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

Tahsin N. Khan, Eric B. Wong, and Ziaur S. M. Rahman
Department of Microbiology and Immunology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107

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 (TBMs). 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 (TFH) 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 immunohistochemistry of spleen tissue obtained on day 14 (A), 21 (B) and 80 (C) post-NP-OVA immunization of WT and Mer−/− mice. Splenocytes 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) overlap 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 for each genotype for each time point.

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+anti-CD95/Fas+ GC B cells are shown in rectangular gates (A and B, left panels) and the percentage of B220+PNA+anti-CD95/Fas+ 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+anti-CD95/Fas+ GC B cells are shown in rectangular gates (C). Splenocytes obtained from WT (D) and (E) and Mer−/− mice (E and G) on days 21 and 80 were stained with anti-IgD and PNA. Low (35x) and high magnification (200x) representative images are shown.

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

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 per 1x10⁶ 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.

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 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. These data were obtained from age- and sex-matched five to six mice of each genotype.

Figure 5. B cell activation was evaluated by flow cytometric analysis of splenocytes obtained on days 14 (A) and 21 (B) post-immunization of WT and Mer−/− mice with NP-OVA. Splenocytes 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 percentages of B220−CD80+ (left panels) and B220−CD86+ (right panels) cells in WT and Mer−/− mice are 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-IgG 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.

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

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

Figure 6. The percentage of CD4+CD44hiCD62Llo (middle panels) and CD4+CD62Llo (right panels) T cells in WT and Mer−/− mice is shown in (A) and (B). (C, D) The percentage of CD4+CD80+ T 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.

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

Figure 7. Anti-IgD (A), anti-histone (B) and anti-nucleosome (C) Abs were measured by ELISA in serum samples collected 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.

Figure 8. IgG-producing secondary AFCs in the spleen were 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 Tfh 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

We acknowledge support for this study from the National Institute of Health grant (RO1AI091670) to Z.S.M.R.