The Role of Mer in Apoptotic Cell Clearance in the Germinal Center

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Abstract

Germinal centers (GCs) are specialized micro-environments that generate high affinity Ab-forming cells (AFCs) and memory B cells. Many B cells undergo apoptosis during clonal selection in GCs. The TAM (Tyro-3, Axl, and Mer) family receptor tyrosine kinases, including Mer, facilitate macrophage clearance of apoptotic cells. We previously showed that tingible body macrophages (TBMφs) in GCs express Mer. We observed that apoptotic cells (ACs) accumulated in GCs of mice deficient in Mer (Mer-/-), after immunization with T-dependent Ag. Accumulation of ACs in GCs of Mer-/- mice resulted in significantly increased AFCs, GCs, and Th1-skewed IgG2c Ab responses. We report here that increased GC response in Mer-/- mice compared to controls is due to increased proliferation of GC B cells. We also found that AC accumulation in Mer-/- GCs is not due to increased B cell apoptosis. We show that TBMφs express two other members (Tyro-3 and Axl) of TAM family receptors, which are similar in both Mer-/- and controls. TBMφs in GCs of both strains express similar levels of milk fat globule EGF factor 8 (Mfge8) and T cell immunoglobulin 4 (Tim-4), which are believed to aid in AC clearance. These data indicate the critical role for Mer in the clearance of ACs in GCs. This is further strengthened by the efficient clearance of ACs from GCs in mice deficient in Axl (Axl-/-) in the presence of Mer. Together, these data demonstrate a pivotal role of Mer in regulating B cell response and in the maintenance of B cell tolerance.

Methods and Results

Augmented anti-NP GC response in Mer$^{-/-}$ mice

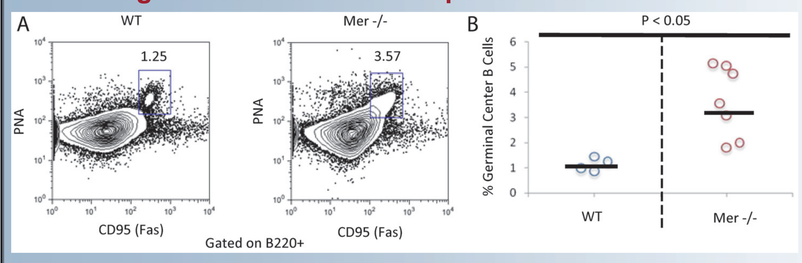

Figure 1. (A) B6/129.Mer$^{-/-}$ (Mer$^{-/-}$) and wild-type control (WT) mice were immunized with the T-dependent Ag (TD Ag) NP-OVA. Flow cytometric analysis was performed on splenocytes obtained 21 days postimmunization and gated on GC B cells (B220$^+$PNA$^+$CD95$^+$). (B) The percentage of WT and Mer$^{-/-}$ GC B cells is shown. n= 4 for WT and 7 for Mer$^{-/-}$

Enhanced primary (short-lived) AFC responses in Mer$^{-/-}$ mice

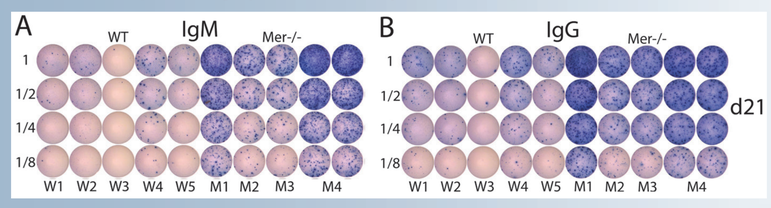

Figure 2. Splenic NP-specific IgM (A) and IgG-secreting (B) AFCs were measured by ELISpot. Representative images of an assay obtained on day 21 of anti-NP response is shown. n= 4-5 mice of each genotype.

Elevated Th1-skewed IgG2 Ab responses in Mer$^{-/-}$ mice

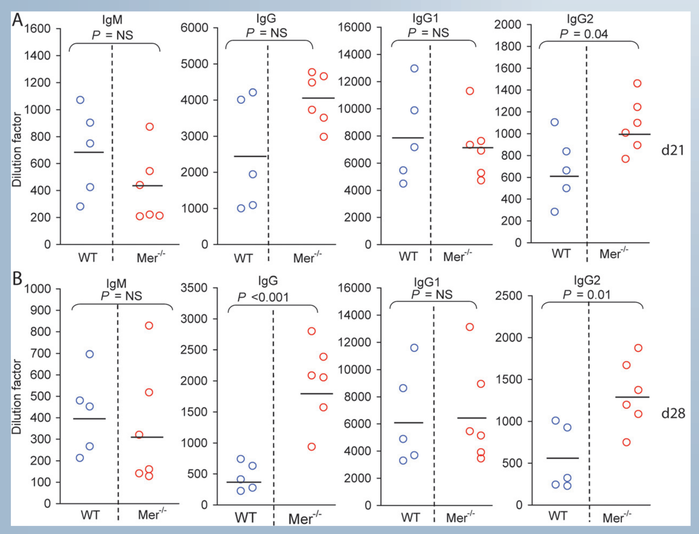

Figure 3. TD Ag-induced anti-NP total IgM, IgG, IgG1 and IgG2 titers were measured by ELISA in WT and Mer$^{-/-}$ serum samples obtained on days 21 (A) and 28 (B) postimmunization. n=5 for WT and 7 for Mer$^{-/-}$

Significantly increased number of apoptotic cells (ACs) accumulate in Mer$^{-/-}$ GCs

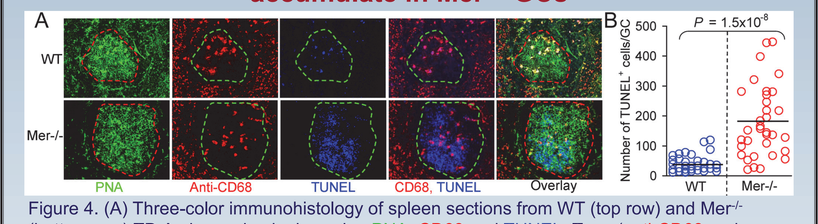

Figure 4. (A) Three-color immunohistology of spleen sections from WT (top row) and Mer$^{-/-}$ (bottom row) TD Ag immunized mice using PNA, CD68 and TUNEL. Two- (anti-CD68 and TUNEL) and three-color (PNA, anti-CD68 and TUNEL) overlay images are shown in the fourth and fifth columns, respectively. GCs are shown in dotted red lines in the first column and defined by the presence of PNA$^+$ cells. Magnification: 200x. (B) TUNEL$^+$ cells were counted in randomly selected GCs from four to five mice of each genotype. Horizontal bar indicates average value.

Accumulation of ACs is not due to increased cell death in Mer$^{-/-}$ GCs

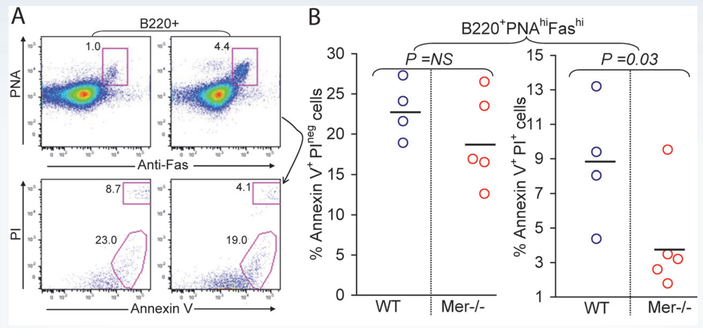

Figure 5. Flow cytometric analysis of splenocytes obtained on day 14 postimmunization. Staining performed with Annexin V and PI, gated on GC B cells (B220$^+$IgD$^-$Fas$^+$). The percentage of GC B cells undergoing cell death appears to be lower in Mer$^{-/-}$ mice compared to the wild-type control.

Significantly increased number of proliferating B cells in Mer$^{-/-}$ GCs

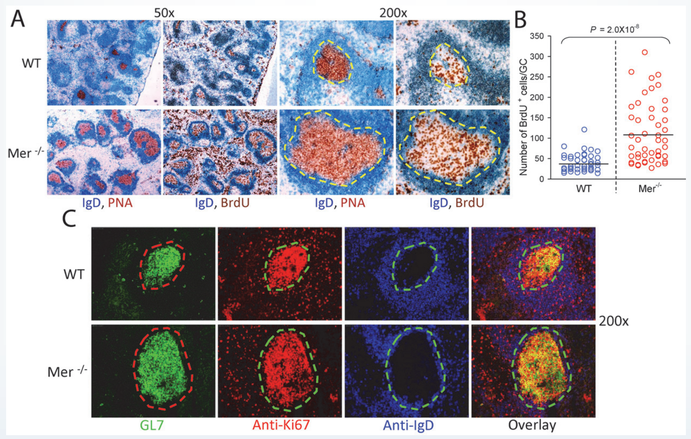

Figure 6. (A) Spleen sections of WT (top row) and Mer$^{-/-}$ (bottom row) mice from day 21 postimmunization were stained with IgD, PNA and BrdU. (B) Average number of BrdU$^+$ cells per randomly selected GC in WT and Mer$^{-/-}$ mice. (C) Spleen sections stained with GL-7, anti-Ki67, and anti-IgD showing GC B cell proliferation in WT and Mer$^{-/-}$ mice.

Expression of milk fat globule EGF factor 8 (Mfge8), a dual-function bridging molecule involved in the integrin pathway to clear ACs is not compromised in Mer$^{-/-}$ mice

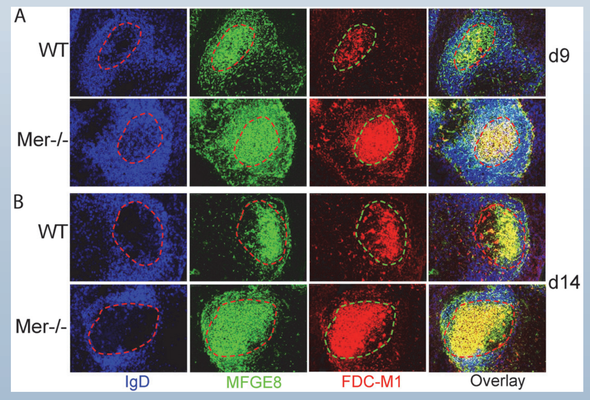

Figure 7. Multicolor immunohistological analysis of spleen sections from WT and Mer$^{-/-}$ mice on days 9 (A) and 14 (B) postimmunization using anti-IgD, MFGE8, and FDC-M1. GCs are shown in dotted red lines in the first column and defined by the presence of IgD$^-$ cells. Magnification: 200x. n= 4-5 mice of each genotype.

Similar expression levels of Tim-4, a molecule that directly binds phosphotidylserine (PS) on ACs and mediates phagocytosis in WT and Mer$^{-/-}$ GCs

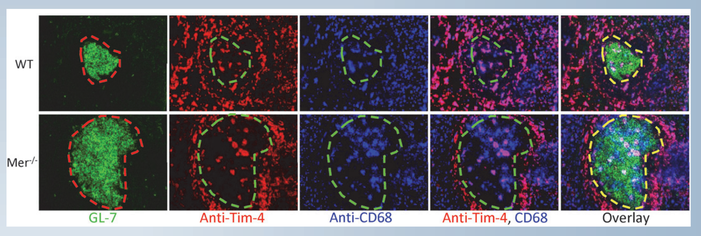

Figure 8. Spleen sections of WT (top row) and Mer$^{-/-}$ (bottom row) mice from day 14 postimmunization with TD Ag were stained using GL-7, Anti-Tim-4 and Anti-CD68. Two- (anti-Tim-4 and CD68) and three-color (GL-7, anti-CD68 and CD68) overlay images are shown in the fourth and fifth columns, respectively. GCs are shown in dotted red lines in the first column and defined by the presence of GL-7$^+$ cells. Magnification: 200x. n=4-5 mice of each indicated genotype.

Other members of the TAM family receptor tyrosine kinases are not altered in Mer$^{-/-}$ mice

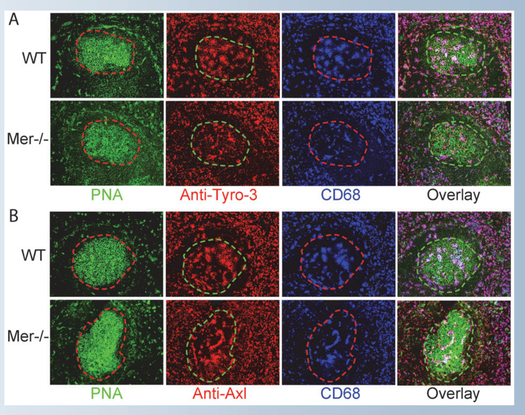

Figure 9. Immunohistology of spleen sections of WT and Mer$^{-/-}$ mice from day 14 postimmunization. (A) Splenic tissue was stained with PNA, anti-Tyro-3 and CD68. (B) Spleen frozen sections were stained with PNA, anti-Axl and CD68. The expression levels of Tyro-3 and Axl are similar in both the presence and absence of Mer. Magnification: 200x

No significant accumulation of ACs in GCs of Axl deficient (Axl$^{-/-}$) mice in the presence of Mer

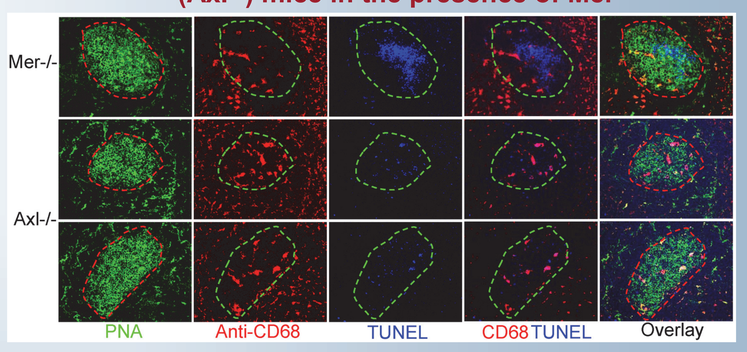

Figure 10. Similar immunofluorescent staining of spleen sections as in Figure 4 was performed with PNA, CD68 and TUNEL using tissue from Mer$^{-/-}$ (top row) and Axl deficient (Axl$^{-/-}$) mice (bottom two rows). Significant number of ACs accumulate in Mer$^{-/-}$ GCs, whereas ACs are largely cleared in Axl$^{-/-}$ GCs, in the presence of Mer.

Conclusion

- Significantly higher number of apoptotic cells (ACs) accumulate in GCs of Mer deficient (Mer$^{-/-}$) mice
- Augmented anti-NP GC, AFC, and Th1-skewed IgG2 Ab responses in Mer$^{-/-}$ mice
- Significantly increased number of proliferating B cells in Mer$^{-/-}$ GCs
- Accumulation of ACs in Mer$^{-/-}$ GCs is not due to increased cell death
- ACs are largely cleared from GCs of Axl deficient (Axl$^{-/-}$) mice, in the presence of Mer

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