Helicobacter bilis monoassociation in gnotobiotic Swiss mice stimulates B cell hyperplasia and cross-reactive immune responses to Altered Schaedler Flora

MT Whary, C Faber, Y Feng, Z Ge, N Parry, S Muthupalani and JG Fox

We reported that *Helicobacter bilis* (Hb) infection of 42 germfree (GF) Swiss Webster mice for 3 to 12 months resulted in concurrent mild, multifocal typhlocolitis that correlated with peak Hb colonization levels at the cecal-colic junction. Compared to 39 germfree control mice, Hb infection resulted in robust IgG and IgA responses to Hb accompanied by dramatic hypertrophy of gut-associated lymphoid tissues (GALT). To characterize the immune response to Hb in the absence of other microbiota, cecal-colic tissue obtained from 3 GF and 3 Hb infected mice 9 months post infection were stained for CD3 (pan T cell), B220 (pan B cell), F4/80 (macrophage) and Ki67 (cell proliferation) using immunohistochemistry. Positively stained cells were counted for each marker in 5 fields of well-defined lymphoid follicles and 10 intestinal crypts. Sera from 3 GF controls, 6 Hb infected mice and 5 barrier-maintained SPF mice were screened by ELISA for IgG reactivity to Hb, 4 members of ASF and as a control, *H. trogontum*, a helicobacter isolated from rats. Compared to GF, Hb infected mice had more prominent B cell staining co-localized with Ki67, with a lower level of T cell staining, resulting in a higher B:T cell ratio in Hb infected mice. GF and Hb infected mice had minimal macrophages in lymphoid follicles and Ki67 staining was low in GF GALT. More than 50% of intestinal epithelial cells stained positive for Ki67 in GF mice but the percentage was higher in Hb infected mice. Hb seropositive mice had IgG cross-reactivity with *H. trogontum* and ASF antigens, consistent with literature demonstrating Hb may cause seroconversion to other microbiota and contribute to inflammation. This GF Swiss mouse model will allow for further investigation of the mechanism responsible for cross-reactive immune responses initiated by Hb and may be important to further understanding colitis models in research mice.