Abstract

Characterization of Human Age-Associated B Cells (ABCs) in Normal and Lupus Peripheral Blood

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Background

Age-associated B cells (ABCs) are associated with autoantibody production in lupus-like mice. This population is expanded upon exposure to self-antigen and interferon-γ. ABCs in mice are identified as CD11c+, CD21− and T-bet+. However, the characterization of these cells and factors promoting their generation in human SLE is unclear. The purpose of this study was to define ABCs in human SLE patients and their association with interferon (IFN) status.

Methods

Peripheral blood leukocytes were purified by density gradient centrifugation from 26 lupus patients with a range of disease activity (n=10 with flaring disease, n=16 controlled disease) and 6 normal healthy donors. Surface and T-bet intracellular antibody staining was analyzed in a blinded fashion by flow cytometry. A putative population of ABCs was defined as CD3− CD19+ CD11c+ CD21− cells. Samples were divided into groups regardless of disease status based upon percentage of B cells with putative ABC phenotype. B cell subset analysis was completed on the groups expressing minimal (≤2%), intermediate (2%–7%), or high (≥8%) levels of ABCs. In addition, the B cell subset distribution (switched memory, unswitched memory, total naïve, and CD27- IgD- double negative) and phenotypic markers (CXCR3, T-bet, CD24, IgD, and CD27) of putative ABCs were assessed. IFN-α, IFN-β, and IFN-λ levels were quantitated by ELISA from sera drawn at the same time.

Results

Five samples were identified as having high putative ABC levels (range 8.4%–20.5% of B cells, mean 14.9%). These CD11c+CD21− CD19+CD3− cells were predominantly CD24− CD27− IgD−. Across all 32 samples, the percentage of putative ABCs positively correlated with percentage of T-bet+ B cells (r=0.819, p=5.4×10−9), IgD− CD27− (double negative) B cells (r=0.59, p=0.0003), and CD24− double negative cells (r=0.74, p=8.72×10−7) with a high degree of statistical significance. Paired serum samples had a range of type I and type III interferon levels (mean ±SEM for all samples): 6.2±2.1 pg/m (IFN-α), 4.0±0.6 pg/ml (IFN-β), and 44.1±9.6 pg/ml (IFN-λ). Analysis including correlation between B cell subsets, interferon status, clinical features and disease activity is in progress.

Conclusions

An expanded putative ABC population (CD11c+ CD21− CD19+ CD3−) was identified in a subset of human peripheral blood samples. This subset is positively correlated with IgD−CD27−CD24− B cells (DN2 cells), whose expansion has been described previously in lupus patients.