Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

B cell function impacts the efficacy of IFN-β therapy in EAE

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Abstract

Recent studies identified that interferon beta (IFN-β) treatment skewstowards a regulatory phenotype in multiple sclerosis. To assess B cell involvement during IFN-β therapy, we compared IFN-β treatment in a B cell-independent model and a B cell-dependent model of experimental autoimmune encephalomyelitis (EAE). We show that in B cell-independent EAE, IFN-β ameliorates neuroinflammation. Conversely, in B cell-dependent EAE, IFN-β has no effect on disease. Effective IFN-β therapy in B cell-independent EAE was associated with reduced inflammatory T cells in the CNS and skewed splenic B cells towards an immature population and away from a germinal center population. These immune cell populations were unchanged in B cell-dependent EAE. Finally, we found that IFN-β increased marginal zone B cells in both EAE models. These findings indicate that B cell function impacts IFN-β efficacy during neuroinflammation.

1. Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disorder characterized by inflammation of the central nervous system (CNS) and neuronal demyelination. For years, T cells were considered the predominant mediators of inflammatory damage in MS (Chitnis, 2007), but clinical studies have now identified that B cells are also key players of MS pathology (Castillo-Trivino et al., 2013). Relapsing-remitting MS (RRMS) is the most common form of MS and is characterized by alternating episodes of relapses and recovery. Interferon beta (IFN-β) was the first disease-modifying drug developed for RRMS. IFN-β has a strong safety profile and significantly reduces disease activity in the general MS population (Group, T.I.M.S.S, 1993). However, approximately 30% of RRMS patients respond poorly to IFN-β. The therapeutic mechanism of IFN-β in MS remains unclear. Studies from our lab and others indicate that IFN-β has either pro-inflammatory or anti-inflammatory effects, depending on disease context (Axtell et al., 2010; Hegen et al., 2016; Palace et al., 2010; Paty and Li, 1993).

Previous studies indicate that IFN-β therapy drives a regulatory B cell phenotype in RRMS and in experimental autoimmune encephalomyelitis (EAE) induced in C57BL/6 with rodent myelin-oligodendrocyte glycoprotein (MOG) peptide 35–55 (Dooley et al., 2016; Schubert et al., 2015). EAE can be induced by immunization with various myelin antigens in different strains of mice and each EAE model mimics different aspects of MS. For instance, different forms of MOG antigen dictate the impact of B cells on disease development in C57BL/6 mice (Lyons et al., 1999). EAE induced with rodent MOG35–55 peptide (rpMOG) is widely thought to be B cell-independent. In fact, B cell depletion prior to the induction of EAE exacerbates disease, indicating that certain subsets of B cells are regulatory in this model (Matsushita et al., 2008; Weber et al., 2010). However, in EAE induced with recombinant human MOG1–125 protein (hMOG), B cell depletion ameliorates EAE, indicating an inflammatory role of B cells in this model (Weber et al., 2010). The difference in B cell requirement in rpMOG versus hMOG-induced EAE has been attributed to differences in antigen presentation and antigen-specific antibody secretion (Molnarfi et al., 2013).

Many previous studies have shown that IFN-β treatment ameliorates rpMOG-induced EAE (Axtell et al., 2010; Guo et al., 2008; Inoue et al., 2016; Prinz et al., 2008). However, the effect of IFN-β treatment on hMOG-induced EAE is currently unknown. Since we previously showed that IFN-β drives regulatory B cell functions in rpMOG-induced EAE (Schubert et al., 2015), we sought to examine whether IFN-β would have similar regulatory effects in hMOG-induced EAE where B cells drive disease. Therefore, we compared the effects of IFN-β therapy on B cell-independent (rpMOG) and B cell-dependent (hMOG) models of EAE. We show that IFN-β ameliorates rpMOG-EAE, re-affirming our previous observations of IFN-β having a regulatory role in B cell-
independent EAE (Schubert et al., 2015). In contrast, we show that IFN-β has no effect on hMOG EAE, indicating the poor efficacy of IFN-β in B cell-driven EAE. Additionally, IFN-β reduced CNS infiltration of inflammatory cells in rpMOG-induced but not hMOG-induced EAE. Our findings indicate that B cells impact the efficacy of IFN-β treatment during neuroinflammation and provides insights into the varied responsiveness to IFN-β therapy in the MS patient population.

2. Materials and methods

2.1. Mice

Eight to ten-week-old female C57BL/6 were purchased from Jackson Laboratory and held in the Oklahoma Medical Research Foundation animal facility. All animals were housed and treated in compliance with the institutional IACUC.

2.2. EAE induction

C57BL/6 were immunized subcutaneously with 150 μg of rodent MOG p35–55 (rpMOG) (Genemed Synthesis Inc.) or human recombinant MOG protein1-125 (hMOG) (Anaspec) emulsified in Complete Freund’s adjuvant (5 mg/ml heat killed M. tuberculosis), followed by intraperitoneal injection of 250 ng Bordetella pertussis toxin (List Biological Laboratories Inc.) at day 0 and day 2 following immunization. Paralysis was monitored daily using a standard clinical score: 1) Loss of tail tone, 2) incomplete hind limb paralysis, 3) complete hind limb paralysis, 4) forelimb paralysis and 5) moribund/dead. Mice were treated every second day with IFN-β (10,000 U/dose; PBL) or vehicle (PBS), starting at day 6 post-immunization. Spinal cords from EAE mice were fixed and sectioned for histological analysis using H&E staining.
2.3. Analysis of CNS immune cell infiltration by flow cytometry

At disease endpoint, CNS infiltrating cells were isolated from the spinal cords of perfused animals. CNS homogenates were incubated with collagenase (4 mg/ml; Roche) and DNAse (5 μl/ml; Sigma) at 37°C for 1 h. Cells were isolated by Percoll gradient and analyzed by flow cytometry. Cells were stimulated with PMA (Sigma-Aldrich), ionomycin (Sigma-Aldrich) and monensin (BD Biosciences) for 4 h prior to intracellular staining. Cells were stained with a fixable viability dye (Biolegend) and treated with Fc block before staining with anti-mouse CD4 (Biolegend) and anti-mouse CD19 (Biolegend). Cells were then fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) before staining with IL-17 (Biolegend), IFNγ (Biolegend) and GM-CSF (Biolegend) (Suppl. Fig. 1). All flow cytometric data was collected using LSRII (BD Biosciences) and analyzed using FlowJo (Tree Star Inc.).

2.4. Analysis of splenic B cells by flow cytometry

Spleens were harvested from rpMOG-EAE and hMOG-EAE mice 15 days post-immunization. Spleens were processed and cells were stained with viability dye (Biolegend) and treated with Fc block prior to staining with the following antibodies; anti-mouse CD19 (Biolegend), anti-mouse CD21/35 (eBioscience), anti-mouse CD23 (BD Biosciences), anti-mouse PNA (Vector Laboratories), Streptavidin (eBioscience), anti-mouse GL-7 (eBioscience), IgM (Invitrogen) and IgD (Invitrogen) (Suppl. Fig. 2).

3. Results

3.1. IFN-β is effective for B-cell independent EAE but has no effect on B-cell dependent EAE

Several reports have shown that IFN-β treatment ameliorates rpMOG-induced EAE (rpMOG-EAE) (Axtell et al., 2010; Guo et al., 2008; Inoue et al., 2016; Prinz et al., 2008), a B-cell-independent model, and we have previously published that the efficacy of IFN-β requires regulatory B cell phenotype in this EAE model (Schubert et al., 2015). Currently the effect of IFN-β on hMOG-induced EAE (hMOG-EAE) is unknown. In order to better understand the effects of IFN-β treatment on B cell function in EAE, we directly compared efficacy of IFN-β treatment in the B cell-dependent disease model (hMOG-EAE) and the B cell-independent disease model (rpMOG-EAE). In both EAE models, mice were treated with IFN-β (10,000 units/dose) or vehicle every other day starting at day 6 and continuing until the termination of the experiment (Fig. 1). Consistent with our previous observations, our data show that IFN-β treatment significantly reduced the severity of paralysis in rpMOG-EAE mice (Fig. 1a). On the other hand, we found that IFN-β had no effect on the paralysis of hMOG-EAE mice (Fig. 1b). Similarly, linear regression analysis showed that IFN-β ameliorated disease in rpMOG-EAE (Fig. 1c) but had no impact on hMOG-EAE (Fig. 1d). In agreement with disease scores, our data show that IFN-β reduces cellular infiltration in the cerebellum and spinal cords of rpMOG-EAE mice (Fig. 1e). However, IFN-β did not reduce cellular infiltration in hMOG-EAE mice (Fig. 1f).
infiltration in the cerebellum and spinal cords of hMOG-EAE mice (Fig. 1f). Therefore, these data demonstrate that IFN-β treatment is efficacious in B cell-independent EAE but is ineffective in B cell-dependent EAE.

3.2. IFN-β does not impact CNS immune cell populations in B-cell driven EAE

To assess the impact of IFN-β on CNS-infiltrating immune cells during rpMOG-induced EAE and hMOG-induced EAE, immune cell populations in the CNS of rpMOG-EAE and hMOG-EAE mice were analyzed. EAE mice treated with IFN-β or vehicle were sacrificed at disease endpoint (EAE day 25) to assess the infiltration of immune cells in the CNS. Congruent with disease scores, IFN-β significantly reduced immune cell infiltration in the CNS of rpMOG-EAE mice (Fig. 2). The number of CD4+ T-helper (TH) cells and CD19+ B cells was significantly reduced in the spinal cords of IFN-β-treated mice (Fig. 2a). Additionally, IFN-β treatment also reduced inflammatory immune T-cell populations, CD4+ IFNγ+ (TH1), CD4+ IL-17+ IFNγ+ (TH1/TH17) (Fig. 2b) and CD4+ GM-CSF+ in the CNS of rpMOG-EAE mice (Fig. 2c).

In contrast, IFN-β had no effect on immune cell infiltration in the CNS of hMOG-EAE mice (Fig. 3). There was no significant impact on TH cells and B cells in the spinal cords of hMOG EAE mice treated with IFN-β (Fig. 3a). Additionally, IFN-β treatment did not significantly impact the generation of IFNγ, IL-17 (Fig. 3b) and GM-CSF by TH cells (Fig. 3c). These data provide further evidence that IFN-β is efficacious in reducing neuroinflammation in B cell-independent EAE but having no effect on neuroinflammation in B cell-dependent EAE.

3.3. IFN-β differentially alters B cell subsets in B cell-dependent versus B cell-independent EAE

Previously, we have shown that IFN-β alters B cell populations in the spleens of mice with rpMOG-induced EAE (Schubert et al., 2015). To study the effects of IFN-β therapy on B cell subsets in B cell-independent and B cell-dependent EAE, splenic B cell populations from rpMOG-EAE and hMOG-EAE mice were analyzed after treatment with IFN-β or vehicle (Fig. 4). In rpMOG-EAE, we found that IFN-β significantly increased the immature/transitional (IgM+ IgDlo) and marginal zone (MZ) B cell populations and decreased germinal center (GC) B cells (Fig. 4a). No effect was seen on mature B cells, follicular (FO) B cells (Fig. 4a), class-switched memory B cells and total splenic B cells with IFN-β treatment of rpMOG-EAE (Suppl. Fig. 3a).

In contrast, IFN-β treatment of hMOG-EAE only affected the MZ B cell population, as it was increased with this therapy (Fig. 4b). These data demonstrate that IFN-β treatment differentially affects B cell populations in rpMOG- and hMOG-EAE which correlates with the efficacy of treatment in reducing disease severity.

4. Discussion

Taken together, our data suggest that different B cell functions play a key role in determining the outcome of IFN-β therapy in neuroinflammation. Similar to our previous reports, we found that IFN-β...
ameliorates rpMOG-induced EAE, a model in which B cells can have anti-inflammatory effects (Schubert et al., 2015). We also show for the first time that IFN-β has no effect on hMOG-induced EAE, where B cells play an inflammatory role. In agreement with its effects on disease, we observe that IFN-β reduces immune cell infiltration in the CNS of rpMOG-EAE mice, whereas treatment with IFN-β has no effect on immune cell populations in the CNS of hMOG EAE mice. We also show that IFN-β promotes the expansion of an immature/transitional B cell population in rpMOG-EAE but not hMOG-EAE. In our study, we assessed the effects of IFN-β on B cells in the spleen and T cells in the CNS at different timepoints. Hence, the sequential immunological events of IFN-β are not entirely resolved. However, immature B cells are known to have anti-inflammatory functions. In MS, immature and transitional B cell populations also undergo expansion following treatment with different therapies, including IFN-β, fingolimod, natalizumab and alemtuzumab in MS patients and rituximab in rheumatoid arthritis patients (Chiarini et al., 2015; Dooley et al., 2016; Haas et al., 2011; Heidt et al., 2012; Krumbholz et al., 2008; Leandro et al., 2006; Schubert et al., 2015). Therefore, our current data re-affirm the function of immature/transitional B cells in mediating the immunoregulatory effects of IFN-β during neuroinflammation (Schubert et al., 2015) and we hypothesize that IFN-β is expanding an immature B cell population in the peripheral lymphoid organs which then inhibits inflammatory T cell responses in the CNS.

Additionally, we show that IFN-β reduces splenic GC B cells in rpMOG-induced EAE but has no effect on this B cell subset in hMOG-EAE. The GC B cell population is a key developmental checkpoint which leads to the generation of high affinity plasma cells and memory B cells (Mesin et al., 2016). Our data indicate that IFN-β only inhibits B cell responses in the B cell-independent EAE model. Finally, in both rpMOG-induced EAE and hMOG-induced EAE, IFN-β increases the splenic MZ B cell population. Thus, IFN-β promotes MZ B cell expansion regardless of whether B cells drive anti-inflammatory or inflammatory effects in EAE. Since MZ B cells have the capacity to secrete either anti-inflammatory IL-10 (Lee and Kung, 2012) or pro-inflammatory IL-6 (Barr et al., 2012), a possible explanation could be that MZ B cells have either inflammatory or regulatory properties depending on disease context.

Previous reports show that MOG-specific T cells and B cells cooperate to induce a spontaneous, severe form of EAE in which inflammatory lesions are primarily distributed in the optic nerves and spinal cord, closely replicating human neuromyelitis optica (NMO). Similar to MS, NMO is a neuro-inflammatory disorder involving demyelination of the CNS. Here, we observe that pathological and clinical phenotypes of NMO are mimicked in the hMOG-EAE model where B cells drive pathogenesis and IFN-β therapy shows poor efficacy. It was previously also shown that MOG-specific B cells can also drive MOG-specific T cell proliferation and activation by enhancing antigen presentation. In addition to driving T cell responses in the peripheral lymphoid tissue, B cells can also form lymphoid follicle-like structures in the meninges, which can enhance T cell responses locally in the CNS. Whether similar mechanisms are affected by IFN-β treatment in the hMOG-EAE model still needs to be addressed.

The differing roles of B cells in affecting IFN-β therapy outcome during rpMOG or hMOG-induced EAE reflects the nuanced nature of the EAE model and highlights the importance of choosing the appropriate EAE model for testing different MS therapies. Therefore, careful consideration in choosing which EAE model to use in preclinical studies will be key for the translation of new therapies into MS patients. Altogether, these findings give insight into the role B cells play in the efficacy of IFN-β and may have implications determining response to this therapy.

Disclosures

Dr. Axtell has consulted for and is on the bureau of speakers for EMD-Serono.

Declaration of Competing Interest

Dr. Axtell has consulted for and is on the bureau of speakers for EMD-Serono.

Acknowledgements

This manuscript was funded by grants awarded to Dr. Axtell from the National Multiple Sclerosis Society (RG-1602-07722) and the
National Institutes of Health (R01AI137047 and R01EY027346).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.577106.

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