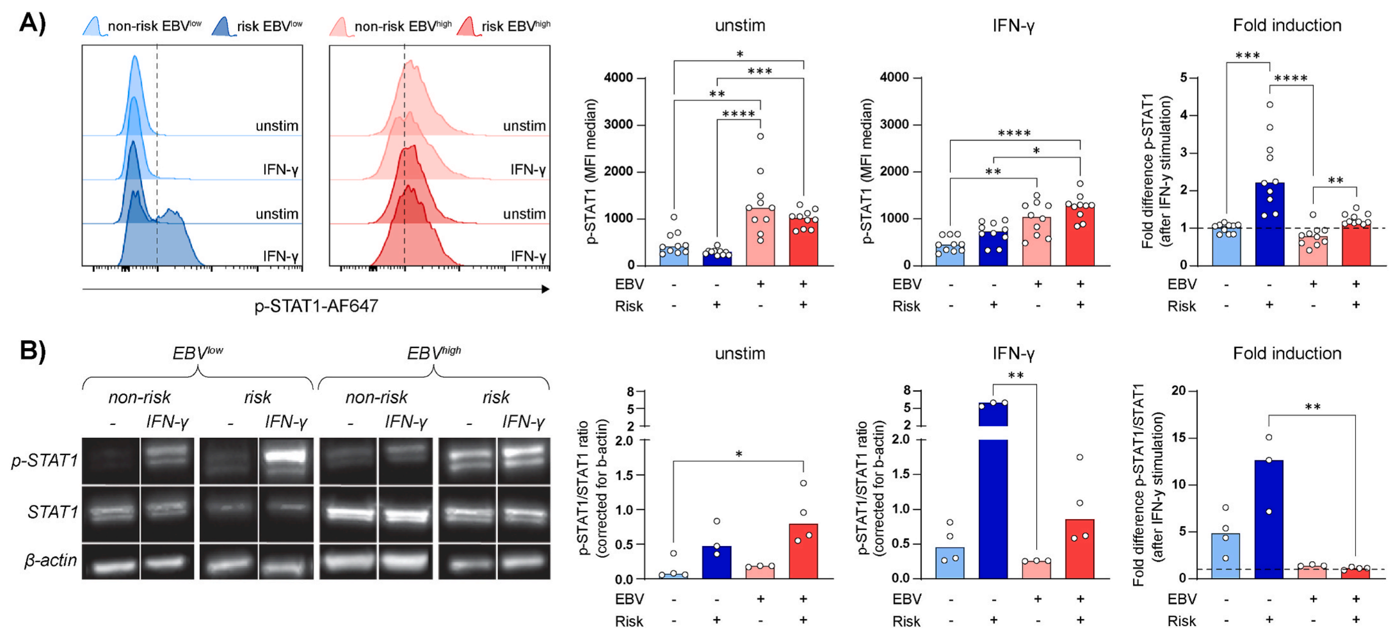

Fig. 1. STAT1 phosphorylation is influenced by EBV infection and the presence of rs9808753 in human B-cell lines. (A) Phosphorylated STAT1 (p-STAT1) levels were measured at baseline and after 10 min of IFN-γ stimulation in 2 EBV^{low} and 2 EBV^{high} B-LCLs with and without rs9808753 (non-risk: AA, risk: GG, n = 10) using phosphoflow. (B) Immunoblotting was performed for total and phosphorylated STAT1 protein levels in lysates from the same B-LCLs (n = 3–4) at baseline and after 10 min of IFN-γ stimulation. (A–B) Data were analyzed using Kruskal-Wallis and Dunn's post-hoc tests. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$; ****$p < 0.0001$.

These data imply that the risk SNP is associated with impaired differentiation of T1 (p-$STAT1^{high}$) into T2 (p-$STAT1^{low}$) transitional B cells. T1 transitional B cells have been proposed as targets of negative selection and seem to depend on STAT1 for further development in the spleen [28]. Hence, it is tempting to speculate that due to MS risk SNPs such as rs9808753, p-$STAT1^{high}$ T1 transitional B cells have an enhanced capacity to escape from selection and interact with IFN-γ-producing T follicular helper (T_{FH}) cells to promote subsequent maturation in the periphery [2,29,30]. Transitional B cells highly express the type I IFN receptor, which may further promote the survival of autoreactive populations via another coding MS risk SNP in *TYK2* [31–33]. In type I IFN-treated MS patients, changes in circulating transitional B cells are also associated with risk of active MRI lesions [34]. Not only IFNGR, but also CD40 plays a critical role in regulating T cell-mediated B-cell tolerance in humans [35] and is downregulated on B cells of people carrying an MS risk variant in *CD40* [36].

This work has some limitations. First, the used human B-cell lines and B cells of MS patients were specifically genotyped and/or selected for the risk SNP in *IFNGR2*, without considering any other risk variants that could impact the IFN-γ signaling pathway (see also above). Second, we do not have information on the genotype of healthy individuals who were included as a control group. However, also given the low odds ratio for individual (non-HLA) risk SNPs, we did not aim and expect to find disease-specific effects of this SNP. Third, we show a clear association of this risk SNP with changes in IFN-γ signaling, but do not prove causality. Fourth, the very low number of circulating EBV-infected B cells did not allow for a proper analysis of EBV as an independent determinant of T-bet $^{+}$ B cells *ex vivo*. Previously, we and others did find that T-bet^{+} B-cell differentiation is especially induced by EBV during the acute phase of infection [15,37,38], which underlines the impact of EBV years before the diagnosis of MS and reflects the EBV load analyzed in the current study. Despite these limitations, our findings contribute to a better understanding of the increased sensitivity of B cells to IFN-γ in MS [5,29].

4. Conclusion

These findings indicate an independent role of the *IFNGR2* risk SNP and EBV in regulating the IFN-γ signaling pathway in human B cells, in which the risk SNP has a more selective effect by promoting signaling through phosphorylation of STAT1. The association of rs9808753 with higher p-STAT1 levels in transitional B cells, the earliest emigrants from the bone marrow, provides a first clue to why B cells are more sensitive to IFN-γ and how these cells are alternatively selected in the periphery of people with MS. One could further speculate that through the interaction with IFN-γ-producing $CD4^{+}$ T helper cells, the *IFNGR2* risk SNP is one of the genetic hits and EBV infection is a secondary environmental hit for driving brain-homing B cells in MS. In such a model, during an acute infection, EBV exploits CD21 to infect and trigger the IFN-γ signaling pathway in naïve B cells. Together with the effect of rs9808753 and other risk SNPs, this could promote the survival of autoreactive $CD21^{low}$ (T-bet^{+}) B cells in people who develop MS [2,39]. Work by others indeed support the concept that T-bet is upregulated as a result of an early response to EBV infection to affect the B-cell differentiation program [37,38,40]. This could explain their propensity to become $CXCR3^{+}$ class-switched B cells that enter and terminally differentiate into antibody producers in the MS brain [5,7,15]. Obviously, more research is needed to further unveil this underlying B cell-restricted mechanism and how this differs in MS compared to other autoimmune diseases.

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CRediT authorship contribution statement

Laurens Bogers: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Jasper Rip:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **Liza Rijvers:** Formal analysis, Investigation, Methodology, Validation. **Jamie van Langelaar:** Formal analysis, Investigation, Methodology, Validation. **Steven C. Koetzier:** Formal analysis, Investigation, Methodology. **Kirsten L. Kuiper:**

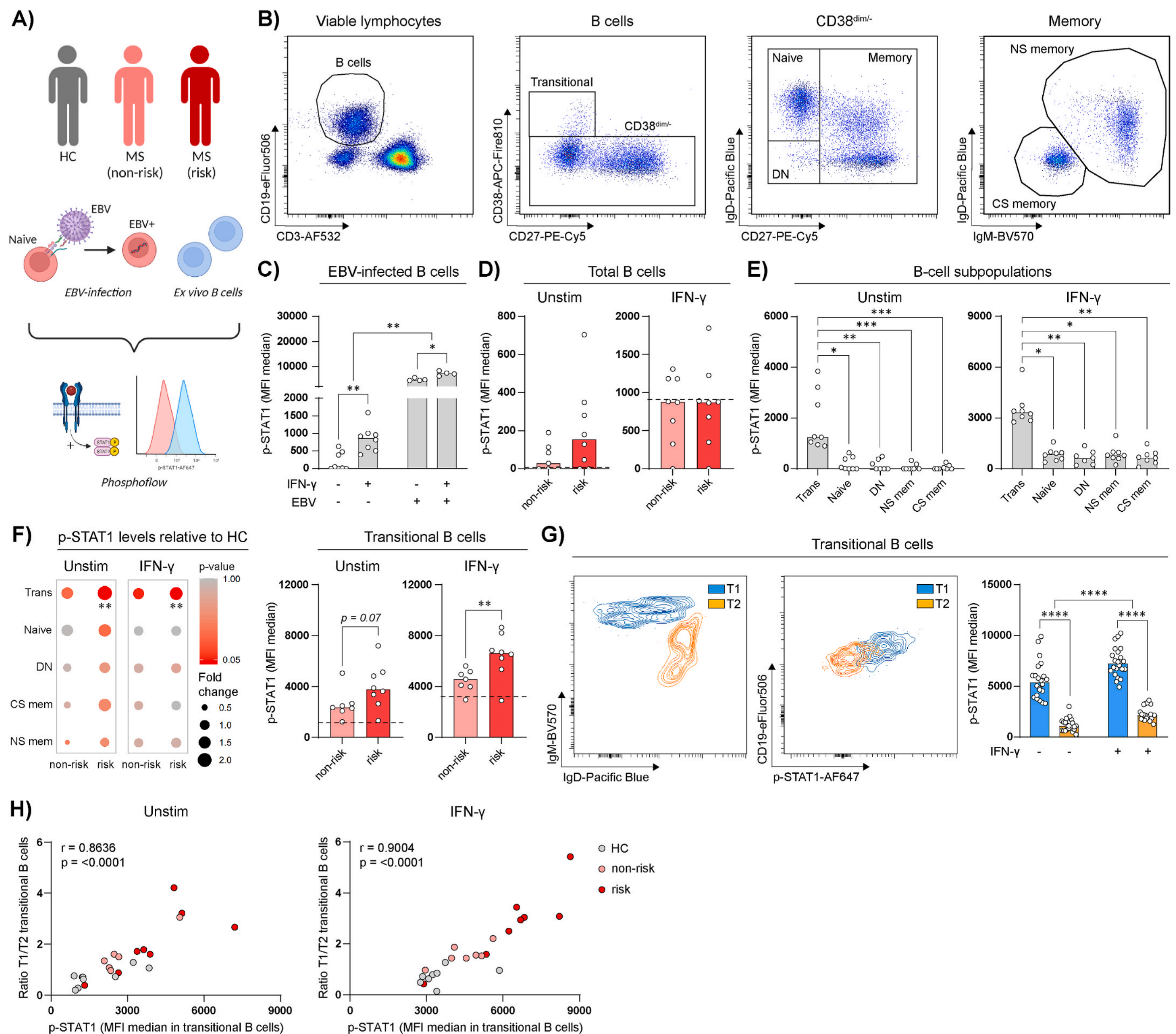

Fig. 2. EBV infection and the presence of rs9808753 induce STAT1 phosphorylation in primary B cells. (A) Peripheral blood mononuclear cells (PBMCs) were isolated from healthy controls (HC, n = 8) and natalizumab-treated MS patients genotyped and selected for the presence of rs9808753: non-risk (AA, n = 8) or risk (AG/GG, n = 8). FACS-sorted naive mature B cells were infected with EBV *in vitro*. Phosphoflow was performed on total and EBV-infected B cells to analyze phosphorylated STAT1 (p-STAT1) before and after IFN-γ stimulation for 10 min. (B) Within total B cells ($CD19^{+}CD3^{-}$), we analyzed transitional ($CD38^{high}CD27^{-}$), naive mature ($CD38^{dim/-}CD27^{-}IgD^{+}$), double negative ($CD38^{dim/-}CD27^{-}IgD^{-}$), non-switched memory ($CD38^{dim/-}CD27^{+}IgD/IgM^{+}$), and class-switched memory ($CD38^{dim/-}CD27^{+}IgD^{-}IgM^{-}$) populations. (C) p-STAT1 levels in B cells from healthy control blood before and after *in vitro* infection with EBV. (D) p-STAT1 expression in total B cells from the blood of healthy controls (dotted line) and both non-risk and risk patients groups. (E) p-STAT1 expression in B-cell subsets from healthy control blood. (F) Dot plot illustrating p-STAT1 levels in B-cell subsets among non-risk and risk patient groups relative to healthy controls. Dot size corresponds to the fold change in p-STAT1 expression between patients and healthy controls, while dot color signifies the p-value associated with this fold change. In addition, p-STAT1 expression in transitional B cells was compared between healthy controls (dotted line) and non-risk and risk patient groups. (G) p-STAT1 expression in T1 ($IgM^{high}IgD^{low}$) and T2 ($IgM^{dim}IgD^{+}$) transitional B cells from all groups. (H) Correlations between T1 versus T2 ratios and p-STAT1 expression in transitional B cells. Data were analyzed with (C, D, F) Mann-Whitney U tests, (E) Kruskal Wallis and Dunn's post-hoc tests, (G) Wilcoxon signed-rank tests, and (H) Spearman correlation coefficients. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$; ****$p < 0.0001$.

Investigation. **Veronique Meerdink:** Investigation. **Annet F. Wierenga-Wolf:** Investigation. **Marie-José Melief:** Investigation. **Ana M. Marques:** Investigation. **Joost Smolders:** Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Marvin M. van Luijn:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

Marvin M. van Luijn received research support from EMD Serono, Merck, Novartis, GSK and Idorsia Pharmaceutical Ltd. Joost Smolders received lecture and/or consultancy fees from Biogen, Merck, Novartis, Sanofi-Genzyme and Roche. The remaining authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2024.103279.

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