Are MYD88 mutations by themselves sufficient to cause WM?

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Waldenström's Macroglobulinemia (WM) is an incurable lymphoplasmacytic lymphoma characterized by bone marrow infiltration of IgM-secreting lymphoplasmacytic cells. Activating mutations in MYD88, present in 93-97% of WM patients, trigger the NF-kB pro-survival signaling. To this day, there is no bona fide mouse model for WM. We therefore generated conditional transgenic mice overexpressing the human MYD88 mutated and wild-type (WT) protein to investigate the oncogenic role of $MYD88^{L265P}$ in WM. We generated compound transgenic mice by mating MYD88 transgenic mice with those expressing Cre recombinase under the control of Activation-Induced Cytidine Deaminase (*AID*) gene promoter to induce MYD88 expression in germinal center (GC) and post-GC B cells.

Transgenic mice were euthanized at different ages and lymphoid organs were rigorously examined. All $AID^{Cre/-}$;$MYD88^{WT/-}$ and $AID^{Cre/-}$ mice were phenotypically and histologically unremarkable. Compared to controls, $AID^{Cre/-}$;$MYD88^{L265P/-}$ mice had significantly increased serum IgM levels. Histological analysis of 19 $AID^{Cre/-}$;$MYD88^{L265P/-}$ mice sacrificed frequently demonstrated a nodular lymphoid infiltrate of small atypical cells variously associated with CD138+ plasma cells in both lymphoid and non-lymphoid tissues, with enhanced expression of MYD88 and NF-κB activation (p65). Serum protein electrophoresis showed diffuse polyclonal

increase in γ-globulins. Rouleaux formation was documented in the peripheral blood. Notably, 3 additional old $AID^{Cre/-}$;$MYD88^{L265P/-}$ mice developed a more aggressive monoclonal disease, resembling human large B cell lymphomas and showing a prominent lymphoplasmacytic infiltrate and enhanced MYD88 expression. Southern blot analysis revealed clonal bands of the IgH gene only in 2/3 mice harboring aggressive lymphomas. All three mice showed increased serum CXCL13 levels. Starting from 8 weeks of age, $AID^{Cre/-}$;$MYD88^{L265P/-}$ mice showed high frequency of submandibular dermatitis and/or alopecia, varying from mild to severe phenotype. Those sacrificed because of severe dermatitis had enlarged cervical lymph nodes and splenomegaly. Increased serum IL-6 levels (>49 pg/ml) were measured in 8 mice, 6 of which had intermediate or severe dermatitis. By IHC, the skin lesions revealed an infiltrate of macrophages/histiocytes and T cells.

These results indicate that MYD88L265P gives rise to a low-grade mature polyclonal B-cell lymphoproliferative disorder that shares many features of human WM that with time can transform into an aggressive lymphoma.