Check for updates

understood. Similarly, the application of genomic technologies for identification of novel, functionally-important platelet genes and proteins remains a high priority.[2]

Raslova and colleagues describe cellular mRNA profiling to specifically dissect genetic changes occurring during an in vitro model of megakaryocyte differentiation and ploidization. Cells were flow-sorted by modal ploidy, and gene changes were compared between 2 cellular subsets: aggregate 2N+4N MKs versus aggregate 8N+16N MKs. Of interest, transcript changes were limited to approximately 350 genes across all the subsets, 106 of which were consistently down-regulated and 248 of which were consistently up-regulated between the 2 groups. Further analysis highlighted additional differences between the up-regulated and down-regulated subsets; specifically, members of the latter subset not infrequently (24/105) corresponded to genes involved in DNA replication (arrest) and recombination repair, while a majority of the former corresponded to genes important to platelet biogenesis, viability, and function (ie, actin and microtubule cytoskeleton, glycoproteins, and signaling/transport proteins). It is important to note, however, that when the gene subsets are carefully analyzed by gene ontology functional classification, considerable overlap exists between these 2 groups, confounding detailed interpretations. Nonetheless, the data do support a role of ploidization in modulating gene expression, although a direct, regulatory role in platelet biogenesis remains speculative.

The study by Macaulay and colleagues adapts a nearly-identical in vitro strategy of MK differentiation, coupled with a bioinformatic strategy to specifically identify novel transmembrane domain–containing MK receptor proteins. An initial gene list of 151 transcripts was assembled using paired, comparative expression profiling with CD34$^+$-differentiated erythroblasts, and the list further pared using strict criteria to identify putative, functionally-relevant platelet proteins. Five of 8 highly-selected genes were characterized by transcript and protein expression studies, 3 of which were shown to be platelet restricted *(G6b, G6f,* and *LRRC32),* and another of which *(SUCNR1)* encoded the G protein–coupled succinate receptor.[3] More detailed functional studies established that succinate (a key component of the citric acid cycle) exhibited costimulatory effects on platelet aggregation induced by various platelet agonists (adenosine diphosphate, thrombin receptor activating peptide, and a glycoprotein VI–specific collagen peptide). The latter functional data are especially insightful in that they identify a novel, cocoupling signal transduction pathway in platelets, while opening new avenues of research linked to platelet hyperreactivity.[4]

While both study designs overlap in their initial in vitro differentiation strategies using megakaryocytes, the conclusions, future directions, and ability to compare data sets are distinct and limited. One restriction inherent in cross-experimental microarray data-sharing is the disparate platforms used among investigators, well exemplified in these studies, that used nonoverlapping oligonucleotide or cDNA probe sets for their analyses.[5,6] This limitation does not minimize results, although it emphasizes the importance of validation strategies of transcript differences initially identified by microarray. The identification of a costimulatory succinate receptor on platelets represents a discrete end product of integrated MK transcriptomic studies, coupled with a concrete hypothesis and sophisticated experimental design to characterize novel functional receptors. Likewise, the application of microarray technology to dissect MK ploidization is highly novel, and although the results are less focused in scope, they are likely to yield broader implications in the foreseeable future. Finally, such unique data sets open up exciting opportunities for sophisticated data mining likely to provide unexpected insights into molecular mechanisms of MK and platelet function.[7]

The author declares no competing financial interests. ■

REFERENCES

1. Wright JH. The origin and nature of blood platelets. Boston Med Surg J. 1906;154:643-645.
2. Gnatenko DV, Dunn JJ, McCorkle SR, Weissmann D, Perrotta PL, Bahou WF. Transcript profiling of human platelets using microarray and serial analysis of gene expression. Blood. 2003;101:2285-2293.
3. He W, Miao FJ, Lin DC, et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature. 2004;429:188-193.
4. Boos CJ, Lip GY. Platelet activation and cardiovascular outcomes in acute coronary syndromes. J Thromb Haemost. 2006;4:2542-2543.
5. Brazma A, Hingamp P, Quackenbush J, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet. 2001;29:365-371.
6. Gnatenko DV, Perrotta PL, Bahou WF. Proteomic approaches to dissect platelet function: half the story. Blood. 2006;108:3983-3991.
7. Denis MM, Tolley ND, Bunting M, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. Cell. 2005;122:379-391.

IMMUNOBIOLOGY

Comment on León-Ponte et al, page 3139

Serotonin: a real blast for T cells

John Gordon and Nicholas M. Barnes UNIVERSITY OF BIRMINGHAM MEDICAL SCHOOL

Mouse T lymphocytes unexpectedly produce the classic neurotransmitter serotonin, which—upon binding the constitutively expressed 5-HT$_7$ G$_s$-coupled receptor-subtype—signals ERK1/2 phosphorylation and NFκB activation to boost their stimulation.

Serotonin/5-hydroxytryptamine (5-HT), often perceived as the brain's "happy chemical," is—increasingly—assuming a prominent role in immune regulation. In fact, this "neurotransmitter" is primarily a product of the periphery, with gut enterochromaffin cells the principal factories. Intestinal lymphocytes could certainly be exposed directly to their output, though platelets are typically proffered as the source of the monoamine within the immune system: these 5-HT–loaded reservoirs delivering their potent cargo at sights of inflammation and immunologic reactivity.[1,2]

In this issue of *Blood,* León-Ponte and colleagues not only consolidate serotonin's importance to T-cell activation but also tender a paradigm whereby the monoamine is provided by the immune cell itself. Critical to any outcome from 5-HT exposure—whether the source be autocrine or paracrine—is a capacity for target cells to sense the monoamine. This could be via the serotonin transporter (SERT) or one or more of 14 receptor subtypes (13 in

Downloaded from http://ashpublications.org/blood/article-pdf/109/8/3130/1291111/zh800807003130.pdf by guest on 16 June 2021

Receptor subtype	Signal	Antagonist[1]	T cells, mouse*		Splenocytes, rat[3]		DCs, human[4]		Monocytes, human[5]	
			R	A	R	A	I	M	R	A
5-HT_{1A}	$G_{i/o}$	WAY100635	–	–	–	–	–	–	–	–
5-HT_{1B}	$G_{i/o}$	SB236057	+	–§	+	+	+	±	–	–
5-HT_{1D}	$G_{i/o}$	BRL15572	–	–	–	–	–	–	–	–
5-HT_{1E}	$G_{i/o}$	None	n/a¶	n/a¶	n/a¶	n/a¶	+	±	+	+
5-HT_{1F}	$G_{i/o}$	None	–	–	+	+	–	–	–	–
5-HT_{2A}	$G_{q/11}$	MDL100907	–	+	+	+	+	+	+	+
5-HT_{2B}	$G_{q/11}$	RS127445	–	–	+	+	+	±	–	–
5-HT_{2C}	$G_{q/11}$	SB242084	–	–	–	–	–	–	–	–
5-HT_{3}	Ion chan.	Ondansetron	–	–	–	+	+	+	+	+
5-HT_{4}	G_{S}	SB204070	–	–	–	–	±	+	+	+
5-ht_{5A}	Multiple?	SB699551A	–	–	–	–	–	–	–	–
5-ht_{5B}	Unknown	None	–	–	–	–	n/a#	n/a#	n/a#	n/a#
5-HT_{6}	G_{S}	SB271046	–	–	–	–	–	–	–	–
5-HT_{7}	G_{S}	SB656104	+	+	+	+	±	+	+	+

Reported expression of 5-HT receptor transcripts in immune cells. Synthesis of findings from studies by *León-Ponte et al in this issue of *Blood* and from Stefulj et al,[3] Idzko et al,[4] and Durk et al[5] highlighting the repertoire of 5-HT receptor subtype of mRNA expressed in immune cells. R indicates resting; A, activated; I, immature; M, mature; Ion chan., ligand-gated ion channel; –, negative; +, strong signal; and ±, weak signal. §5-HT1B protein increased on activation. ¶Absent from mouse and rat. #Truncated transcript in human. Illustration by Frank Forney.

rodents).[1,2] Using reverse transcription–polymerase chain reaction (RT-PCR), the authors ruled out SERT and all but 3 receptor subtypes: only 5-HT_{1B}, 5-HT_{2A}, and 5-HT_7 receptors remaining to deliver the 5-HT hit. While not excluding contributions from 5-HT_{1B} and 5-HT_{2A} receptors, the authors homed in on 5-HT_7 as the major player: this 7-transmembrane domain G protein–coupled receptor (GPCR) assisted T-cell stimulation by promoting ERK1/2 phosphorylation and canonical NFκB activation. Not only were the authors able to attenuate 5-HT–dependent change with a selective 5-HT_7 receptor antagonist but, by using a 5-HT_7 receptor agonist, were able to reverse otherwise defective T-cell stimulation arising as a consequence of having blocked tryptophan hydroxylase (TPH), the rate-limiting enzyme in the conversion of tryptophan to 5-HT. Tryptophan is also the catabolic substrate for indolamine-2,3-dioxygenase (IDO), and the relative abundance/activity of TPH, IDO, and, additionally, metabolizing monoamine oxidases (MAOs) could determine 5-HT output from immune cells.

While the present study marks a significant advance, the authors themselves address seeming inconsistencies between this and isolated reports on the relative importance of individual 5-HT receptor subtypes to T-cell function, and rightly posit additional questions. Issues include strain and species differences, tissue and subset heterogeneity, and methodological concerns.

Few studies have attempted such an extensive survey of an immune cell's 5-HT receptor repertoire as the one here. The table summarizes and compares the study's salient features with 3 others of merit: a pioneering study on rat splenocytes[3] and, from Idzko and colleagues, separate analyses of receptor subtype distribution on human dendritic cells (Idzko et al[4]) and monocytes (Durk et al[5]). Of the discordant and common themes, the near-universal expression of 5-HT_7 receptors—irrespective of cell type or animal species—is striking. A built-in knowledge of the pharmacology and signaling properties of 5-HT receptors (see table), driven by and gained largely through their contribution to CNS pathways, holds promise for the ready translation of 5-HT receptor therapeutics to immunology and hematology clinics once a more robust integration of their place in immune physiology—and pathology—has been secured.

The authors are directors and cofounders of Celentyx Ltd, a spinout company whose activities include the profiling of serotonergic compounds against immune cells. ■

REFERENCES

1. Meredith EJ, Chamba A, Holder MJ, et al. Close encounters of the monoamine kind: immune cells betray their nervous disposition. Immunology. 2005;115:289-295.

2. Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? Trends Immunol. 2003;24:438-443.

3. Stefulj J, Jernej B, Cicin-Sain L, et al. mRNA expression of serotonin receptors in cells of the immune tissues of the rat. Brain Behav Immun. 2000;14:219-224.

4. Idzko M, Panther E, Stratz C, et al. The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. J Immunol. 2004;172:6011-6019.

5. Durk T, Panther E, Muller T, et al. 5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different 5-HTR subtypes. Int Immunol. 2005;17:599-606.

● ● ● NEOPLASIA

Comment on Zhao et al, page 3432

The CBFB-MYH11 butterfly effect in hematopoiesis

Andre J. van Wijnen and Gary S. Stein UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

As the butterfly flaps its wings, so can a small latent change within hematopoietic cells have major effects on a pathological outcome such as leukemia.

The paper by Zhao and colleagues in this issue of *Blood* indicts a leukemia-related chimeric protein for selectively killing one cell type while sparing another to cause a cell type–specific disease.[1] The runt-related transcription factor 1 (RUNX1/AML1) and its cofactor core-binding factor subunit β (CBFβ) are essential regulators of hematopoiesis. Genetic

Downloaded from http://ashpublications.org/blood/article-pdf/109/8/3130/1291111/zh800807003130.pdf by guest on 16 June 2021