Genetic predisposition to Waldenström's macroglobulinaemia is associated with B-cell activation outside the germinal centre.

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Background: The importance of genetic factors in the development of Waldenström's macroglobulinaemia (WM) is uncertain and no risk-associated genes have been identified. Familial predisposition is well documented and the study of such families can provide insights into relevant biological pathways. We have previously described an Icelandic family with 4 cases of WM and/or Non-Hodgkin's lymphoma and one case of IgM-MGUS. *In vitro* testing of peripheral blood B cells revealed a functional phenotype of increased production of immunoglobulins in response to stimulation with poke-weed mitogen (PWM) in fourteen healthy family members, referred to as hyper-responders (HRs).

Aim: To explore potentially aberrant B-cell activation pathways in hyper-responders (HR) by analysing acquired genomic changes, phenotypic characteristics and signalling pathways.

Methods: Acquired genomic changes in B-cells were detected by array-based comparative genomic hybridization (CGH) using DNA isolated from neutrophils from the same blood sample as reference. Phenotypes were analysed by multi-parameter flow cytometry, immunoglobulins were determined by ELISA. The role of TLR in PWM stimulation was examined using specific inhibitors.

Results: The expected deletions at immunoglobulin loci on chromosomes #14 (heavy chain), #2 and #22 (light chains) were detected in all samples, but samples from HR showed significantly fewer random gains and losses than samples from unrelated controls or normal responder family members. Peripheral-blood B cells of HR were enriched for marginal zone B cells, defined as IgD+ IgM+CD27+, 9.35% of B cells *vs*. 4,38%, $p<0.05$ compared with normal donors. Furthermore, the subclass distribution of plasma IgG was different between HR and controls, with a significantly lower proportion of IgG1 (the classical T-dependent antibody against protein antigens), 58,2% *vs.* 82,9%, $p<0,001$, and a higher proportion of IgG2 (anti-polysaccharide antibodies), 45,2 *vs.* 34,0%, $p=0,02$. Inhibition studies indicated that stimulation of antibody production by PWM is predominantly mediated by TLR2.

Conclusions: B cells with MZ phenotype are significantly increased among PB B cells from HR. The IgG subclass profile in PB from HR is skewed towards T-cell-independent activation. PB B cells from HR show low random genomic variability, indicating less exposure to gene-modifying AID activity in the germinal centre. Together, these results indicate a preference for B-cell activation outside the germinal centre in HR which is of interest in light of the discussion of the cell of origin of WM, proposed to be a marginal zone/B1 B cell. Stimulation of IG production by PWM appears to be mediated mostly through TLR2. It remains to be tested whether HR B-cells show enhanced TLR signalling upon PWM stimulation. Potential involvement of TLR signalling is relevant in view of the prevalent acquired *Myd88* mutation in WM.