lymph nodes, tonsils, and intestinal Peyer's patches.[8]

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.[10]

*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, Bleesing 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, Florkin 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.

© 2014 by The American Society of Hematology

---

● ● ● ■ **IMMUNOBIOLOGY**

Comment on Yang et al, page 1610

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

**Cosima T. Baldari**   UNIVERSITY OF SIENA

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.[1]

COXs, 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.[2] 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.[2] 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,[3] 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 $E_2$ ($PGE_2$)–dependent transition from the double negative ($CD4^-CD8^-$) to the double positive ($CD4^+CD8^+$) stage.[4] At nonimmunosuppressive concentrations, $PGE_2$ also modulates the differentiation of $CD4^+$ T cells in the periphery, impacting on the T-helper (Th)1/Th2 balance and promoting their polarization to Th17 effectors.[3] 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 $PGE_2$ receptors EP2 and EP4.[5,6] As with T cells, $PGE_2$ affects peripheral B-cell differentiation, promoting their maturation to immunoglobulin (Ig)E-secreting cells[7] and participating in interleukin (IL)-21–dependent B-cell death during germinal center selection.[8] 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*.[9] 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 $COX-1^{-/-}$ (but not $COX-2^{-/-}$) 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 $COX-1^{-/-}$ 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 prostanoids. The maturation of pro-B to pre-B cells is controlled by the cytokine IL-7, which promotes expression of the master transcription factor
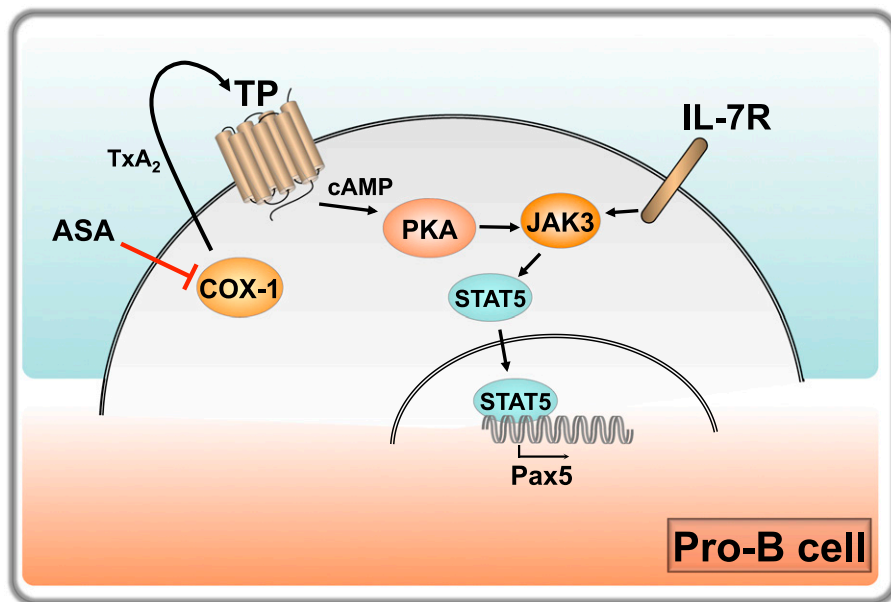
Regulation of B-cell development by COX-1. IL-7 receptor engagement on pro-B cells triggers JAK/STAT5 signaling, resulting in translocation of STAT5 to the nucleus and transcription of target genes. These include the master transcription factor Pax5, which drives the pro-B to pre-B cell transition. COX-1 is expressed at high levels in pro-B cells, where it catalyzes the production of $TxA_2$. Released $TxA_2$ triggers its receptor TP in a cell autonomous manner, promoting the accumulation of cAMP and activation of PKA, which enhances JAK3/STAT5 signaling and Pax5 expression, thereby cooperating with the IL-7 receptor in driving the pro-B to pre-B maturation step. COX-1 inhibition by aspirin (ASA) in healthy volunteers results in a reduction in $TxA_2$ production, which correlates with impaired JAK3/STAT5 signaling and Pax5 expression. Professional illustration by Laura Patrussi.

Pax5 through Janus kinase (JAK)3/signal transducer and activator of transcription (STAT)5 signaling. Based on the finding that COX-1$^{-/-}$ B cells have a defect in Pax5 expression, Yang et al address the potential modulation of IL-7–induced JAK3/STAT5 signaling by COX-1 in in vitro experiments with BM B cells, demonstrating that COX-1 participates in this pathway upstream of STAT5. To identify the underlying mechanism the authors examine the prostanoid profiles in COX-1$^{-/-}$ mice, identifying thromboxane $A_2$ ($TxA_2$) as the main prostanoid altered by COX-1 deficiency and providing evidence that $TxA_2$ and its receptor TP, which is abundantly expressed in developing B cells with a peak at the pro-B stage, participates in B-cell development downstream of COX-1. Finally, they show that $TxA_2$ regulates JAK/STAT5 signaling in B cells by promoting cyclic adenosine monophosphate (cAMP) accumulation and protein kinase A (PKA) activation. Of note, the authors show that healthy volunteers subjected to a low-dose aspirin regimen have a reduction in the number of circulating B cells correlating with decreased levels of urine $TxA_2$ metabolites (see figure).

The report by Yang et al provides important new insights into the IL-7–dependent pathway that regulates a key step in B-cell development. The authors not only implicate COX-1 in the pro-B to B-cell transition but establish a functional link between COX-1 and JAK/STAT5 signaling mediated by the $TxA_2$/TP axis, identifying cAMP as the second messenger responsible for this function. Taken together with the finding that COX-1 is required for the generation of an effective humoral response to infection,[9] these data identify COX-1 as a central player in the B-cell compartment. It is noteworthy that the function of COX-1 appears to be mediated by different prostanoids in BM ($TxA_2$) and peripheral ($PGE_2$) B cells. Because immune cells express both TP and the $PGE_2$ receptors EP2 and EP4,[10] these results underscore the importance of a lipidomic analysis of the prostanoids to which these cells are physiologically exposed to establish unequivocally which prostanoid is responsible for the specific biological end point. Furthermore, this report shows that, although COX-1 expression is indeed constitutive, it is also dynamic, such that the levels can be substantially different, as exemplified by pro-B and pre-B cells. This must be kept in mind when addressing the function of COX-1. Finally, although the results obtained on healthy volunteers subjected to a low-dose aspirin regimen are limited to a very small number of individuals, they have profound implications for the B-cell response of individuals undergoing preventive antithrombotic therapy. It will be interesting to see whether the reduction in peripheral B cells documented in this report will be confirmed in a larger cohort.

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

## REFERENCES

1. Yang Q, Shi M, Shen Y, et al. COX-1 derived thromboxane $A_2$ plays an essential role in early B-cell development via regulation of JAK/STAT5 signaling in mouse. *Blood*. 2014;124(10):1610-1621.

2. Rossi Paccani S, Boncristiano M, Baldari CT. Molecular mechanisms underlying suppression of lymphocyte responses by nonsteroidal antiinflammatory drugs. *Cell Mol Life Sci*. 2003;60(6):1071-1083.

3. Kalinski P. Regulation of immune responses by prostaglandin E2. *J Immunol*. 2012;188(1):21-28.

4. Rocca B, Spain LM, Puré E, Langenbach R, Patrono C, FitzGerald GA. Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. *J Clin Invest*. 1999;103(10):1469-1477.

5. Kabashima K, Saji T, Murata T, et al. The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest*. 2002;109(7):883-893.

6. Zasłona Z, Okunishi K, Bourdonnay E, et al. Prostaglandin $E_2$ suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells. *J Allergy Clin Immunol*. 2014;133(2):379-387.

7. Fedyk ER, Phipps RP. Prostaglandin E2 receptors of the EP2 and EP4 subtypes regulate activation and differentiation of mouse B lymphocytes to IgE-secreting cells. *Proc Natl Acad Sci USA*. 1996;93(20):10978-10983.

8. Magari M, Nishikawa Y, Fujii Y, et al. IL-21-dependent B cell death driven by prostaglandin E2, a product secreted from follicular dendritic cells. *J Immunol*. 2011;187(8):4210-4218.

9. Blaho VA, Buczynski MW, Dennis EA, Brown CR. Cyclooxygenase-1 orchestrates germinal center formation and antibody class-switch via regulation of IL-17. *J Immunol*. 2009;183(9):5644-5653.

10. Hirata T, Narumiya S. Prostanoids as regulators of innate and adaptive immunity. *Adv Immunol*. 2012;116:143-174.

© 2014 by The American Society of Hematology