Effects of apoptotic cell accumulation caused by Mer deficiency on germinal center B cells and follicular helper T cells

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Abstract

Mer (MerTK), a member of the Tyro-3/Axl/Mer subfamily receptor tyrosine kinases, expression on phagocytes facilitates their clearance of apoptotic cells (ACs). Mer expression in germinal centers (GCs) occurs predominantly on tingible body macrophages (TBMφs). B and T cells do not express Mer. Mer deficiency (Mer-/-) results in the accumulation of ACs in GCs and augmented antibody-forming cell (AFC), GC and IgG2 Ab responses against T-dependent (TD) Ag. Here, we show that AC accumulation in GCs and elevated AFC, GC and IgG2 Ab responses in Mer-/- mice lasted for at least 80 days after immunization with NP-OVA. Enhanced responses and AC accumulation in Mer-/- GCs were associated with increased activation and proliferation of B cells and activated effector helper T cells, including follicular T (T_{FH}) cells. Secondary IgG-producing AFC, total IgG and IgG2 Ab responses were also increased in Mer-/- mice. Augmented B and T cell responses and long-term AC accumulation in Mer-/- GCs were linked to high titers of anti-nuclear antibodies (ANAs) in Mer-/- mice compared to wild type (WT) controls. Together, these results highlight the important role of AC clearance by Mer in regulating GC B cell, helper T cell and autoantibody responses and in maintaining peripheral B cell tolerance.

Methods and Results

Long-term accumulation of ACs in Mer-/- GCs

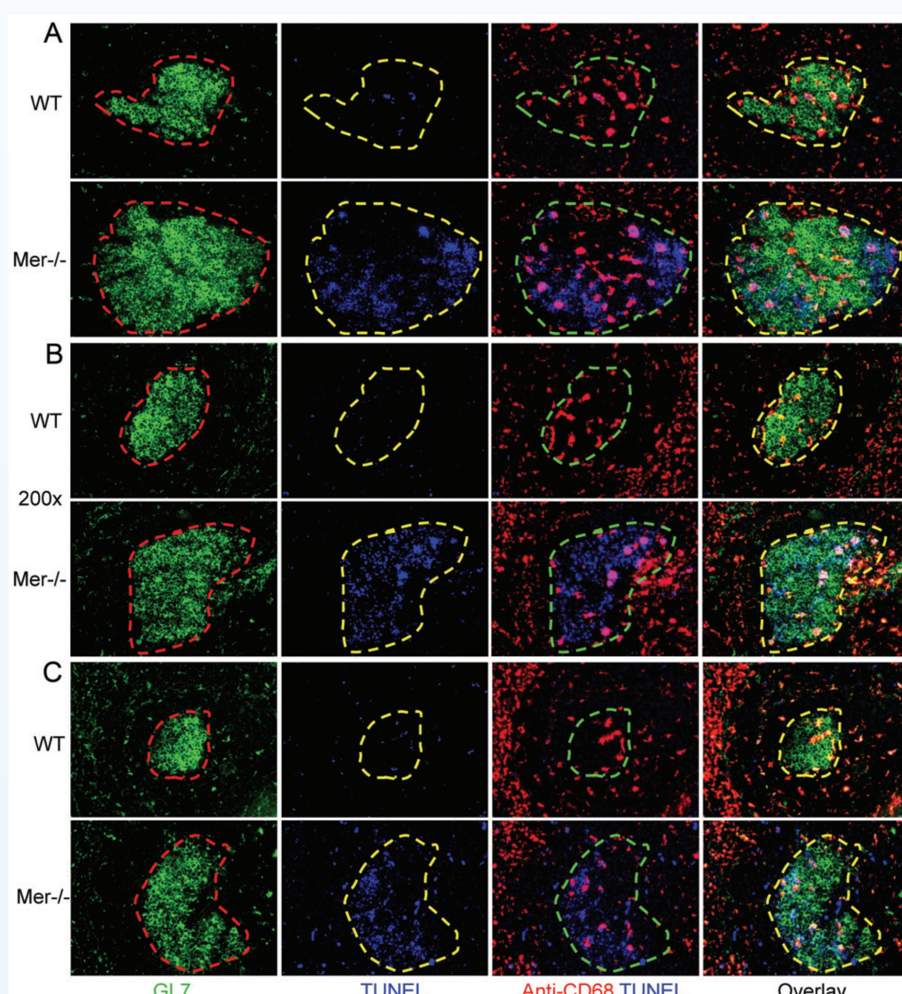

Figure 1. Three-color immunohistology of spleen tissue obtained on day 14 (A), 21 (B) and 80 (C) post-NP-OVA immunization of WT and Mer-/- mice. Spleen sections were stained with GL7, TUNEL and anti-CD68. GCs are defined by the presence of $GL7^+$ cells. Two- (TUNEL and anti-CD68) and three-color (GL7, TUNEL and anti-CD68) overlay images are shown in the 3rd and 4th columns, respectively. Original magnification of images was 200x. These data represent age- and sex-matched seven to eight mice of each genotype for each time point.

Primary GC response in the presence and absence of Mer

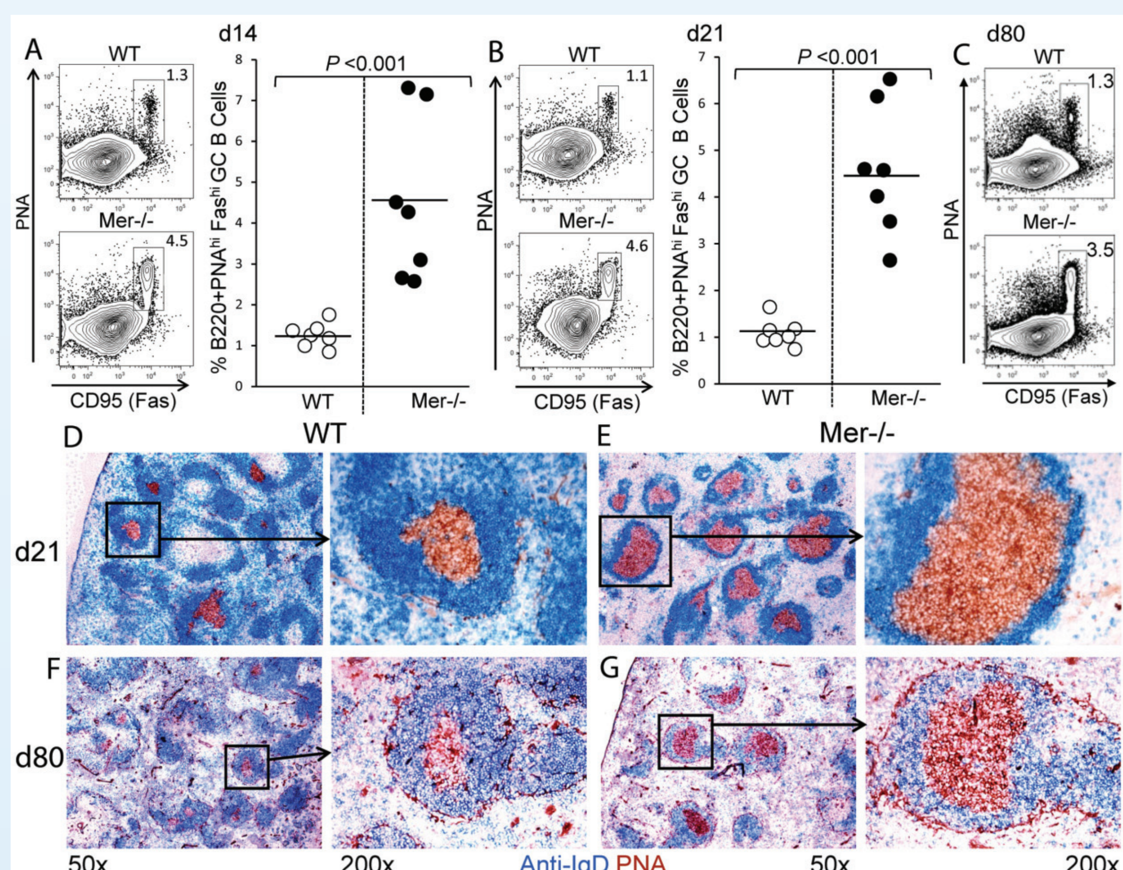

Figure 2. Flow cytometric analysis of splenocytes obtained from WT and Mer-/- mice on days 14 (A), 21 (B) and 80 (C) post-immunization with NP-OVA. Cells were stained with GC B cell markers (B220, PNA and anti-CD95/Fas). $B220^+PNA^{hi}Fas^{hi}$ GC B cells are shown in rectangular gates (A and B, left panels) and the percentage of $B220^+PNA^{hi}Fas^{hi}$ GC B cells in WT (open circle) and Mer-/- (closed circle) mice is shown in scatter plots (A and B, right panels). Analysis on day 80 was performed on pooled samples of seven WT control (top) and four Mer-/- (bottom) mice where $B220^+PNA^{hi}Fas^{hi}$ GC B cells are shown in rectangular gates (C). Spleen sections obtained from WT (D and F) and Mer-/- mice (E and G) on days 21 and 80 were stained with anti-IgD and PNA. Low (50x) and high magnification (200x) representative images are shown.

Enhanced long-lasting AFC response in Mer-/- mice

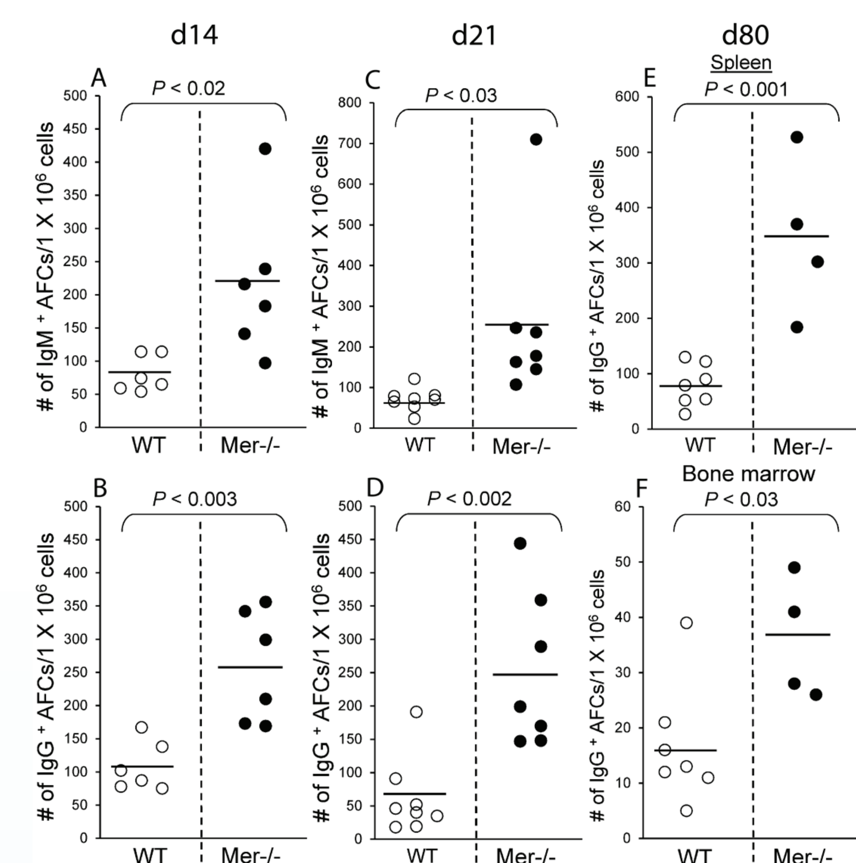

Figure 3. The number of short-lived splenic NP-specific IgM (A and C) and IgG (B and D) secreting AFCs were measured by ELISpot assay 14 and 21 days after immunization of WT (open circle) and Mer-/- (closed circle) mice with NP-OVA. The number of long-lived splenic (E) and bone marrow-derived (F) NP-specific IgG-producing AFCs were measured 80 days post-immunization. Each circle represents the number of AFCs per $1x10^6$ splenocytes obtained from an individual mouse. Horizontal bars represent the average values. These data were obtained from age- and sex-matched four to seven mice of each genotype. Statistical analysis was performed by Student's t-test.

Augmented IgG and Th1-IgG2 Ab responses in Mer-/- mice over time

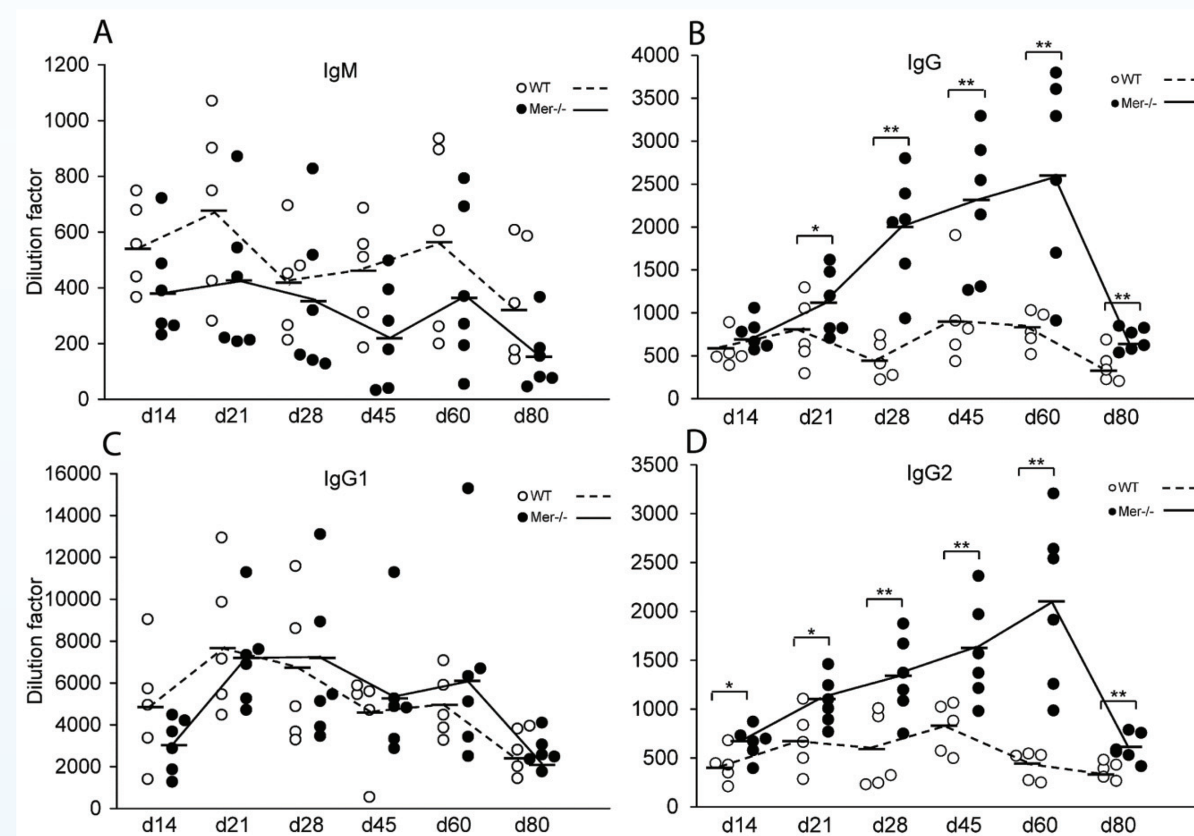

Figure 4. Anti-NP IgM (A), IgG (B), IgG1 (C) and IgG2 (D) Ab titers were measured by ELISA in WT (open circle) and Mer-/- (closed circle) serum samples obtained on multiple time points (days 14, 21, 28, 45, 60 and 80) post-immunization of these mice with NP-OVA. The dashed lines represent WT and solid lines represent Mer-/- mice. Each circle represents an individual mouse and horizontal bars represent the mean values. Statistical analysis was performed by the Student's t-test. P values of <0.05 and <0.01 are depicted as * and **, respectively. These data were obtained from age- and sex-matched five to six mice of each genotype at each time point.

Significantly increased percentages of activated and proliferating B cells in Mer-/- GCs

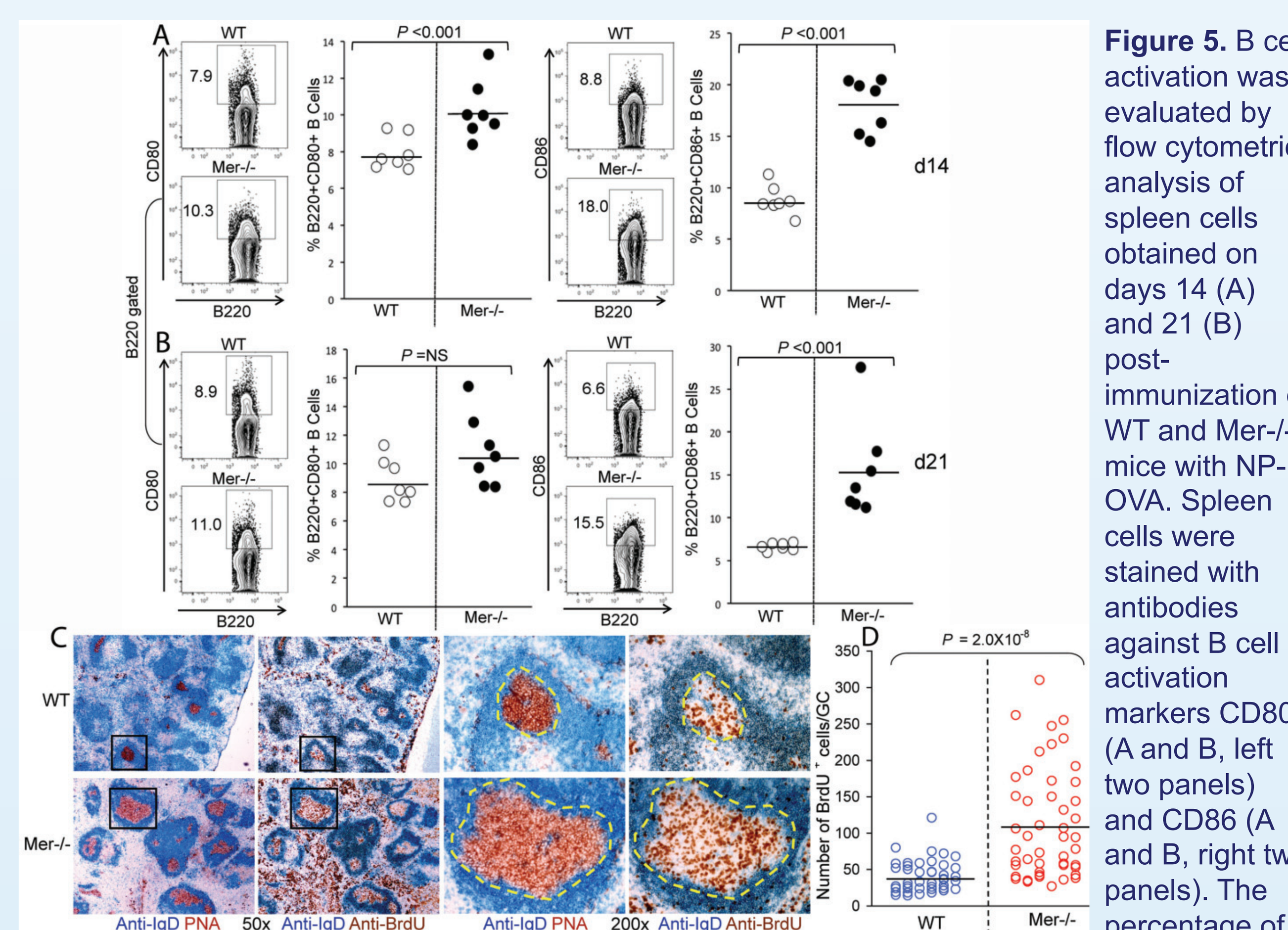

Figure 5. B cell activation was evaluated by flow cytometric analysis of spleen cells obtained on days 14 (A) and 21 (B) post-immunization of WT and Mer-/- mice with NP-OVA. Spleen cells were stained with antibodies against B cell activation markers CD80 (A and B, left two panels) and CD86 (A and B, right two panels). The percentage of $B220^+CD80^+$ (left panels) and $B220^+CD86^+$ (right panels) cells in WT and Mer-/- mice is shown in scatter plots. (C): Immunohistological analysis was performed on two consecutive spleen sections obtained from WT (top row) and Mer-/- (bottom row) mice on day 21 after NP-OVA immunization: one stained using anti-IgD and PNA and the other with anti-IgD and anti-BrdU. (D): Semi-quantitative analysis of the number of $BrdU^+$ proliferating B cells per GC was performed by counting $BrdU^+$ cells in 45-50 representative GCs from seven WT and eight Mer-/- mice.

Elevated $CD4^+$ helper T cell responses in Mer-/- mice

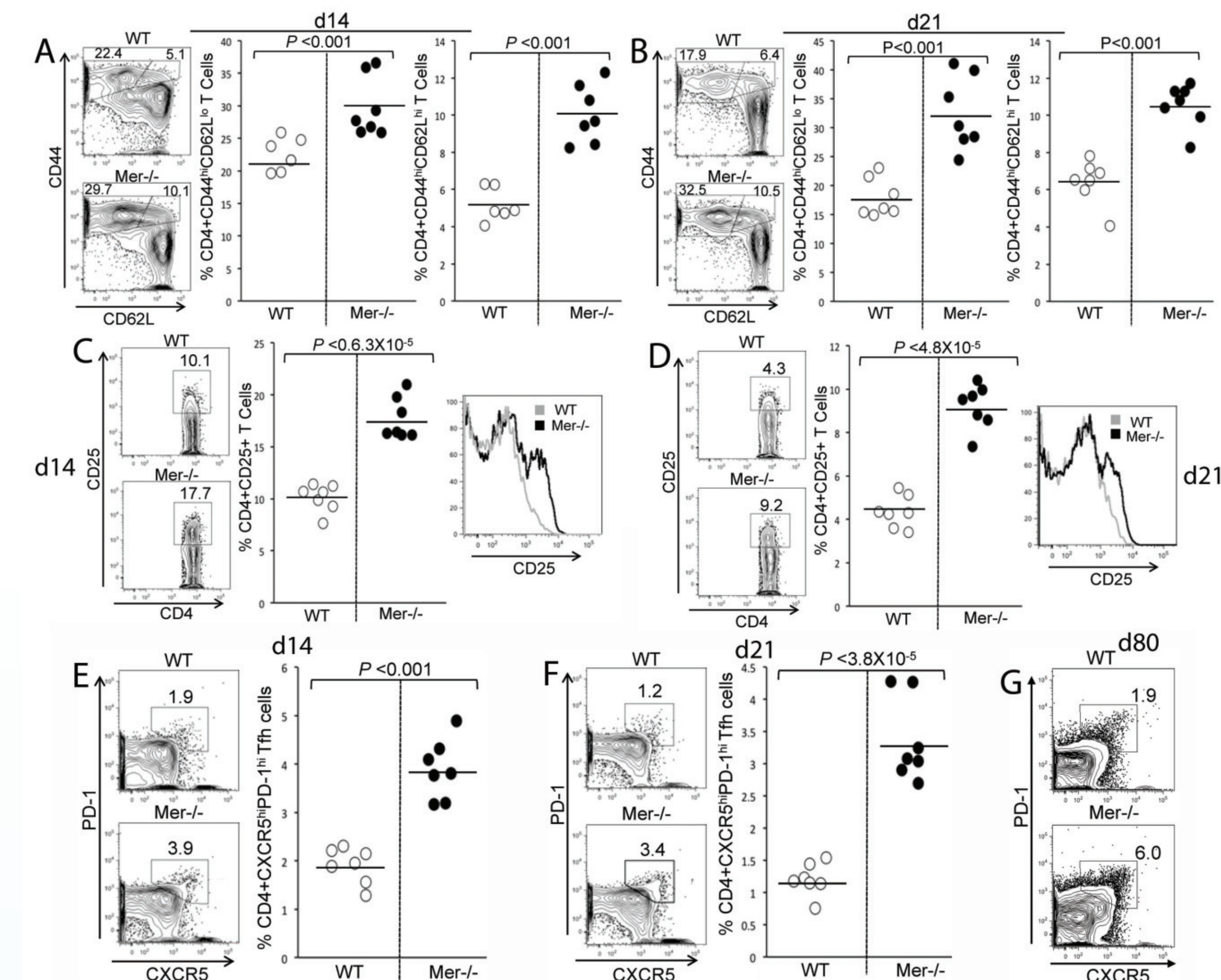

Figure 6. The percentage of $CD4^+CD44^{hi}CD62L^{lo}$ (middle panels) and $CD4^+CD44^{hi}CD62L^{hi}$ (right panels) T cells in WT and Mer-/- mice is shown in (A) and (B). (C, D): The percentage of $CD4^+CD25^+$ T cells in WT and Mer-/- mice. (E, F): The percentage of $CD4^+CXCR5^{hi}PD\text{-}1^{hi}$ T_{FH} cells in WT and Mer-/- mice is shown in right panels (E, F). Statistical analysis was performed by Student's t-test. These data were obtained from sex and age-matched seven mice of each genotype.

Long-term accumulation of ACs in Mer-/- GCs associated with high titers of ANAs

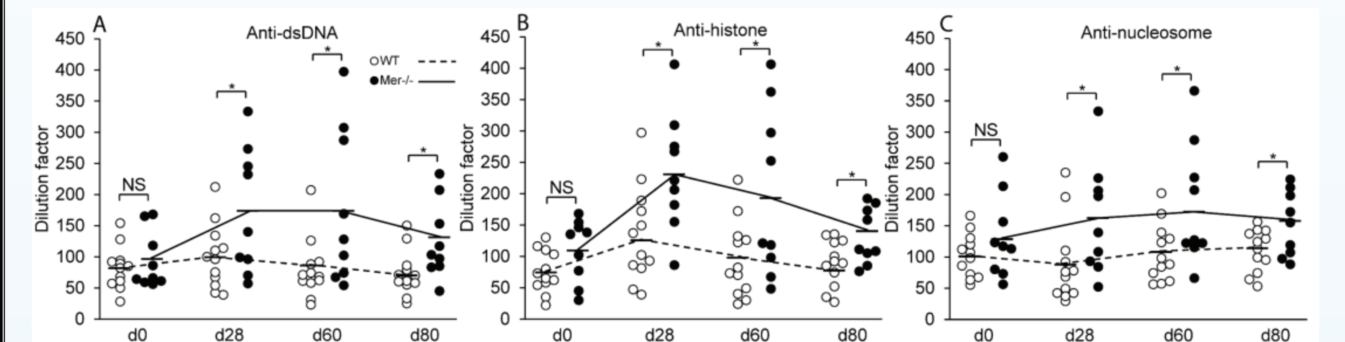

Figure 7. Anti-dsDNA (A), anti-histone (B) and anti-nucleosome (C) Abs were measured by ELISA in serum samples obtained from a group of WT (open circle, n =12) and Mer-/- (closed circle, n = 9) mice on multiple time points before (d0) and after (d28, d60 and d80) immunization with NP-OVA. The dashed lines represent WT and solid lines represent Mer-/- mice. Each circle represents one individual mouse and bars represent the mean values. Statistical analysis was performed by Student's t-test. P values of <0.05 and <0.01 are depicted as * and **, respectively.

Augmented secondary AFC and IgG2 Ab responses in Mer-/- mice

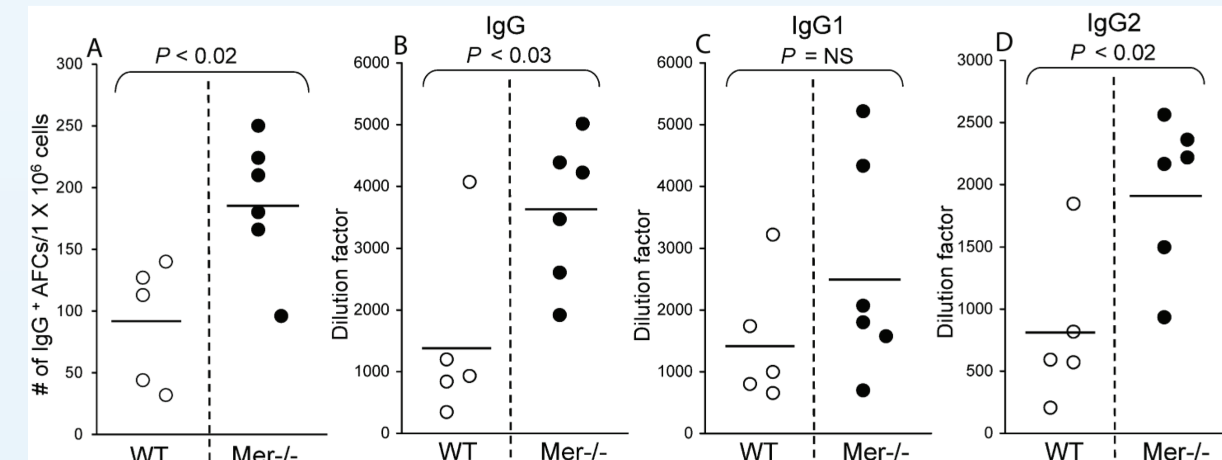

Figure 8. IgG-producing secondary AFCs in the spleen was measured by ELISpot assay four days after boosting WT and Mer-/- mice with NP-OVA. The anti-NP IgG (B), IgG1 (C) and IgG2 (D) Abs were measured by ELISA in serum samples collected from mice described in (A). Age- and sex-matched five to six mice of each genotype were used to generate these data.

Conclusion

- Long-term accumulation of ACs in GCs in the absence of Mer associated with enhanced primary GC and AFC responses
- Elevated total IgG and Th1-IgG2 Ab responses in Mer-/- mice
- Significantly increased percentages of activated B and helper T cells, including T_{FH} cells, and proliferating GC B cells in the absence of Mer
- Long-term AC accumulation in Mer-/- GCs associated with high titers of ANAs
- Augmented secondary AFC and IgG2 Ab responses in Mer-/- mice

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