JEM Journal of Experimental Medicine

INSIGHTS

Natural history of MZ B cells

David Nemazee

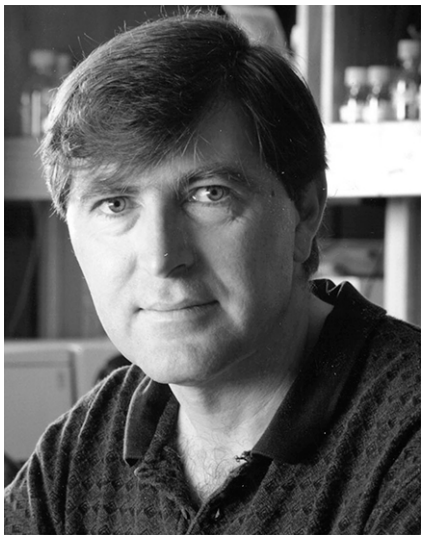
In this issue, Tull et al. (https://doi.org/10.1084/jem.20202001) and Kibler et al. (https://doi.org/10.1084/jem.20201952) track human marginal zone B cell development from early progenitors to the memory compartment, addressing changes in age and autoimmunity, the sequence of development in the gut-associated lymphoid tissue, and clonal sharing among memory cells.

The saga of cellular immunology is marked by the progressive definition of subtypes, starting with the delineation of B and T lymphocytes, followed by their progressive splitting into subsets marked by distinct markers, transcription factor control, and cytokine profiles. The identification of B lymphocyte subsets with distinct yet overlapping functions and developmental origins (Hayakawa et al., 1984; Linton et al., 1989) inspired the concept of a layered immune system (Herzenberg and Herzenberg, 1989), whose functions and meaning remain to be sorted out. Translating this knowledge to the human condition still requires considerable field work, with each cell subset like an exotic pachyderm whose habits and behavior must be tracked in the wild.

The elephant at issue, the marginal zone B cell (MZB), does not fall neatly into innate or adaptive compartments but displays elements of both. Here, we highlight two papers on human MZB development and memory (Tull et al., 2021; Kibler et al., 2021), which look at both ends of the beast. By applying single-cell RNA sequencing (RNA-seq), flow spectrometry, and antibody gene mutational analysis techniques, these studies provide a higher-resolution view of these enigmatic compartments.

Identified first based on their anatomical location outside the follicular areas of the spleen in rodents, and later in humans, MZBs are in intimate contact with the blood and functionally important in the response to blood-borne encapsulated bacteria (Weill et al., 2009). MZBs are considered to be specialized to make rapid antibody responses to infection and are also believed to be specialized for the response to T-independent antigens. They are particularly responsive in vitro to innate type signals such as Toll-like receptor ligands. However, MZBs clearly can also participate in the T cell–dependent antibody response, where they may undergo considerable V-region somatic mutation and affinity maturation.

MZ transcription is regulated to a large extent by external stimuli, hence MZBs might be targetable by treatment modalities. Unlike other B cell types, MZBs require signals through the Notch pathway for their development and have distinct requirements for homing and trafficking to their niche (Cinamon et al., 2004; Cinamon et al., 2008). The MZB cell surface phenotype is also distinct from those of other B cells: in humans, MZBs are $IgM^+IgD^+CD21^{2+}CD23^-CD1c^+CD27^+$, indicative of interactions with the complement fragment C3b, NK-T cells, and CD70. CD27 expression is a marker of memory—indeed, most MZBs in humans carry somatic mutations. However, somatic mutations are present in the MZBs of infants before they are fully functional, and diversification is also seen in MZBs of individuals with mutated CD40L, who lack germinal center reactions and a class-switched memory compartment (Weill et al., 2009). So, human MZBs likely undergo preimmune hypermutation. In addition to their presence in the spleen, human MZ-like B cells are found in regions distinct from those of other B cells within the lymph nodes, Peyer's patch, and tonsil, where they may receive specific or innate stimulation (Spencer et al., 1985; Zhao et al., 2018).



Insights from David Nemazee.

MZBs develop from transitional B cells, a population in blood and the spleen derived from bone marrow emigrants, which quickly either die or differentiate to naive follicular or MZB subsets. Predictably, these transitional B cells have been further subdivided into T1, T2, T3 sub-subsets (Allman

Scripps Research.

David Nemazee: nemazee@scripps.edu.

and Pillai, 2008), with T1 cells being apparent recent bone marrow emigrants and T2 cells a subsequent developmental stage. T2-like cells have been suggested to be the precursors of MZBs in many studies.

Our first band of intrepid explorers, Tull et al. (2021) noted that human T2 cells express α4β7 integrins necessary for migration to the gut-associated lymphoid tissue (GALT), and that this expression is reduced in systemic lupus erythematosus (SLE; Vossenkämper et al., 2013), leading them to study these B cell subsets in normal and lupus patients. Flow spectrometry and single-cell RNA-seq analysis identified two apparent lineages of transitional B cells in the blood differing in cell surface marker and RNA expression. The T2 IgM^{hi} branch was preferentially present in the GALT, whereas the T2 IgM^{lo} population was underrepresented. The differences were related to T2 cell migration to GALT, which correlated with higher T2 B cell expression of integrin β7 and lower CCR7, along with higher expression of activation markers CD69 and CD80. These data implicate GALT as an important site for MZB differentiation and strengthen the view that MZBs represent a distinct lineage from naive follicular B cells. The authors tie together these findings with previous observations that T2 IgM^{hi} cells may be MZB precursors and that lupus patients tend to have lower numbers of T2 and MZ subsets, particularly in GALT. The authors confirmed that patients with lupus nephritis have strikingly reduced MZB frequencies along with a reduced number of putative MZ precursor cells, suggesting a possible developmental or trafficking defect in these patients. The study implies that normalization of MZ precursor migration might be a possible avenue of therapeutic exploration for lupus nephritis. Also consistent with an immune deficit in lupus nephritis patients was a dearth of class-switched IgA+ cells. The authors show that T2 IgM^{hi} cells also appear to more easily express IL-10, speculating that in lupus nephritis a reduction in IL-10–producing B reg cells might contribute to disease. Tull et al. also suggest that dysregulated stimulation by TLR ligands might represent a link between gut MZB dysregulation and lupus nephritis. While MZB development is normal in *MyD88*$^{-/-}$*Trif*$^{-/-}$ mice, which lack all TLR signaling (Gavin et al., 2006), and in UNC93b-deficient humans lacking all endosomal TLR signaling (Weller et al., 2012), it is quite possible that excessive TLR signaling may augment MZB development or function. These pathways are clearly therapeutically inhibitable. In summary, these studies very much support the notion that MZBs represent a distinct developmental lineage whose dysregulation may underlie systemic lupus erythematosus.

Meanwhile, naturalist Kibler and colleagues (Kibler et al., 2021) evaluated changes in the human splenic B cell compartment with age, comparing the B cell receptor diversity and clonal abundance of peripheral blood and splenic memory B cells from the same donors. 141 individuals of various ages were assessed by flow cytometry for the composition of the splenic and peripheral blood B cell compartments. 17 donors were assessed by deep antibody H-chain gene sequencing, whereby clones were tracked and sharing between the peripheral blood and memory B cells and other subsets was assessed. Clonal relatives of peripheral blood expanded clones were invariably found in the spleen. The most striking finding was the extensive clonal sharing between splenic and peripheral blood memory B cells with blood memory cells characterized by sequences with more mutations, and, therefore, likely more recent activation. In many cases, blood memory sequences represented a sub-branch of a clonally related tree. The data suggest remarkable clone size and rather comprehensive archiving of the memory population in the spleen. A feature of this study is that repeated identical sequences were collapsed, which might underestimate the apparent clone sizes. More subtly, the data suggest that many memory B cells must be relatively sessile until activated. The authors observed a trend of increased clone size and reduced diversity with age.

Surprisingly, the sequence data also showed shared clone members present in both MZ and non-MZ memory fractions. This would not be expected from a lineage model supported by the companion paper or some previous studies (Zhao et al., 2018) but agrees with the authors' previous finding that some MZBs carry mutations in *BCL6*, indicative of passage through a germinal center B cell stage (Seifert and Kuppers, 2009). Based on data showing that stimulation with the Notch ligand DLL1 upregulates CD21 in memory B cells, the authors suggest that the MZ environment, rather than longer-term imprinting, controls their phenotype. Further studies would be needed to confirm this view.

To reconcile all of these data with each other is difficult. Possible, though still improbable, explanations might be invoked. As pre–B cells with identical H-chain variable exons proliferate before giving rise to immature B cells, they might seed both follicular and MZ linages generating parallel populations, possibly with distinct light chains, that subsequently undergo similar initial mutations, depending upon activation-induced cytidine deaminase hot spots. Such a phenomenon may also explain the apparently large clone sizes and odd branching behavior of the clonal trees. Another possibility is that naive follicular B cells of a clone can convert to an MZ type under homeostatic conditions before antigen encounter (Kraal and Mebius, 2006). Clearly, we are not out of the woods yet.

References

Allman, D., and S. Pillai. 2008. *Curr. Opin. Immunol.* https://doi.org/10.1016/j.coi.2008.03.014

Cinamon, G., et al. 2004. *Nat. Immunol.* https://doi.org/10.1038/ni1083

Cinamon, G., et al. 2008. *Nat. Immunol.* https://doi.org/10.1038/ni1542

Gavin, A.L., et al. 2006. *Science.* https://doi.org/10.1126/science.1135299

Hayakawa, K., et al. 1984. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.81.8.2494

Herzenberg, L.A., and L.A. Herzenberg. 1989. *Cell.* https://doi.org/10.1016/0092-8674(89)90748-4

Kibler, A., et al. 2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20201952

Kraal, G., and R. Mebius. 2006. *Int. Rev. Cytol.* https://doi.org/10.1016/S0074-7696(06)50005-1

Linton, P.J., et al. 1989. *Cell.* https://doi.org/10.1016/0092-8674(89)90761-7

Seifert, M., and R. Kuppers. 2009. *J. Exp. Med.* https://doi.org/10.1084/jem.20091087

Spencer, J., et al. 1985. *Clin. Exp. Immunol.* 62: 607–612.

Tull, T.J., et al. 2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20202001

Vossenkämper, A., et al. 2013. *J. Exp. Med.* https://doi.org/10.1084/jem.20122465

Weill, J.C., et al. 2009. *Annu. Rev. Immunol.* https://doi.org/10.1146/annurev.immunol.021908.132607

Weller, S., et al. 2012. *Blood.* https://doi.org/10.1182/blood-2012-07-440776

Zhao, Y., et al. 2018. *Nat. Commun.* https://doi.org/10.1038/s41467-018-06089-1