Type I interferon (IFN-I) and T helper 17 (TH17) drive pathology in neuromyelitis optica spectrum disorder (NMOSD) and in TH17-induced experimental autoimmune encephalomyelitis (TH17-EAE). This is paradoxical because the prevalent theory is that IFN-I inhibits TH17 function. Here we report that a cascade involving IFN-I, IL-6 and B cells promotes TH17-mediated neuro-autoimmunity. In NMOSD, elevated IFN-I signatures, IL-6 and IL-17 are associated with severe disability. Furthermore, IL-6 and IL-17 levels are lower in patients on anti-CD20 therapy. In mice, IFN-I elevates IL-6 and exacerbates TH17-EAE. Strikingly, IL-6 blockade attenuates disease only in mice treated with IFN-I. By contrast, B-cell-deficiency attenuates TH17-EAE in the presence or absence of IFN-I treatment. Finally, IFN-I stimulates B cells to produce IL-6 to drive pathogenic TH17 differentiation in vitro. Our data thus provide an explanation for the paradox surrounding IFN-I and TH17 in neuro-autoimmunity, and may have utility in predicting therapeutic response in NMOSD.
IgG antibodies against the astrocyte water channel protein, 
Rituximab-treated NMOSD (NMO-Ritux) patients had many 
fact, when compared with healthy controls, we found that 
expression and away from genes associated with NMOSD etiology. In 
therapy and 17% of patients were not on disease-modifying 
patients were on rituximab, 21% were on non-B-cell-depleting 
with healthy controls. In our cohort of NMOSD patients, 62% of 
signatures that are associated with NMOSD disease compared 
healthy controls (Supplementary Fig. 1).

In lupus, there is an association between the expression of IFN-
signature genes and variations in clinical features. Therefore, 
sought to determine whether IFN-I signatures can distinguish clinical 
differences in the NMOSD population. We found that 
pathologies that reflect NMO-DS and MS, respectively.

Like NOMOSD, EAE induced with TH17 cells manifests with 
severe optic neuritis, involves neutrophil infiltration into the CNS 
and has elevated levels of IL-17. Even more striking are the 
differential effects of IFN-β treatment on TH17-EAE and TH1-
EAE. TH17-EAE mice had increased paralysis and increased 
immune cell infiltration in the spinal cords when treated with 
IFN-β. Conversely, IFN-β treatment of TH1-EAE mice 
significantly reduced paralysis and inflammation in the CNS. 
These observations position TH17-EAE models as useful tools to 
study how IFN-1 and TH1 drive pathology in diseases such as NMOSD.

The cooperative effects of TH17 cells and IFN-I in NOMOSD 
and TH17-EAE were unexpected observations. The prevailing 
theory is that IFN-I inhibits the differentiation of TH17 cells and 
it has been speculated that the efficacy of this therapy in MS is 
achieved by inhibiting the function of the TH17 pathway. This 
paradox represents a major knowledge gap in the field of 
neurology.

In this study, we perform biomarker studies in NOMOSD 
patients and experiments in mice with TH17-EAE to resolve this 
paradox. Our data suggest the mechanism by which IFN-I con-
tributes to the pathogenicity of TH17 cells is through the 
induction of IL-6 in B cells.

**Proteomic signatures in IFN-high and IFN-low NOMOSD.** We 
next determined which inflammation-related protein biomarkers 
were associated with the IFN-I transcriptional signature. We used a multiplex approach (OLINK) to assess the 
levels of 91 inflammatory proteins in the IFN-high patients and 
IFN-low patients compared with healthy volunteers. Using 
multivariate analysis of variance, we found that 26 inflammatory 
proteins were significantly elevated (with adjusted p values of <0.05 and Log2FC > 0.5) in the IFN-high NOMOSD patients 
compared with healthy controls (Fig. 1f, Supplementary Data 4). In comparison, only three proteins were elevated in the 
IFN-low NOMOSD patients compared with healthy controls (Fig. 1f, Supplementary Data 4). As expected, we found that 
chemokines induced by IFN-I (CXCL9, CXCL10, CXCL11, 
MCP-3/CCL7) were elevated in the IFN-high patients but not in 
the IFN-low patients. We also found that IL-17A, the prototypic TH17 cytokine, and CCL20, a chemokine that promotes 
TH17 trafficking into inflamed tissue, were elevated in the IFN-
high patients but not the IFN-low patients. Finally, we observed that IL-6 was among the most elevated proteins in the IFN-high 
patients (Fig. 1f). These data show that patients with high IFN-I also display elevated levels of serum IL-6 and proteins 
associated with the TH17 pathway.
Fig. 1 NMOSD patients stratify into two groups based on IFN-I gene expression. RNA profiles of a untreated patients (NMO-untreated; n = 7), b Rituximab-treated patients (NMO-Ritux; n = 24) and c patients on other therapies (NMO-Other Tx; n = 7) were compared with healthy volunteers (n = 18). d Venn diagram of differentially expressed genes of the NMO-Untreated vs healthy, NMO-Ritux vs healthy, and NMO-Other Tx vs healthy. e Heatmap depicts relative levels of IFN-I genes in NMOSD patients (Red = NMO-Untreated, Yellow = NMO-Other Tx, Green = NMO-Ritux). Patients were stratified into two groups, IFN-low and IFN-high, based on IFN-I gene expression. Yellow represents relative high expression and blue represents relative low expression. f Heatmap depicts the differentially abundant serum proteins in IFN-high NMOSD (NMO-Ritux vs healthy). g Heatmap of differentially expressed serum proteins in NMOSD patients of different EDSS range (EDSS 4–6.5, IFN-low NMOSD n = 22, and healthy controls n = 18). Yellow represents relative high serum levels; blue represents relative low serum levels. Comparison of h number of relapses 2 years prior to sample collection, i age, and j autoantibody status of IFN-high and IFN-low NMOSD patients. Two-tailed Student’s t tests and Chi-square tests were used to determine statistical significance. k MCP-3 and l IL-6 levels in NMOSD patients of different EDSS range (EDSS 4–6.5: n = 15, EDSS 2.5–3.5: n = 9, EDSS 0–2: n = 16). P values were determined using two-tailed Kruskal-Wallis tests with multiple comparisons corrected by the Dunn’s method. Bar graphs represent the mean and error bars are the S.E.M. Source data are provided as a Source Data file.

Fig. 2 Correlation between TH17 and disability in NMOSD patients. Correlations between EDSS and %TH17, %TH17.1, %TH17 + %TH17.1, and %TH1 cells in NMOSD patients (n = 6). Two-tailed Pearson correlations were used to determine statistical significance. P values < 0.05 were considered significant and P values > 0.05 were not significant.

NMOSD patients (Supplementary Table 2) and determined whether TH17 cells (CXCR3+CCR6+CD161+), TH17.1 cells (CXCR3+CCR6+CD161+), or TH1 cells (CXCR3+CCR6−CD161−) correlated with EDSS. We found no clear correlation between TH17 and TH17.1 with EDSS (Fig. 2a, b). However, combined frequencies of TH17 and TH17.1 showed a significant positive correlation with EDSS (Fig. 2c). We did not observe a positive correlation with EDSS and TH1 cells (Fig. 2d). In addition, we found that the percentage of TH17 cells, but not TH1 or TH17.1, was higher in the NMOSD patients compared to healthy volunteers (Supplementary Fig. 1).

Taken together, these data provide evidence that transcriptomic, proteomic, and Cell-Based-Assays can stratify NMOSD patients based on disability. Furthermore, these data suggest that
the cooperative effects of IFN-I, TH17, and IL-6 drive excessive CNS tissue damage that result in severe disability in NMOSD.

**Effects of B-cell-depleting therapy in NMOSD.** A popular therapy for NMOSD is B-cell depletion with anti-CD20 antibody. In our cohort, 62% of the patients were on rituximab at the time of serum sample collection (Supplementary Table 1). To determine the transcriptional effects of rituximab treatment, we compared DEGs in patients treated with rituximab with patients not treated with rituximab (Supplementary Data 5). Since we found no significant difference in the transcriptomes of NMO-Un treated and NMO-Other Tx patients, we combined these patients for this comparative analysis. Based on the expression of DEGs, patients were clustered into two groups, which we defined as B-cell-deficient and B-cell-sufficient (Fig. 3a). We found that all patients in the B-cell-deficient group were on rituximab therapy. We also found that 14 of the 18 patients in the B-cell-sufficient group were on rituximab or other B-cell-depleting therapies (Fig. 3a). Based on similar expression patterns in the population, the DEGs were divided into four gene clusters (Fig. 3a, b). Genes in clusters 1–3, which were reduced in the B-cell-deficient group, were determined to be predominantly expressed in B cells using the cell-specific RNA database (http://www.proteinatlas.org) (Fig. 3b). Conversely, genes elevated in the B-cell-deficient patients in cluster 4 were expressed in cell types other than B cells (Fig. 3b).

To determine whether B-cell depletion affects the IFN-I signature of NMOSD patients, we assessed the distribution of IFN-high and IFN-low NMOSD patients in the B-cell-deficient and B-cell-sufficient groups (Fig. 3c). The percentage of IFN-high and IFN-low NMOSD patients were similar in both B-cell-deficient and B-cell-sufficient patients (Fig. 3c). In addition, composite IFN scores, defined as an average read count of IFN-I genes, were not different between B-cell-deficient and B-cell-sufficient patients that were IFN-high or IFN-low (Fig. 3d). These data indicate that B-cell depletion with rituximab treatment does not impact IFN-I gene expression in NMOSD.

However, we did observe that serum protein profiles were significantly different in B-cell-deficient patients compared with B-cell-sufficient patients (Fig. 3e, Supplementary Data 6). Interestingly, we found that serum levels of IL-6, IL-17, and MCP-3 are highest in the IFN-high B-cell-sufficient NMOSD patients and were reduced in the B-cell-deficient IFN-high group (Fig. 3e, Supplementary Data 6). We also compared levels of IL-6, IL-17 and MCP-3 in NMO-Un treated, NMO-Other Tx, NMO- Ritux, and healthy controls. Serum IL-6 levels were elevated in NMO-un treated and NMO-Other-Tx patients, but not in NMO-Ritux patients; serum IL-17 was elevated in the NMO-Other Tx patients; and MCP-3 was elevated in the NMO-Un treated patients (Fig. 3f). These data suggest that B cells are a key cell type in elevating IL-6, IL-17, and MCP-3 in NMOSD patients.

Recent studies have suggested that the efficacy of B-cell depletion differs in AQP4-IgG+ and MOG-IgG+ NMOSD patients. In our cohort, we compared annualized relapse rates in AQP4-IgG+ and MOG-IgG+ B cell-sufficient and B-cell-deficient patients. In the B-cell-sufficient patients, we found no
the S.E.M. Source data are provided as a Source Data file.

Fig. 4 Effects of B-cell depletion on AQP4-IgG+ and MOG-IgG+ NMOSD. a Comparison of relapse rates in AQP4-IgG+ (n = 12) and MOG-IgG+ NMOSD (n = 5) who are B-cell-sufficient. b Comparison of relapse rates in AQP4-IgG+ (n = 13) and MOG-IgG+ (n = 7) NMOSD who are B-cell-deficient. P values were determined using two-tailed Mann-Whitney tests. Serum IL-6 and MCP-3 levels in B-cell-sufficient AQP4-IgG+ patients (n = 13), B-cell-deficient MOG-IgG+ patients (n = 5) and B-cell-deficient MOG-IgG+ patients (n = 7) were compared with healthy controls (n = 18). P values were determined using one-way ANOVA tests with multiple comparisons corrected by the Tukey’s method. Error bars indicate the S.E.M. Source data are provided as a Source Data file.

IFN-I drives expression of IL-6 in human memory B cells. The data above suggest that B cells are the major producers of IL-6 in NMOSD. We next questioned whether IFN-I drives IL-6 expression in B cells. In B-cell-sufficient NMOSD, we found significant positive correlations between IL-6 and IFN scores and between IL-6 and CXCL11 protein (Fig. 5a). In contrast, no correlation between IL-6 and IFN scores or between IL-6 and CXCL11 was seen in B-cell-deficient NMOSD patients (Fig. 5a). In healthy controls, no correlation was observed between IL-6 and IFN scores but there was a significant correlation between IL-6 and CXCL11 protein (Fig. 5a). Together, these correlations suggest that IL-6 is induced by IFN-I in B cells. To directly test this hypothesis, we isolated CD27− naive and CD27+ memory B cells from PBMCs of healthy donors. Both B-cell subsets were activated through CD40 and B-cell receptor in the presence or absence of IFN-β. We found that IFN-β stimulation did not alter CD80 and CD86 expression in naive B cells but significantly increased their expression in memory B cells (Fig. 5b, c). We also found that IFN-β stimulation did not alter IL-6 expression in naive B cells but significantly increased IL-6 expression in memory B cells (Fig. 5b, c). Thus, these data from patient sera and B-cell cultures from healthy donors provide strong evidence that IFN-I drives memory B cells to produce high levels of IL-6 in NMOSD.

IFN-I exacerbates disease and elevates IL-6 in TH17-EAE. Animal models are necessary to identify disease mechanisms which cannot be experimentally addressed in humans. We and others have reported that TH17-EAE mimics several features of NMOSD9,13,14. Like NMOSD, we previously identified that IFN-β treatment is not an effective therapy for TH17-EAE and instead exacerbates disease14. However, the mechanism by which this occurred was not identified. Consistent with our previous observations, IFN-β exacerbated paralysis (Fig. 6a) and increased the infiltration of immune cells and demyelination in the spinal cords of mice with TH17-EAE (Fig. 6b). Since IFN-I signatures and serum IL-6 were associated with increased disease burden in NMOSD patients (Fig. 1g, i), we tested if IFN-β treatment elevated IL-6 in mice with TH17-EAE. We found that IFN-β treatment significantly elevated serum levels of IL-6 (Fig. 6c). In addition, we found that numbers of T helper cells were elevated in the spinal cords of IFN-β treated mice (Fig. 6d). T-helper cells co-expressing IL-17 and granulocyte-macrophage colony-stimulating factor (GM-CSF) were also elevated in the CNS of TH17-EAE mice treated with IFN-β (Fig. 6e). We also measured B cells in the spinal cords of TH17-EAE mice treated with vehicle or IFN-β. We found that IFN-β treatment did not alter the number of B cells in the CNS of TH17-EAE mice (Fig. 6f). Our EAE data indicate that treatment of TH17-induced disease with IFN-I is associated with high levels of IL-6 and increased numbers of CNS infiltrating, inflammatory TH17 cells.

Blocking IL-6 ameliorates IFN-I-treated TH17-EAE. IL-6 is a potent inflammatory cytokine that is critical for the induction and pathogenic function of TH17 cells24,25. However, it has been reported that blocking IL-6 has little effect on TH17-EAE26. We observed that IFN-β treatment induces higher levels of IL-6, so we hypothesized that blocking IL-6 would ameliorate disease in TH17-EAE mice treated with IFN-β. To address our hypothesis, TH17-EAE mice were treated with IFN-β or vehicle as well as with an antagonistic anti-IL-6R antibody or isotype control. We found that treatment with anti-IL-6R did not ameliorate TH17-EAE in vehicle-treated mice (Fig. 7a). In agreement with the
IL-17 (Fig. 7b) and no difference in the number of neutrophils or difference in the number of T helper cells secreting GM-CSF or adoptive transfer EAE26,27. In contrast, we found that treatment reports showing that inhibition of IL-6 does not effectively reduce cords of vehicle-treated mice. This result is similar to previous clinical course of vehicle-treated mice, we found no significant difference in the number of T helper cells secreting GM-CSF or IL-17 (Fig. 7b) and no difference in the number of neutrophils or inflammatory monocytes (Supplementary Fig. 3a) in the spinal cords of vehicle-treated mice. This result is similar to previous reports showing that inhibition of IL-6 does not effectively reduce adoptive transfer EAE26,27. In contrast, we found that treatment with both IFN-β and anti-IL-6R significantly attenuated TH17-induced EAE (Fig. 7c). Reduced numbers of GM-CSF+ and IL-17 + TH cells (Fig. 7d) and neutrophils (Supplementary Fig. 3b) was observed in the CNS of mice treated with both IFN-β and anti-IL-6R. These data thus show that IFN-I drives an inflammatory function of IL-6 in TH17-EAE.

B-cell-deficiency reduces TH17-EAE regardless of IFN-I. Several studies support the importance of B cells in driving TH17-induced neuroinflammation28–30. However, how Type I IFN affects the function of B cells during TH17-EAE is not known. To address this question, we induced TH17-EAE in C57BL/6 mice and in B-cell-deficient, µMT mice and then treated with either vehicle or IFN-β. We found that vehicle-treated µMT mice had a significant delay in the onset of TH17-EAE disease as compared to vehicle-treated C57BL/6 mice (Fig. 8a). However, we found that at the experimental endpoint, µMT mice had similar disease scores to C57BL/6 mice. In fact, at disease endpoint, we found no significant difference in the number of T helper cells secreting GM-CSF or IL-17 in the spinal cords of vehicle-treated µMT and C57BL/6 mice (Fig. 8b). IFN-β-treated µMT mice had significantly attenuated disease severity throughout the entire course of disease in comparison with C57BL/6 mice (Fig. 8c). In agreement with the disease course of IFN-β-treated mice, there was a significant reduction in the number of T helper cells expressing GM-CSF or IL-17 in the spinal cords of µMT mice (Fig. 8d). Together, these findings show

![Image](https://example.com/image.png)

**Fig. 5** Type I IFN drives IL-6 production in human memory B cells. a Correlation between composite IFN scores and serum CXCL11 levels with IL-6 in B cell-sufficient NMOSD patients (n = 18), B-cell-deficient NMOSD patients (n = 20) and healthy volunteers (n = 18). R and p values were determined using two-tailed Pearson correlation coefficient tests. Direct effect of IFN-β on human naive and memory B cells was assessed by stimulating purified human naive (CD27−) and memory B cells (CD27+) from healthy donors (n = 4) with CD40L, anti-Ig ± IFN-β. Representative flow cytometric plots and frequency of live b naive CD19+ B cells and c memory B cells expressing CD80, CD86, and IL-6 are shown. Statistical significance was determined using two-tailed Student t tests. P values <0.05 were considered significant.
that B cell-deficiency reduces TH17-EAE disease severity, regardless of IFN-I treatment. Furthermore, our data also show that protection of disease by B cell-deficiency is prolonged in IFN-I-treated mice compared with vehicle-treated mice.

**IFN-β stimulates B cells to drive pathogenic TH17 cells.** Our data demonstrate a link between IFN-I, B cells, and IL-6 to elevated inflammatory TH17 responses in neuro-inflammation. These observations led us to hypothesize that B cells are the inflammatory mediator between IFN-I and TH17 during inflammation. To test this hypothesis, we designed the following culture experiment. We first stimulated B cells isolated from healthy or EAE mice in the presence or absence of IFN-β, washed the B cells of IFN-β, then co-cultured these B cells with CD4+ T cells from 2D2 mice (Fig. 9c) in the presence of the myelin peptide antigen, MOG35–55 (Supplementary Fig. 4a).

Prior to treatment with IFN-β, we observed that B cells isolated from EAE mice had a more mature phenotype (IgMhiIgDhi and IgMloIgDhi) compared with B cells from healthy mice (Supplementary Fig. 4b). We also evaluated the expression of IFN-α receptor (IFNAR) and found no difference in IFNAR expression in B cells from healthy and EAE mice (Supplementary Fig. 4c). We found that IFN-β directly enhanced the expression of CD80, CD86, and MHCIi on B cells isolated from both healthy and EAE mice (Fig. 9a, Supplementary Fig. 4d), suggesting that IFN-β enhances the antigen-presenting function of B cells. IFN-β stimulation of B cells isolated from healthy mice had marginal effects on IL-6, IL-12/IL-23p40, and IL-10 secretion (Fig. 9c).

Strikingly, IFN-β stimulation of B cells from EAE mice led to an abundance in secretion of IL-6 and IL-12/IL-23p40 but not IL-10 (Fig. 9c). These data suggest that IFN-β has a direct effect on a population of mature B cell that results in its skewing towards a more inflammatory phenotype.

We next assessed for 2D2 T-helper cell proliferation and cytokone production from the co-culture assay. We observed that there was no significant effect on T-cell proliferation following co-culture with IFN-β-stimulated B cells from healthy mice (Fig. 9d, Supplementary Fig. 4e). In contrast, IFN-β stimulation of EAE-derived B cells significantly increased T-cell proliferation (Fig. 9e, Supplementary Fig. 4e). In addition, IFN-β-stimulated B cells from healthy mice did not impact the secretion of IL-17, GM-CSF and IL-10 by T helper cells (Fig. 9f). However, we found enhanced secretion of GM-CSF and IL-17, but not IL-10, from T helper cells co-cultured with IFN-β-stimulated B cells isolated from EAE mice (Fig. 9f). These cell culture assays demonstrate that IFN-β acts directly on antigen-experienced B cells to elevate their expression of CD80, CD86, MHCIi, IL-6, and IL-12/IL-23p40, which in turn drive the proliferation of inflammatory T helper cells that secrete elevated levels of IL-17 and GM-CSF.

**Discussion**

The complex interplay between IFN-I and TH17 cells plays a significant role in the pathology of certain autoimmune diseases, notably, MS, NMO, psoriasis, systemic lupus erythematosus and ulcerative colitis. The ability of IFN-I to drive or inhibit inflammation relies on the disease context. IFN-β remains a
widely prescribed treatment for MS. As a therapy, IFN-I reduces relapse rates and lesion formation in MS patients and a predominant theory behind its efficacy is through the inhibition of TH17 differentiation and function\(^\text{37}\). Paradoxically, strong evidence from NMOSD and TH17-EAE indicate that IFN-I and TH17 cells cooperate to drive disease progression\(^\text{10,33-35}\). Our study now defines a mechanism by which IFN-I cooperates with TH17 to drive severe disease in NMOSD.

We now show that IFN-I signatures stratify NMOSD patients into two subsets: IFN-high & IFN-low. Our data indicate that IFN-high NMOSD patients have elevations in IL-6 and cytokines related to the TH17 pathway. Most strikingly, IFN-I signatures and serum IL-6 stratify patients based on disability highlighting their potential utility in clinical tests for the prognosis of NMOSD. In addition, we found that patients treated with rituximab had reduced IL-6 and IL-17 levels in IFN-high NMOSD patients. Currently, the precise mechanisms through which rituximab mediates its therapeutic effects is unclear, but these data suggest that the therapeutic mechanism is through the reduction of IL-6. This observation is congruent with previous reports showing that reducing IL-6 expressing B cells is critical for the therapeutic effects of rituximab in mice with EAE\(^\text{36}\). Therefore, we speculate that IL-6 levels could be used to monitor treatment response to B cell-depleting therapies in NMOSD. Our data also indicate that IFN-I stimulation is responsible for the elevated IL-6 production from memory B cells. In NMOSD, we found a significant correlation between IFN-I signature expression and IL-6 levels, and this correlation is absent in patients treated with rituximab. B cell cultures also determined that the human memory B cell population produces high levels of IL-6 after IFN-I stimulation. In summary, these data suggest an inflammatory cascade that is initiated by IFN-I to induce IL-6 from memory B cells, which then affects other inflammatory pathways, such as, the generation of inflammatory TH17 cells and autoantibody production.

Classification of MOG seropositive patients in the NMO spectrum is currently being re-evaluated\(^\text{37}\). In our cohort, we find that both AQP4-IgG\(^+\) and MOG-IgG\(^+\) patients have elevated IFN-I signatures compared to healthy controls. This suggests that IFN-I pathway drives disease in both MOG-IgG\(^+\) and AQP4-IgG\(^+\) patients and perhaps therapeutic strategies that block IFN-I would be effective in both patient subtypes. Recent studies have identified that MOG-IgG\(^+\) NMOSD patients do not respond equally well to B cell depletion compared to AQP4-IgG\(^+\) patients\(^\text{23}\). In addition, another study suggested that IL-6R inhibition might be more effective in AQP4-IgG\(^+\) patients than in AQP4-IgG\(^-\) patients\(^\text{38}\). Our data revealed that relapse rates of B cell depleted MOG-IgG\(^+\) patients were significantly higher than relapse rates of B cell depleted AQP4-IgG\(^+\) patients. We did not observe differences in the transcriptomes or serum proteins in B cell depleted AQP4-IgG\(^+\) or MOG-IgG\(^+\) patients. Interestingly, in B cell-sufficient patients, we found elevated serum levels of IL-6 and the IFN-I chemokine, MCP-3 in AQP4-IgG\(^-\) but not MOG-IgG\(^+\) patients. Although longitudinal studies are needed, we
speculate that IFN-I and IL-6 pathways are involved in the responsiveness to B cell depletion and IL-6R inhibition in AQP4-IgG+ NMOSD patients.

The TH17-EAE model in C57BL/6 mice mimics several aspects of NMOSD9,13, which demonstrates the usefulness of this animal model for mechanistic studies of NMOSD. Here, we found that the results from our TH17-EAE experiments were congruent with the observations made with the NMOSD patient specimens. We found that creating an IFN-I-high TH17-EAE model, with IFN-β injections, resulted in increased serum IL-6, elevated TH17 responses and exacerbated paralysis in mice. Clinical trials demonstrate that IL-6R inhibition and B cell depletion are promising therapies for NMOSD39. To address how IL-6 blockade would affect IFN-β treatment of TH17-induced disease, TH17-EAE mice were treated in vivo with or without IFN-β as well as with anti-IL-6R or an isotype control. Surprisingly, IL-6 blockade in TH17-EAE without IFN-β treatment did not ameliorate disease. A possible explanation for this observation is that IL-6 is required for the generation of inflammatory TH17 cells and blockade is no longer effective in the adoptive transfer model of EAE where TH17 are already activated. In striking contrast, IL-6 blockade significantly attenuated TH17-EAE treated with IFN-β, demonstrating that IL-6 is a critical inflammatory mediator induced by IFN-I which exacerbates disease. Contrary to IL-6R inhibition of TH17-EAE, we found that B cell-deficiency attenuates disease in TH17-EAE regardless of IFN-β treatment. These data suggest that B cells play a key role in initiating disease in TH17-EAE mice, which is not mediated through a IFN-I/IL-6 cascade but likely through antigen presentation40,41. The differences in efficacy of IL-6 inhibition and B cell-deficiency in IFN-β treated TH17-EAE may provide insights into how IFN-high and IFN-low NMOSD patients will respond to these therapies. In this study, we use the adoptive transfer of MOG-specific TH17 cells to induce disease that reflects many aspects of NMOSD, especially in regards to IFN-I. The development of a mouse model with a neuro-autoimmune disease that targets AQP4 has been a challenge. Recent developments have shown that T cells from AQP4-deficient mice recognize distinct AQP4 epitopes and these AQP4-specific T cells require TH17 programming to induce severe optic-spinal inflammation42. These data confirm the importance of the TH17 pathway in driving NMOSD-like disease in mice. However, how IFN-I affects the AQP4-specific animal model remains to be tested.

One predominant theory behind the efficacy of IFN-β is that this therapy reduces disease by inhibiting TH17 differentiation and function16–18. However, MS and NMOSD patients with high TH17 signatures and mice with TH17-induced EAE have exacerbated disease when treated with IFN-β4,5,8,10,11,14,43. Our cell culture experiments provide key insights into how IFN-β paradoxically increases TH17 pathology (depicted in Fig. 10). IFN-β indirectly

**Fig. 8** B cell-deficiency attenuates IFN-β-treated TH17-EAE. TH17-EAE was induced in either C57BL/6 or muMT mice and treated with IFN-β or vehicle. a Clinical scores of vehicle-treated C57BL/6 (n = 10) and muMT (n = 10) mice with TH17-EAE. Data were pooled from two independent experiments. Mann–Whitney tests were performed to determine statistical significance (P < 0.05). b Number of CD4+ T cells that express GM-CSF and IL-17 in spinal cords of vehicle-treated mice (day 29). c Clinical scores of IFN-β-treated C57BL/6 (n = 10) and muMT (n = 11) mice with TH17-EAE. Data were pooled from two independent experiments. Mann–Whitney tests were performed to determine statistical significance (P < 0.05). d Number of CD4+ T cells that express GM-CSF and IL-17 in spinal cords of IFN-β-treated mice (day 29). Statistical analysis was performed using two-tailed Mann–Whitney tests. Error bars indicate the S.E.M. Results are compiled from two independent experiments. Source data are provided as a Source Data file.
IFN-I alters both the transcriptional and cytokine profiles towards an inflammatory phenotype during TH17-mediated disease. IFN-I-driven TH17 pathogenicity occurs in an indirect manner and is partly attributed by Wingerchuk et al. All findings broaden our understanding of what biological pathways drive severe disease in NMOSD and provide potential markers for the clinical management of these patients. Further studies from larger cohorts are underway to confirm the clinical relevance of IFN-I and TH17 biomarkers in this devastating neuro-inflammatory disease.

**Methods**

**Clinical classification of NMOSD.** We obtained serum and PAXGene tubes from 42 patients with NMOSD from the Charité-Universitätsmedizin Berlin (Supplementary Table 1). Serum proteins were measured in all 42 patients. Thirty-eight of the 42 NMOSD RNA samples passed quality control and were analyzed by RNAseq. Of the 42 patients, EDSS was available for 40 patients and relapse rates were available for 41 patients. We obtained PBMCs from seven NMOSD patients, six of which had EDSS scores, from the University of Michigan (Supplementary Table 2). NMOSD diagnosis was fulfilled using clinical criteria defined by Wingerchuk et al. All NMOSD patients were tested for AQP4-IgG or MOG-IgG using cell-based assays (CBA). No patient was on steroid therapy during blood draw. Serum and PAXgene tubes were obtained from 18 healthy volunteers, 18 passed quality control and were used in transcriptomic and proteomic analysis. PBMCs were obtained from 13 healthy volunteers and used for FACS analysis. Written informed consent was obtained from individuals prior to participation in the study, which was approved by the Charité Universitätsmedizin Berlin, University of Michigan and the Oklahoma Medical Research Foundation’s Institutional Review Boards. Assays on the samples were performed blinded from the clinical data.
Peripheral blood RNA transcript isolation. Whole blood was obtained by venipuncture into PAXGene tubes (BD company) and RNA was extracted with on-column DNAse digestion (Qiagen). Excess globin transcripts were removed using GLOBINclear (Ambion). RNA concentrations were determined using a NanoDrop spectrophotometer and RNA quality was assessed using the RNA 6000 Nano kit on the Bioanalyzer 2100 (Agilent) with quality threshold RIN scores > 8.

RNASequencing and quality control measures. Starting from the raw FASTQ files (2 x 100bp), the quality of raw sequence reads was assessed using FASTQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were then trimmed using Trimmomatic v0.35 to remove low-quality reads. The quality of the reads was then re-assessed using FastQC to confirm quality improvements. All downstream analyses were based on the clean data with the highest quality. The raw FASTQ files are aligned to the human reference genome (GRCh38.p10) and the aligned files were sorted to the bam files using SAMTOOLS v1.9. 1.5 - 2.0 x 10^6 mapped reads were obtained per sample. The sequencing performance was assessed for total number of mapped reads, total number of uniquely mapped reads, strandedness, genes, and transcripts detected, ribosomal fraction known junction saturation, and reads distribution over known gene models with RSeQC v3.0.0. Sample quality control was assessed using ArrayQualityMetrics v3.14.0. In R. Out of a total of 61 samples, five samples were considered poor-quality and removed from subsequent analyses based on: (a) deviation of read counts, assigned to features from mean ± 2 SD of all samples, (b) having strandedness issue detected by RSeQC, and (c) being detected as outliers by ArrayQualityMetrics. Therefore, RNASeq data from 38 patients with NMOSD and 18 healthy controls were used for subsequent analyses. Transcript counts were derived from the uniquely aligned unambiguous, strand-specific (reverse-stranded reads by Subread) feature v1.6.3, yielding 58,052 transcripts per sample. To assess cell type-based RS expression, we used a Genome-wide RNA database (www.proteinatlas.org). IFN scores were calculated as a log2 average read count of the 25 IFN genes identified as elevated in NMOSD (Fig. 1d).

Serum protein profiling of NMOSD and RRMS patients. Protein arrays were performed on sera drawn during stable disease from 42 NMOSD patients and 18 healthy volunteers. Concentrations of 91 proteins were assessed by proximity extension assay (Olink Bioscience, Sweden) using the inflammation panel. The assay uses oligonucleotide-labeled antibody pairs allowing for pair-wise binding to target proteins. When antibody pairs bind target antigens, corresponding oligonucleotides form an ampiclon allowing for quantification of protein expression by high-throughput real-time PCR. Data are presented as normalized protein expression values, Olink Proteomics’ arbitrary unit on a log2 scale.

Flow cytometric analysis of T helper cells in NMOSD patients. Separation of CD4^+ T cells from healthy or EAE mice (10 days after immunization with M. bovis, CFA and PTX) were harvested (1000 U/ml; PBL) or PBS on days 0, 2, 4, 6, 8, and 10. Recipient mice also received Ptx on days 0 and day 2 post transfer. Mice were monitored daily for clinical scores. Paralysis was assessed using the following standard clinical score: (0) healthy, (1) loss of tail tone, (2) partial hind limb paralysis, (3) complete hind limb paralysis, (4) forelimb paralysis, and (5) moribund/dead. Transfer EAE mice were killed on day 15 and spinal cords were fixed and sectioned for histological analysis using H&E and Luxol fast blue staining. Serum was collected on day 2 post transfer. Treatments were carried out in a blinded experiment. At disease endpoint, CNS infiltration by immune cells was assessed by perfusing EAE mice with PBS and collecting their brains and spinal cords. CNS tissue was homogenized through mechanical disruption and homogenates were incubated with DNAse (5 µl/ml Sigma) and collagenase (4 mg/ml Roche) at 37 °C for 1 hour. Cells were isolated using a Percoll gradient and analyzed by FACS.

Flow cytometry of mouse cells. All cells were stained with Fixable Viability dye (eBioscience, Biolegend) and treated with Fc block (eBioscience) prior to staining with fluorochrome-conjugated anti-mouse mAbs. mAbs were from BioLegend (F4/80, Ly6G, Ly6C, IgD), CD19, CD38, CD80, IFNAR, IgG1) and eBioscience (CD11b, MHCII, IgM).

Intranuclear cytokine staining, cells were stained with PMA (Sigma-Aldrich), ionomycin (Sigma-Aldrich) and monensin (BD Biosciences) for 4 hours. Cells were then stained with anti-mouse CD4 (eBioscience), fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) and stained for IL-17 (BioLegend) and GM-CSF (Biolegend). All flow cytometric data were collected on LSR II (BD Biosciences) and analyzed using FlowJo software (Tree Star Inc.).

For intranuclear staining of Ki-67, cells were stained with anti-mouse CD4 (BD Bioscience), fixed and permeabilized using the Foxp3 Transcription Factor Staining Buffer Set and stained for Ki-67 (Biolegend) to assess for cellular proliferation.

Mouse B cell and T-cell co-culture assays. Splenocytes from either healthy or EAE mice (10 days after immunization with M. bovis, CFA and PTX) were harvested and processed. Purified B cells from splenocytes were obtained using negative sorting with magnetic beads (Milteny). Isolated B cells (2.5 x 10^6 cells/ml) were stimulated with anti-CD40 (1 µg/ml, eBioscience) with or without IFN-β (100 U/ml) for 3 days. B cells from healthy or EAE mice stimulated with or without IFN-β were then washed with PBS (1x) and co-cultured with magnetically sorted TH cells (mAbs) from 2D2 mice. B and TH cells were co-cultured (2.5 x 10^6 cells/ml) at 1:1 ratio with M. bovis SS antigen.

Statistical analysis. For RNA Sequencing, genes with less than one count per million for at least in 1/3rd of samples were considered as non-expressed and not used for differential expression analyses. This resulted in 40,796 transcripts being removed out of a total of 58,052 transcripts, leaving 17,256 for further analysis. Differential expression analyses were performed using DESeq2 v1.24.2, fitting a negative binomial generalized linear model to find significantly DEGs. Genes with a false discovery rate of 0.05 and fold change ≥0.58 or ≤0.57 were considered differentially expressed. All analyses were performed in the R Bioconductor suite.

Data were measurements from distinct biological replicate samples and are presented as means ± s.e.m. at a ≤ p < 0.05 significance level. Data were determined using two-tailed Student’s t tests or Mann–Whitney tests. In the case of three or more data sets, means were compared using two-way analysis of variance with Bonferroni correction or Kruskal–Wallis with a Dunn’s multiple comparison test. Differences were considered significant for P < 0.05. Statistical analyses were performed using Prism 6 (GraphPad). All statistical tests were two-tailed. Cluster analysis of NMOSD patients was performed using hierarchical clustering with Gene Cluster software, where the log2 cytokine values were centered to the mean, then ordered by complete linkage clustering. The clusters were presented as a heat map using TreeView.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability The consent form signed by the participants in this study does not permit public release of potentially identifiable data, which includes the deposit of raw RNAsequencing data. We have provided the read counts in source data file and the raw RNA-sequence data are available from the authors. All other data are provided in the source data file. Source data are provided with this paper.

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Author contributions
A.A., F.P., and R.C.A. conceptualized, designed experiments, interpreted results, and wrote the manuscript. A.A., S.G., G.K., J.I.Q., R.M.K., and R.C.A. executed and analyzed the animal experiments. N.B., K.R., F.P., Y.M.-D. established the patient cohorts. A.A., N.B., S.G., F.P., and R.C.A. analyzed the patient serum data with clinical data. Q.W. analyzed the T-cell subsets in PBMCs from patients. C.J.L. and B.K. performed Q.C. and analysis of the RNAseq data.

Competing interests
R.C.A. has consulted for Roche, Biogen, and EMD serono. Y.M.-D. has consulted for and/or received grant support from: Acorda, Bayer Pharmaceutical, Biogen Idec, EMD Serono, Genzyme, Novartis, Questor, Genentech, and Teva Neuroscience. F.P. has consulted for and/or received speaker honoraria from Bayer, Teva, Genzyme, Merck, Novartis, and MedImmune. All other authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to R.C.A.

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