

While this study is a clear step forward in our understanding of hemogenic endothelium induction, many questions remain. *ncor2* is expressed throughout the aorta and controls expression of arterial markers throughout the vessel, yet loss of *ncor2* only affects hemogenic endothelial formation. What other factors restrict the function of *ncor2* within the presumptive hemogenic endothelial cells? Does *ncor2* regulate expression of *c-fos*, *vegfd*, or Notch targets directly by binding to cell-type specific regulatory elements within these genes? How does *ncor2*, *c-fos*, or *vegfd* alter Notch signaling? *vegfd* is a prolymphangiogenic molecule, so could the effects observed with *vegfd* overexpression result from changes to lymphatic vessel formation? Are lymphatic vessel formation and HSPC emergence coordinated? Although lymphatic endothelial cells are derived from venous endothelial cells<sup>6</sup> and not arterial cells, there is evidence that lymphangiogenic factors might have a supportive role for hematopoietic progenitors during development.<sup>7</sup> Could these endothelial fate changes alter the HSPC niche? The work from Wei et al provides novel insight into the delicate balance of endothelial and hematopoietic fate choices during development, offering new directions in the quest to generate functional blood stem cells in vitro for transplantation therapies.

*Conflict-of-interest disclosure:* The author declares no competing financial interests. ■

## REFERENCES

- Wei Y, Ma D, Gao Y, Zhang C, Wang L, Liu F. *Ncor2* is required for hematopoietic stem cell emergence by inhibiting Fos signaling in zebrafish. *Blood*. 2014; 124(10):1578-1585.
- Pereira CF, Chang B, Qiu J, et al. Induction of a hemogenic program in mouse fibroblasts. *Cell Stem Cell*. 2013;13(2):205-218.
- Sandler VM, Lis R, Liu Y, et al. Reprogramming human endothelial cells to haematopoietic cells requires vascular induction. *Nature*. 2014;511(7509):312-318.
- Richard C, Drevon C, Canto PY, et al. Endothelium-mesenchymal interaction controls *runx1* expression and modulates the notch pathway to initiate aortic hematopoiesis. *Dev Cell*. 2013;24(6):600-611.
- Tang Y, Bai H, Urs S, Wang Z, Liaw L. Notch1 activation in embryonic VE-cadherin populations selectively blocks hematopoietic stem cell generation and fetal liver hematopoiesis. *Transgenic Res*. 2013;22(2):403-410.
- Yang Y, Oliver G. Development of the mammalian lymphatic vasculature. *J Clin Invest*. 2014;124(3):888-897.
- Zou Z, Enis DR, Bui H, et al. The secreted lymphangiogenic factor CCBE1 is essential for fetal liver erythropoiesis. *Blood*. 2013;121(16):3228-3236.

Comment on Neven et al, page 1597

# Marginal zone B-cell dysfunction in ALPS

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In this issue of *Blood*, Neven et al report that patients with autoimmune lymphoproliferative syndrome (ALPS) have poor production of anti-polysaccharide immunoglobulin (Ig) M antibodies and an elevated risk of pneumococcal sepsis and demonstrate that this is caused by infiltration and disorganization of the splenic marginal zone by the prototypical double-negative T cells.<sup>1</sup>

**A**LPS is caused by genetic defects that prevent appropriate lymphocyte cell death.<sup>2,3</sup> Most patients harbor mutations in the FAS (*TNFRSF6*) death receptor gene and present with the predictable consequences of abnormal lymphocyte removal: chronic splenomegaly, lymphadenopathy, autoimmunity, and increased risk of lymphomas.<sup>4,5</sup>

Although under normal conditions these patients can handle perfectly well infections by viral, fungal, and bacterial agents, they seem to be exceedingly sensitive to spleen removal.

Sepsis by encapsulated bacteria is a well-known complication after splenectomy or during functional or congenital asplenia, but splenectomized ALPS patients have even higher complication rates compared with these populations.<sup>1,6,7</sup> In fact, the rate of invasive bacterial infections after splenectomy in ALPS reported here and in a recent publication by the National Institutes of Health ALPS group was as high as 30% and 50%, respectively.<sup>1,6</sup> Currently, postsplenectomy sepsis is the main cause of death in ALPS and not lymphoma development.<sup>5,6</sup>

In their article, Neven et al provide a plausible explanation for these findings and incriminate a cell population typically seen in ALPS: the TCR $\alpha\beta^+$ CD4 $^-$ CD8 $^-$  T cells, also termed double-negative T cells (DN-Ts). They report that DN-Ts infiltrate and disorganize the splenic marginal zone (MZ) during periods of disease activity, leading to abnormal MZ B-cell function.

The splenic MZ is a specialized area at the interface between the circulation and the immune system, thought to be a prime site for the generation of first-line low-avidity IgM antibodies against blood-borne pathogens.<sup>8</sup> These antibodies are produced by local

MZ B cells, which in humans have a IgM<sup>hi</sup>IgD<sup>low</sup>CD1c<sup>+</sup>CD21<sup>hi</sup>CD27<sup>+</sup> phenotype and seem to be particularly important for mounting T-cell independent anti-polysaccharide antigen responses.<sup>8</sup> However, for their proper function, MZ B cells have to be correctly placed within the MZ, as antigens entering the spleen through the perifollicular zone are trapped by neutrophil extracellular trap-like structures emanating from unusual B-cell helper neutrophils inside the MZ.<sup>8,9</sup> The MZ B cells are then exposed to the trapped antigen and initiate antibody production locally, mostly IgM but also IgG or IgA.<sup>9</sup>

Neven et al demonstrate that the spleen MZ in ALPS patients with active disease is packed with DN-Ts, resulting in a paucity of MZ B cells in situ and in the peripheral blood. They go 1 step further by demonstrating that DN-Ts seem to be attracted to and retained within the MZ by the interaction between their  $\alpha 4 \beta 7$  integrin and a thick layer of MAdCAM-1 expressed by MZ stromal cells. This disruption of the normal MZ B-cell responses seems to result in a mild B-cell immunodeficiency that is aggravated by the removal of the spleen. These data could also explain findings of low numbers of circulating memory and MZ-like B cells and reduced serum IgM levels occasionally seen in ALPS patients with active disease.<sup>1</sup>

Although not explored here, it is also plausible that the susceptibility of ALPS patients to pneumococcal sepsis after splenectomy may be further enhanced by DN-T infiltration into additional functional reserves of MZ B cells in humans, such as

lymph nodes, tonsils, and intestinal Peyer's patches.<sup>8</sup>

In addition to shedding light into the pathogenesis of the B-cell defects in ALPS, the data from Neven et al further strengthen the arguments against the removal of the spleen for the treatment of the autoimmune cytopenias seen in this disorder. Alternatives such as mycophenolate mofetil and sirolimus have recently been used with great success for disease control and are viable alternatives to the deadly splenectomy.<sup>10</sup>

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

## REFERENCES

1. Neven B, Bruneau J, Stolzenberg M-C, et al. Defective anti-polysaccharide response and splenic marginal zone disorganization in ALPS patients. *Blood*. 2014;124(10):1597-1609.
2. Bidère N, Su HC, Lenardo MJ. Genetic disorders of programmed cell death in the immune system. *Annu Rev Immunol*. 2006;24:321-352.
3. Oliveira JB, Blessing JJ, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune

lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood*. 2010;116(14):e35-e40.

4. Fisher GH, Rosenberg FJ, Straus SE, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell*. 1995;81(6):935-946.

5. Rieux-Laucat F, Le Deist F, Hivroz C, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science*. 1995;268(5215):1347-1349.

6. Price S, Shaw PA, Seitz A, et al. Natural history of autoimmune lymphoproliferative syndrome associated with FAS gene mutations. *Blood*. 2014;123(13):1989-1999.

7. Neven B, Magerus-Chatinet A, Florin B, et al. A survey of 90 patients with autoimmune lymphoproliferative syndrome related to TNFRSF6 mutation. *Blood*. 2011;118(18):4798-4807.

8. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol*. 2013;13(2):118-132.

9. Puga I, Cols M, Barra CM, et al. B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat Immunol*. 2012;13(2):170-180.

10. Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. *Blood*. 2011;118(22):5741-5751.

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## ● ● ● IMMUNOBIOLOGY

Comment on Yang et al, page 1610

# B-cell development: COX-1 joins the game

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In this issue of *Blood*, Yang et al contribute to fill in the gap of our understanding of cyclooxygenases (COXs) in adaptive immunity by identifying COX-1 as a central player in B-cell development.<sup>1</sup>

**C**OXs, which catalyze the rate-limiting step in the biosynthesis of prostaglandins (PGs) and thromboxanes (TXs), are among the most popular molecules in the biomedical literature, with close to 50 000 references in PubMed since 1975, when the biological activities of these lipids in inflammation and coagulation were first identified. The seminal discovery that COX exists as 2 functionally different isoforms, COX-1 and COX-2, implicated in tissue homeostasis and inflammation, respectively, provided an explanation to the adverse side effects of aspirin on the gastric mucosa, setting the foundations for the development of nonsteroidal anti-inflammatory drugs selectively targeting

COX-2.<sup>2</sup> This finding, however, faced the scientific community with the difficult challenge of elucidating the mechanisms by which COX-1 and COX-2 play different roles using the same toolbox of lipid mediators, which is confounded by accumulating evidence that the homeostatic vs disease-related function of the 2 enzymes is not as black and white as initially inferred from the effects elicited by their pharmacological blockade.<sup>2</sup> Moreover, the widespread expression of COX-1 poses a limit to a full understanding of the growing array of biological functions subserved by this enzyme. The report by Yang et al brings us a step closer to this important objective by implicating COX-1 in the pathway that

regulates B-cell development in the bone marrow (BM), on which the ability of the organism to raise an adaptive immune response to pathogens crucially depends.

Although PGs have long been known to suppress T- and B-cell activation *in vitro*,<sup>3</sup> the role of COX-1 in lymphocyte development, activation, and differentiation has been to date largely limited to the T-cell compartment. COX-1 has been shown to participate in thymocyte development, promoting the prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-dependent transition from the double negative (CD4<sup>-</sup>CD8<sup>-</sup>) to the double positive (CD4<sup>+</sup>CD8<sup>+</sup>) stage.<sup>4</sup> At nonimmunosuppressive concentrations, PGE<sub>2</sub> also modulates the differentiation of CD4<sup>+</sup> T cells in the periphery, impacting on the T-helper (Th)1/Th2 balance and promoting their polarization to Th17 effectors.<sup>3</sup> The relevance of these activities to diseases such as allergic asthma and inflammatory bowel disease has been established with mice lacking the main T-cell PGE<sub>2</sub> receptors EP2 and EP4.<sup>5,6</sup> As with T cells, PGE<sub>2</sub> affects peripheral B-cell differentiation, promoting their maturation to immunoglobulin (Ig)E-secreting cells<sup>7</sup> and participating in interleukin (IL)-21-dependent B-cell death during germinal center selection.<sup>8</sup> In a recent report, the roles of COX-1 and COX-2 in the humoral immune response have been addressed *in vivo* in a model of infection with the Lyme disease pathogen *Borrelia burgdorferi*.<sup>9</sup> This study confirmed the implication of COX-1 in the control of class switching, as assessed by the lack of *Borrelia*-specific IgG in infected COX-1<sup>-/-</sup> (but not COX-2<sup>-/-</sup>) mice, which correlated with defective germinal center formation and production of the cytokines IL-6 and IL-17. The report by Yang et al completes this picture by investigating the function of COX-1 in developing B cells.

Starting with the observation that COX-1<sup>-/-</sup> mice have a reduction in the number of peripheral B cells compared with their wild-type counterparts, which does not result from increased apoptosis, the authors hypothesize an implication of COX-1 in B-cell development, demonstrating that COX-1 regulates the pro-B cell to pre-B cell transition. This was found to correlate with a peak in COX-1 expression in pro-B cells and to be independent of BM stromal cell-derived prostanooids. The maturation of pro-B to pre-B cells is controlled by the cytokine IL-7, which promotes expression of the master transcription factor