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Immunity
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MicroRNA-155 Function in B Cells

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DOI 10.1016/j.immuni.2007.11.010

Vigorito et al. (2007) report (in this issue of *Immunity*) that B cells require microRNA (miR)-155 for normal production of isotype-switched, high-affinity antibodies and for a memory response. They identify transcriptional regulator Pu.1 as a functionally important target of miR-155 in B cells.

MiR-155 is encoded within a region known as *bic*, *B* cell *i*ntegration *c*luster, identified originally as a frequent integration site for avian leucosis virus (Lagos-Quintana et al., 2002; Tam et al., 1997). An important role for miR-155 in B cell malignancies was strongly suggested by the observations that miR-155 is elevated in certain B cell lymphomas (Eis et al., 2005) and that transgenic expression of miR-155 in B cells causes pre-B cell lymphomas in mice (Costinean et al., 2006). Furthermore, the expression pattern of miR-155 in normal B cells suggests a role in differentiation of normal activated B cells because it is induced upon B cell receptor (BCR) ligation of Ramos cells (van den Berg et al., 2003).

Earlier this year, Rodriguez et al. (2007) and Thai et al. (2007) simultaneously reported that mice harboring a germline deletion of miR-155 have normal lymphocyte development but defective T and B immunity, including reduced numbers of germinal center (GC) B cells. However, the cellular nature of these defects was not identified. In the current work, the B cell-intrinsic defects caused by the absence of miR-155 were identified by analysis of purified B cells activated in vitro and chimeric mice in which only B cells lacked miR-155.

Immunization of miR-155-deficient mice with either the T cell independent (TI) antigen DNP-LPS (dinitrophenylated lipopolysaccharide) or with the T cell dependent (TD) antigen NP-KLH (4-hydroxy 3-nitrophenylacetyl keyhole limpet hemocyanin) gave similar results in that IgM responses were normal, but IgG1 responses were substantially reduced in the miR-155-deficient mice, suggesting a particular defect related to formation or maintenance of plasma cells secreting switched isotypes.

TI and TD responses are different in several ways (Figure 1). TI antigens primarily activate marginal zone and B-1 B cells via BCR and Toll-like receptor (TLR) signaling, whereas TD antigens primarily activate follicular B cells in response to BCR, TLR, T cell cytokines, and CD40 signaling. Although both responses cause an extrafollicular, early burst of short-lived plasma cells producing low-affinity antibody, the TD response also leads to the formation of GCs, reviewed recently by Allen et al. (2007). In GCs, B cells undergo intense proliferation, somatic hypermutation (SHM) of the rearranged Ig variable gene segments, and class switch recombination (CSR). Selection, involving competition both for antigen and for T cell help, then provides survival signals for clones making high-affinity BCR, whereas clones with lower-affinity BCR or clones unable to obtain T cell help die by apoptosis. This selective pressure, combined with ongoing somatic mutation of Ig V gene segments, results in "affinity maturation" of GC B cells. Surviving GC B cell clones can either undergo further rounds of proliferation, SHM, CSR, and selection or differentiate and leave the GC. B cells leaving the GC differentiate into plasma cells, which can migrate to survival niches in the bone marrow or into memory B cells. Expression of high-affinity, switched-isotype immunoglobulin is characteristic of plasma cells and memory cells resulting for a GC reaction.

In miR-155-deficient mice, the extrafollicular response to both TI and TD antigens was defective for switched isotypes, but not for IgM (Figure 1). In addition, the number of antibody-secreting cells formed in an extrafollicular response was decreased, although secretion on a per-cell basis was normal, suggesting that formation or maintenance of plasma cells might be defective but that plasma cells differentiated into Ig-secreting cells normally. Multiple defects were observed in the GC response (Figure 1) including decreased numbers of GC B cells, decreased IgG production, decreased affinity maturation, decreased numbers of memory cells, a reduced memory response, and decreased IgG1 plasma cells in the bone marrow.

When the authors explored the mechanisms of these defects, the results were surprising. The miR-155 B cells did not have defects in proliferation, at least as measured in in vitro cultures stimulated with LPS or CD40 and cytokines. Because the antiapoptotic protein Bcl-2 can rescue B cells from apoptosis, the possibility of increased apoptosis in the miR-155-deficient B cells was explored by forced expression of Bcl-2 in in vitro cultures of B cells lacking miR-155. However, this did not alter the reduced numbers of $IgG1^+$ cells in miR-155 B cell cultures, suggesting the defects were not due to increased apoptosis. Activation-induced cytidine deaminase (AID) is required for both SHM and CSR; however, AID mRNA expression was comparable in wild-type and miR-155-deficient B cells. Consistent with this finding, analysis of mutation in a noncoding V_H region not subject to selection showed that the wild-type and miR-155-deficient B cells had a nearly comparable frequency

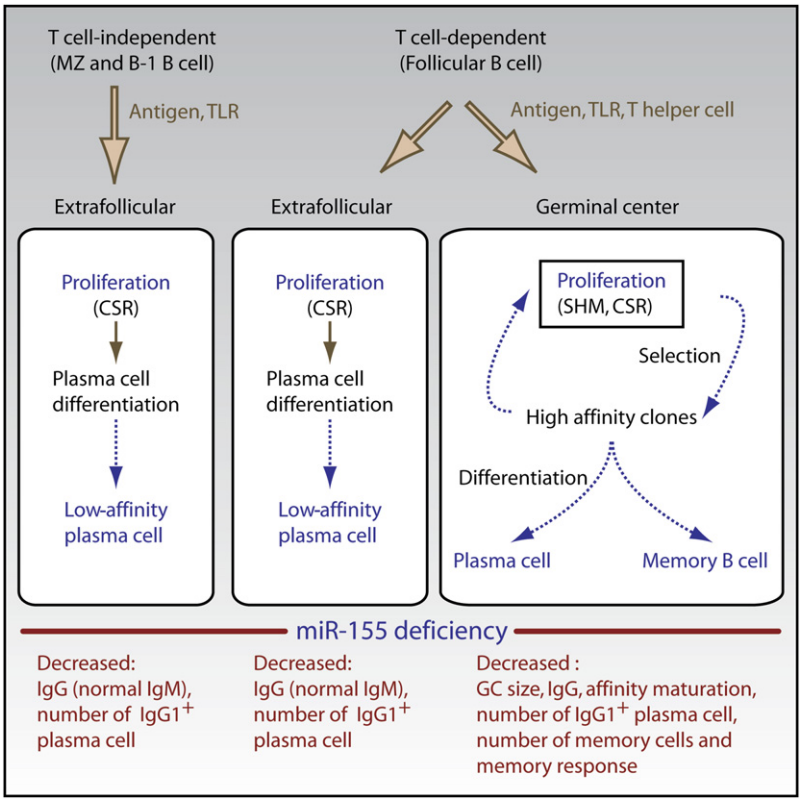

Figure 1. B Cell Responses in the Absence of MiR-155
The important steps in TI and TD B cell responses are summarized above the horizontal line. Processes that might be defective in miR-155-deficient B cells are indicated in blue. Below the horizontal line, the defects observed by Vigorito et al. (2007) in miR-155-deficient B cells are summarized.

and a similar pattern of mutation, suggesting that the SHM mechanisms were intact in miR-155-deficient animals. Finally, the germline transcripts that are required prior to CSR and the switch circle transcripts that result from CSR were present in normal amounts in the miR-155-deficient B cells, showing that the CSR mechanisms were intact.

How can we account for these observations? The authors suggest the reasonable conclusion that selection is defective for the GC B cells of the miR-155-deficient mice. Selection of B cells requires robust expression and function of BCR, MHC class II, CD80, CD86, CD40, complement, Fc, and cytokine receptors so that the B cell can respond to antigen, process and present antigen to T cells, stimulate T cells, and respond to T cells. GC B cells also need to control DNA damage responses. Furthermore, recent studies on the dynamic motions of GC B cells suggest that B migration to encounter antigen and T cells is critical (Allen et al., 2007). Thus, B cells lacking miR-155 might have defects in any of these mechanisms.

One possible molecular mechanism for how miR-155 is working in B cells is by targeting the ets-family transcriptional regulator Pu.1. This paper provides strong evidence that mRNA encoding Pu.1 is one target of miR-155. The authors not only show that Pu.1 mRNA is elevated in miR-155-deficient B cells but they also show that miR-155 directly targets Pu.1 mRNA via a predicted miR-155-binding site in the 3′ untranslated region in a binding-site-dependent way. Finally, they show that forced expression of Pu.1 in cultured wild-type primary B cells recapitulates the miR-155-deficient defect in IgG1 production. Pu.1 is known to be important for early B cell commitment and development; amounts of it are high in GC B cells, but are reduced upon plasmacytic differentiation (Singh et al., 2007). The current observations provide a basis for seeking Pu.1 target genes, and the discovery of these genes might explain some of the observed B cell defects in the miR-155-deficient mice.

In addition, as the authors discuss, Pu.1 might not be the only important target of miR-155 in B cells. The gene-profiling studies the authors carried out identified ~60 genes that are elevated in B cells of the miR-155-deficient mice and that have potential miR-155-binding sites in their 3′ regions. Future work to verify those directly target by miR-155 and functionally important in B cells should yield interesting results. Furthermore, because microRNAs decrease gene expression by inhibiting mRNA translation as well as targeting mRNAs for degradation, some targets might not have been revealed by the microarray studies.

Important mechanistic questions that deserve investigation remain. Why is there a reduction in the number of both extrafollicular plasma cells and GC B cells? The observations that neither B cell proliferation nor apoptosis were defective in vitro seem contradictory with decreased cell numbers in vivo. However, proliferation measured in vitro might not adequately reflect proliferation in vivo, and it is possible that decreased proliferation could indeed be associated with loss of miR-155. Furthermore, there could be abnormalities that cannot be remedied by overexpression of Bcl-2 in vitro in cell death. Another question raised is whether there can be normal amounts of germline gamma 1 transcripts and switch circles but decreased numbers of $IgG1^+$ cells. Additionally, why are IgM responses normal, whereas IgG responses are defective, and why are switched-isotype plasma cells specifically reduced? Answers to these questions might reveal a previously unknown regulatory step in the expression or function of BCRs of the gamma isotype for PC maintenance. Finally, does the reduction of both post-GC plasma cells and memory cells suggest regulation at a common precursor before final differentiation occurs in the GC?

MicroRNAs are an exciting new class of regulators. This work is

intriguing for the new information it provides about the role of miR-155 in B lymphocytes. In addition, the work is also intriguing because the defects in B cells lacking miR-155, although currently not fully clarified at either the functional or molecular level, are quite unusual. Thus, the phenotype of miR-155-deficient B cells might ultimately provide new insights into regulation of poorly understood steps in B cell differentiation, including selection of high-affinity clones during the germinal-center reaction, memory cell formation, and putative differences in homeostasis of plasma cell clones expressing B cell receptors of different isotypes. In addition, it is important to remember that miR-155 is also involved in B cell malignancies. Hopefully, further mechanistic studies in miR-155-deficient B cells will also provide novel insights into B cell lymphomagenesis.

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E Proteins Enforce Security Checkpoints in the Thymus

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In this issue of *Immunity*, two studies define new roles for E proteins in thymocyte development. Agata et al. (2007) show a new role in beta selection; Jones and Zhuang (2007) demonstrate a role in positive selection.

The four E proteins (E12, E47, E2-2, and HEB) are widely expressed basic helix-loop-helix proteins that bind to a canonical site (CANNTG) in DNA as homo- and heterodimers, where they can either activate or repress gene expression. The E12 and E47 molecules were originally identified as proteins that could bind to the immunoglobulin enhancer, and E12 and E47 are splice variants of a single gene (*Tcfe2a*) that is a common breakpoint in B cell acute lymphoblastic leukemia translocations (Mellentin et al., 1989). Consistent with these observations, deletion of *Tcfe2a* in mice by gene targeting results in a complete block early in B cell development (Bain et al., 1994). These results solidified the critical role of E proteins in B cell development. However, in what is perhaps a more surprising result, the E2A- (encompassing both E12 and E47) deficient mice also develop spontaneous T cell leukemias at an early age, and these leukemic cells have the phenotype of immature thymocytes (Bain et al., 1997). This observation has led to numerous fruitful investigations regarding the role of E proteins in thymocyte development.

There is a clear role for E proteins in the very early stages of thymocyte development because reduction in E protein activity limits the maturation of early thymocytes, and E2A also promotes *Tcrg* and *Tcrd* gene rearrangement (Murre, 2005). Beyond these effects, E proteins seem to play a major role in maintaining the phenotype of the double negative 3 (DN3) thymocyte population. DN3 cells are positioned just upstream of a bottleneck in thymocyte development. Development beyond the DN3 stage requires expression of a pre-T cell receptor (pre-TCR), composed of the invariant pre-Tα chain and the rearranged TCRβ chain. Thus, development beyond the DN3 stage requires successful rearrangement of TCRβ (β selection). Once the pre-TCR is formed, the DN3