Transitional B cells are a signature of interferon-β treatment in MS

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Objective

- To define serum levels of B-cell activation factor (BAFF) and specific B-cell phenotypes in remitting multiple sclerosis (RRMS) patients treated with either glatiramer acetate (GA) or interferon-β (IFNβ).
- To establish the link between BAFF, IFNβ, and B-cells in humans.
- To determine whether B cells are necessary for effective treatment with IFNβ in experimental autoimmune encephalomyelitis (EAE).

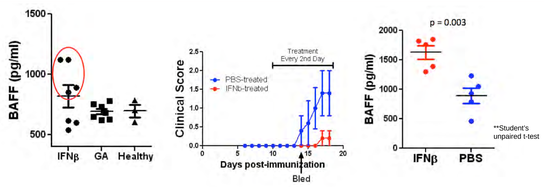
Background

- There are currently no serologic tests that aid in the selection or monitoring of therapy in RRMS. Modulation of B cells by IFNβ has been proposed, yet knowledge of the specific subtypes affected remains lacking. Monitoring of these subtypes may provide a biomarker for treatment effect.
- IFNβ increases both BAFF and the number of circulating B cells in RRMS, but exactly which subtypes and the mechanism whereby IFNβ affects B cells remains unknown.

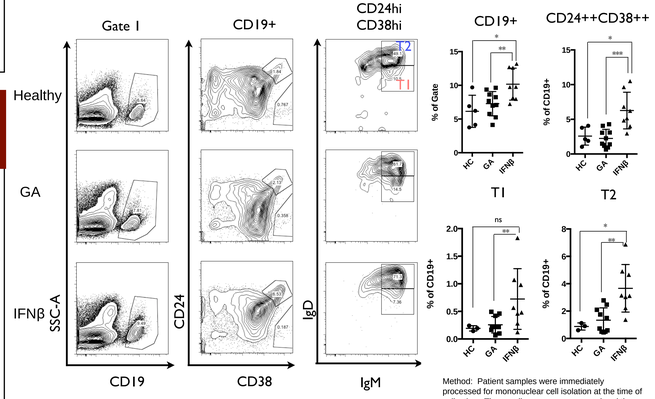
Patients

	Healthy	Glatiramer Acetate	Interferon-β
Number of patients	5	13	8
Average Age (Range)	28.7 (24-36)	37.2 (26-46)	37.2 (24-44)
Sex	100% Male	77% Female	83% Female
Diagnosis	-	RRMS	RRMS
Years since diagnosis (Range)	-	4.2 (1-8)	5.8 (1-8)
EDSS (Range)	-	1.5 (0-4)	1.3 (0-2.5)

B-cell activation factor (BAFF) is elevated in IFNβ treated MS and EAE

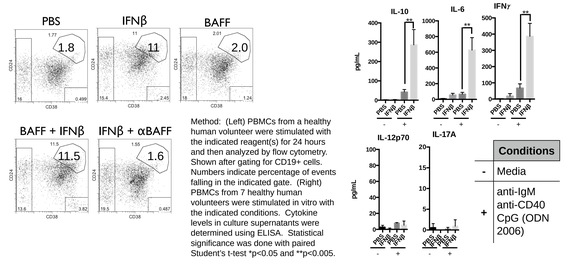


Transitional B cell expansion is a signature of IFNβ therapy in MS



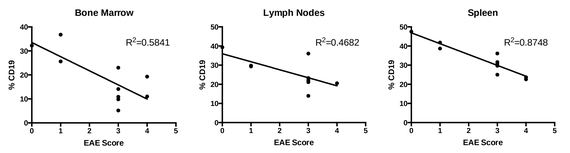
Method: Patient samples were immediately processed for mononuclear cell isolation at the time of collection. These cells were cryopreserved and then thawed for flow cytometry. Statistical significance was determined using Student's unpaired t-test with *p<0.05 and **p<0.005.

IFNβ's effect on B cells requires BAFF and stimulates IL-10, IL-6, and IFNγ production

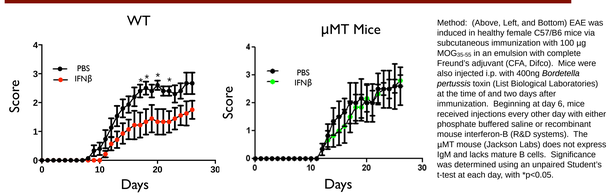


Method: (Left) PBMCs from a healthy human volunteer were stimulated with the indicated reagent(s) for 24 hours and then analyzed by flow cytometry. Shown after gating for CD19+ cells. Numbers indicate percentage of events falling in the indicated gate. (Right) PBMCs from 7 healthy human volunteers were stimulated in vitro with the indicated conditions. Cytokine levels in culture supernatants were determined using ELISA. Statistical significance was done with paired Student's t-test *p<0.05 and **p<0.005.

EAE severity inversely correlates the number of B cells

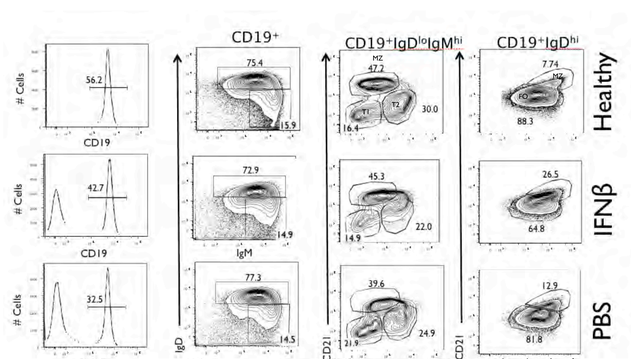


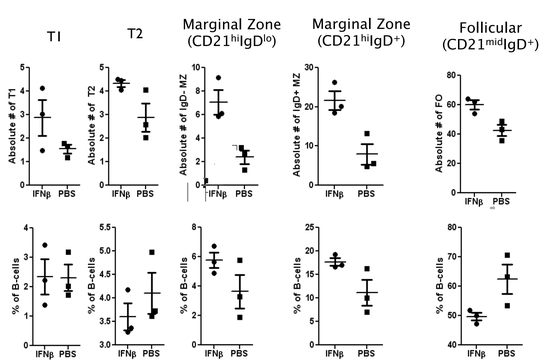
µMT mice are B cell deficient and fail IFNβ therapy in EAE



Method: (Above, Left, and Bottom) EAE was induced in healthy female C57/B6 mice via subcutaneous immunization with 100 µg MOG_{35-55} in an emulsion with complete Freund's adjuvant (CFA, Difco). Mice were also injected i.p. with 400ng *Bordetella pertussis* toxin (List Biological Laboratories) at the time of and two days after immunization. Beginning at day 6, mice received injections every other day with either phosphate buffered saline or recombinant mouse interferon-B (R&D systems). The µMT mouse (Jackson Labs) does not express IgM and lacks mature B cells. Significance was determined using an unpaired Student's t-test at each day, with *p<0.05.

Treatment of EAE with IFNβ expands splenic IgD^{lo} marginal zone B cells





Conclusions

- Transitional B cells are a signature of RRMS patients on IFNβ therapy but not GA. These cells are a more reliable biomarker of treatment than serum BAFF, raising the possibility these cells could useful in treatment monitoring.
- IFNβ treatment induces proliferation of human transitional B cells through a BAFF dependent mechanism.
- IFNβ synergizes with B cell activation to produce IL-10, IL-6, and IFNγ. IL-10 has well-documented anti-inflammatory effects, and may be a critical link between B cells and the successful treatment of RRMS with IFNβ. These cytokines, along with BAFF, are a signature of the splenic marginal zone.
- In EAE, IFNβ preferentially expands marginal zone B cells in the spleen.
- Treatment of EAE with IFNβ requires the presence of B cells. Taken together, these data provide direct evidence that the regulatory effect of IFNβ may be through marginal zone B cell stimulation.

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