THE EFFECT OF BELIMUMAB ON B CELL SELECTION IN HUMAN SLE

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Background Belimumab has a therapeutic benefit in active SLE, especially in patients with high titers of anti-dsDNA antibodies. The current study was designed to address whether the profound loss of naïve B cells in belimumab treated patients is accompanied by a shift in the immunoglobulin repertoire of either mature B cells or plasma cells.

Methods 15 SLE patients who had been continuously treated with belimumab 10 mg/kg monthly for >5 years were matched with 17 SLE controls. 5 SLE patients newly starting on belimumab were studied before and 6 months after drug initiation. B cell phenotyping was performed using flow cytometry. Mature B cells and plasmablasts were sort purified, VH libraries were generated using barcoded primers (iRepertoire) and pooled libraries were sequenced using mSeq. Analyses of unmutated and mutated IgM sequences from mature B cells and all sequences from plasmablasts were performed using customized Perl and R scripts.

Results Phenotyping – novel findings:
1. BAFF regulates the transitional B cell checkpoint with conservation of transitional type 1 cells and >90% loss of transitional type 3 and naïve B cells after chronic belimumab treatment.
2. Neither ‘naïve activated’ B cells nor CD21hi B cells subset are preferentially depleted by belimumab.
3. The early increase in CD27+ class switched cells after belimumab treatment is due to an increase in memory B cells rather than B1 cells.
4. After >5 years of treatment, class switched memory B cells, B1 B cells and plasmablasts are also substantially depleted.

Next Generation Sequencing of VH genes:
1. There was no redistribution of V, D or J family usage among unmutated IgM sequences.
2. There was no effect of belimumab on the frequency of the autoreactive VH4–34 gene or on CDR3 length or composition in unmutated IgM sequences.
3. There was a significantly greater loss of VH4–34 among mutated IgM sequences and plasmablast sequences compared with unmutated sequences in subjects treated with chronic belimumab than in lupus controls.

Conclusions Although BAFF highly regulates survival of naïve B cells past the T1 stage in humans, we were unable to identify an effect of belimumab on VH distribution or CDR3 composition of naïve B cells, suggesting a minimal effect on selection of the naïve B cell repertoire. By contrast belimumab may promote negative selection of autoreactive activated naïve B cells and plasmablasts.