The Role of Foxo3 in B Cell Tolerance

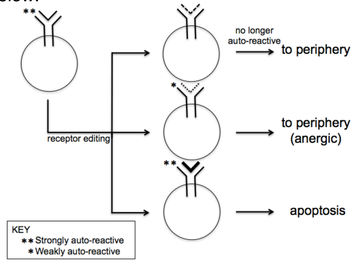
Evan Barrios[1], Kristina Ottens MS[1], Rochelle Hinman PhD[1], Brian Skaug MD, PhD[1], Diego Castrillion MD, PhD[2], and Anne Satterthwaite PhD[1,3]

Departments of [1]Internal Medicine, [2]Pathology, and [3]Immunology

University of Texas Southwestern Medical Center, Dallas, TX

Introduction

Background: B cells help defend the body against pathogens in part by releasing antibodies, which are secreted B cell receptors (BCRs) that recognize antigens and facilitate their destruction by components of the immune system. In order to recognize all potential pathogens, an enormous array of BCRs are required. Some of the generated receptors react against components of the body. The immune system attempts to eliminate or control auto-reactivity in order to prevent autoimmune disease. In the bone marrow, auto-reactive immature B cells recombine components of their receptor in a process called receptor editing shown below:



If the new BCR is no longer auto-reactive, the cell is sent to the periphery to continue in the maturation process. If the B cell receptor is weakly auto-reactive, the cell is sent to the periphery but it is anergized, or functionally inactivated. If the BCR is strongly auto-reactive, the cell dies by apoptosis[1].

Failure to eliminate auto-reactive B cells can contribute to autoimmune diseases, so it is important to understand the molecules that participate in this process. Foxo3 is a transcription factor that is involved in pro-apoptotic pathways in several cell types[2]. Previous work in the lab showed that apoptosis is reduced in immature B cells from Foxo3-/- mice[3], and others have observed decreased levels of Foxo3 in B cells from mouse models of lupus (an autoimmune disease in which B cells produce antibodies reactive against the body's own DNA)[4].

Hypothesis: It is hypothesized that edited cells that remain auto-reactive may survive inappropriately in the absence of Foxo3.

Materials and Methods

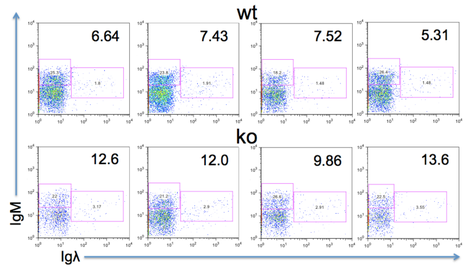
Flow cytometry was used to observe the proportion of Igλ+ B cells in the bone marrow and spleens of Foxo3-/- (ko) and wild type (wt) mice. B cells recombine the Igκ light chain gene first. Thus, cells expressing the Igλ light chain have likely undergone receptor editing[5,6]. Antibodies against B220, CD93, IgM, and Igλ were used to detect Igλ on immature (B220+CD93+IgM+) and mature recirculating (B220+CD93-IgM+) B cells in the bone marrow, and against B220, CD21, CD23 and Igλ to detect Igλ on transitional (B220+CD21-CD23-), follicular (B220+CD21+CD23+), and marginal zone (B220+CD21+CD23lo/-) B cells in the spleen.

RS PCR was utilized to observe whether increased editing occurs in Foxo3-/- B cells compared to wt B cells. Total B cells were purified from mouse spleens using magnetic beads. Then, genomic DNA was isolated and semi-quantitative PCR was conducted in order to detect RS recombination, indicating the amount of editing of BCRs[6,7]. PCR amplification of the Ets1 gene was the loading control. When no difference in editing was discovered between total B cell populations, the cells were separated into Igλ- B cell and Igλ+ B cell subpopulations.

Anti-double-stranded (ds) DNA ELISAs analyzed the auto-reactivity of Foxo3-/- and wt B cells. Total splenic B cells were purified with magnetic beads and stimulated with LPS for 5 days to induce antibody secretion. Supernatants were subjected to ELISA to detect anti-dsDNA antibodies.

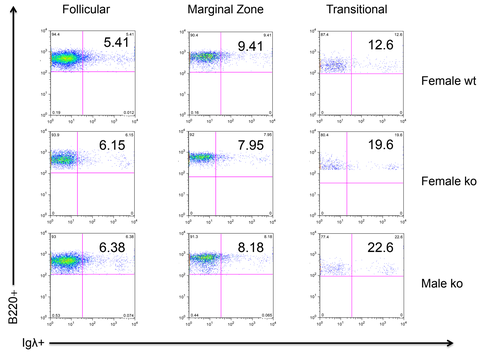
Results

Figure 1. Immature B cells from the bone marrow of Foxo3-/- mice are more likely to express Igλ



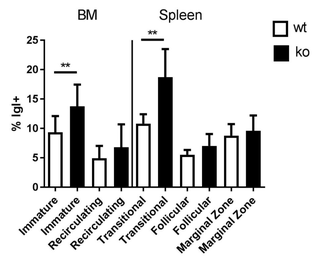
Bone marrow cells were stained with antibodies against B220, Igλ, IgM, and CD93. Shown is the expression of IgM and Igλ on B220+CD93+ cells. Igλ- immature B cells are in the upper left of each panel, and the Igλ+ immature B cells are on the right. The fraction of Igλ+ immature B cells among total immature B cells is indicated in the upper right.

Figure 2. Splenic transitional B cells from Foxo3-/- mice are more likely to express Igλ



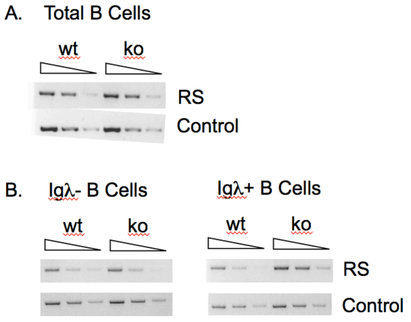
Spleen cells were stained with antibodies against CD21, CD23, B220, and Igλ. B220 and Igλ expression on transitional (recently emerged from the bone marrow), follicular, and marginal zone cells is shown. The frequency of Igλ+ cells among transitional cells is significantly increased in Foxo3-/- (ko) mice and conserved across gender.

Figure 3. Summary of Igλ expression in B cell subpopulations



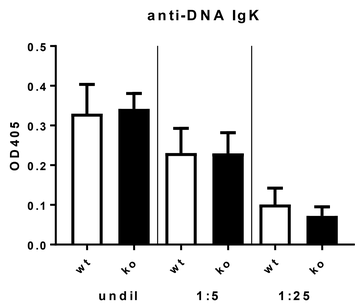
The percentage of Igλ+ B cells in each subpopulation is shown as mean ± SD. n = 9-10 for bone marrow, 5 for spleen. There are significantly more Igλ+ cells in Foxo3-/- (ko) mice in bone marrow immature B cells and splenic transitional B cells.

Figure 4. Foxo3-/- Igλ+ B cells undergo more receptor editing as measured by RS PCR



Serial dilutions of genomic DNA from wt and Foxo3-/- (ko) splenic B cells underwent PCR to detect RS recombination as an indication of receptor editing. Primers for the Ets1 gene were used as a loading control. Total B cells (**A**) and Igλ- (or Igκ+) B cells (**B,** left) showed no difference in the amount of editing between wt and Foxo3-/-. However, among Igλ+ B cells (**B,** right) there is more editing in the Foxo3-/- mice. Results are representative of 3 experiments.

Figure 5. No difference in anti-dsDNA reactivity of B cells from Foxo3-/- and wild type mice



Total splenic B cells were isolated from Foxo3-/- (ko) and wild type mice and stimulated with LPS for 5 days to induce antibody secretion. An ELISA was conducted on the indicated dilutions of the culture supernatants in order to identify the amount of anti-dsDNA Igκ and Igλ antibodies secreted by the B cells. Results are shown for Igκ as mean ± SD, n = 4-6. No difference is seen between the ko and wt mice. Igλ anti-dsDNA antibodies were not detected.

References

[1]Pelanda R and Torres R, Curr. Opin. Immunol. (2006) 18:184-90.
[2]Burgering B and Medema R, J. Leuko. Biol (2003) 73:689-701.
[3]Hinman R, Castrillon D, and Satterthwaite A, Unpublished data.
[4]Nakou M et al, PLoS One (2010) 5(10): e13351.
[5]Hertz M and Nemazee D, Immunity (1997) 4: 429-436.
[6]Panigrahi A et al, J. Exp. Med. (2008) 205: 2985-94.
[7]Vela J et al, Immunity (2008) 28: 161-70.
[8]Hartley S et al, Nature (1991) 353: 765-769.

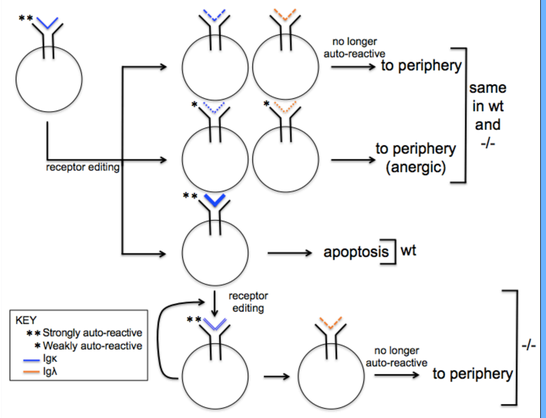
Conclusion

Summary

- Increased receptor editing in Foxo3-/- mice is suggested by
  - Increased frequency of Igλ+ B cells among immature and transitional B cells
  - Increased RS recombination in Igλ+ B cells
- No increase in the overall auto-reactivity of Foxo3-/- B cells, at least as measured by anti-dsDNA reactivity.

Model

- Reduced apoptosis of Foxo3-/- immature B cells allows auto-reactive cells more time to continue editing their BCRs towards Igλ expression and away from auto-reactivity.



Future Directions

1) Do auto-reactive Foxo3-/- B cells survive inappropriately and escape to the periphery when they are not edited?

- Mice containing an anti-Hen Egg Lysozyme (HEL) BCR as a transgene will be used. All BCRs produced by individual B cells recognize HEL in these mice. When developing in the presence of HEL, these B cells are auto-reactive and are deleted by apoptosis[8].
- The transgene is located on a different part of the genome than the coding gene for the BCRs, so receptor editing should not diminish the auto-reactivity of the anti-HEL BCRs[1].
- We hypothesize that auto-reactive Foxo3-/- B cells will survive and be released into the periphery in this system due to their impaired apoptosis.

2) Further analysis of auto-reactivity of Foxo3-/- B cells

- Subpopulations: Differences in auto-reactivity of relatively rare B cell subpopulations, such as the Igλ expressing cells known to be highly edited, may have been missed when total B cells were analyzed.
- Additional auto-antigens: Foxo3-/- B cells may demonstrate altered reactivity to self-antigens other than dsDNA.

Acknowledgments

This project was supported by NIH grant AR067625 and the UT Southwestern Summer Medical Student Research Program. We thank Ian Matthews and Angela Mobley in the UTSW Flow Cytometry Core Facility for assistance.