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LYMPHOID NEOPLASIA

Comment on Vicente-Dueñas et al, page 2003

A "gut feeling" about precursor B-ALL

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In this issue of *Blood*, Vicente-Dueñas et al[1] elegantly demonstrate the interplay of genetic predisposition, altered gut microbiome, and delayed infection in development of precursor B-cell acute lymphoblastic leukemia (pB-ALL) in *Pax5*$^{+/-}$ mice. Many childhood leukemia-initiating events, such as chromosomal translocations, originate in utero (reviewed in Greaves[2]), and studies so far suggest that these "first hits" occur at a far higher frequency than the prevalence of childhood leukemia itself.[3,4] Why only a fraction of children born with such preleukemic "hits" develop full-blown leukemia is a puzzle. Although the causes are likely to be multifactorial,[5] 1 theory put forward has been that of a disordered immune response either upon delayed exposure to infections in childhood or to altered natural microbiota (reviewed in Greaves[5]). Vicente-Dueñas et al show that the gut microbiome is distinct in genetically predisposed mice (*Pax5* heterozygosity or *ETV6-RUNX1* fusion), and that alteration of this microbiome can trigger pB-ALL in *Pax5*$^{+/-}$ mice even in the absence of infection (see figure).

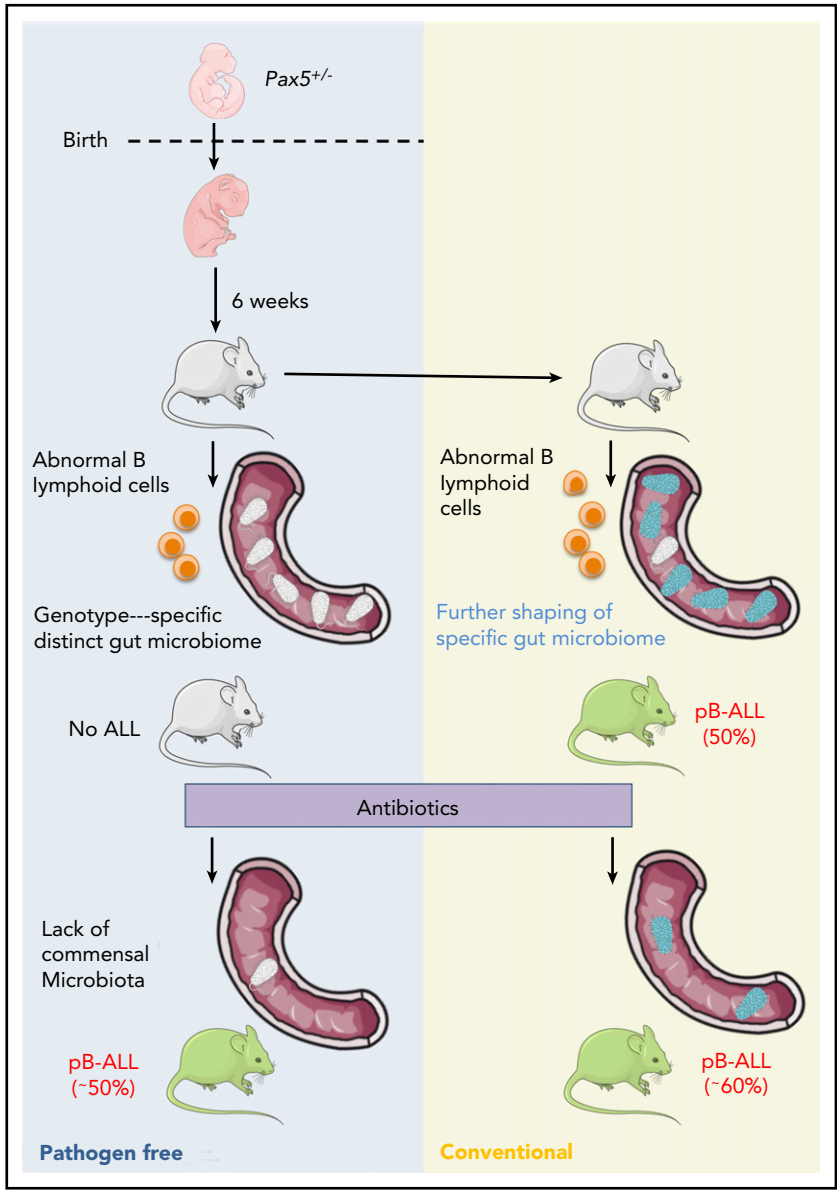
Previous reports by the authors[6] suggested that exposure to infections is a causal factor in the development of pB-ALL in a mouse model of *Pax5*-inherited susceptibility. In this new study, the authors have investigated the role of the gut microbiome in the development of acute lymphoblastic leukemia (ALL) in genetically predisposed mice in specific pathogen-free (SPF) and conventional animal housing facilities, with or without antibiotic treatment. In addition to confirming their previous results that *Pax5*$^{+/-}$ mice moved from SPF to CF conditions develop pB-ALL, the authors demonstrate that the gut microbiome of both wild-type (WT) and *Pax5*$^{+/-}$ mice changed dramatically over time after transfer from SPF to CF facilities. However, the gut microbiome of *Pax5*$^{+/-}$ mice remained different from WT controls in both conditions. The authors were able to use machine learning to predict the genotype of WT and *Pax5*$^{+/-}$ mice from the microbiome data. These differences in the gut microbiome were also seen in mice with *ETV6-RUNX1* fusion, although the small number of mice studied meant that no conclusions could be drawn about pB-ALL development in CF. Hematopoietic cells transplanted from *Pax5*$^{+/-}$ mice to WT mice altered the gut microbiome of recipient mice, suggesting that preleukemic cells could play a specific role in shaping the gut microbiome. In addition, *Pax5*$^{+/-}$ mice had a genotype-specific metabolomics profile that correlated with the altered gut microbiome, when compared with WT controls.

Depleting the gut microbiome by antibiotic treatment did not prevent infection-driven pB-ALL development in *Pax5*$^{+/-}$ mice in CF, but remarkably, this intervention promoted pB-ALL development in an SPF environment, even in the absence of infectious stimuli. This reinforces the fact that an intact microbiome is crucial in preventing leukemic transformation in predisposed mice, with or without exposure to infections.

These results bring us 1 step closer to understanding the potential role of the microbiome and infections in the transformation of preleukemic clones in early childhood. The authors of this study[7] and others (Valeria Cazzaniga, Anthony Ford, Mel Greaves, written personal communication, August 2020) have been using *ETV6–RUNX1*$^{+}$ mouse models to understand the implications of delayed infections in childhood, and how an abnormal immune response to these might provide the "second hit" for leukemic transformation. However, this current study has demonstrated an increased risk of leukemia even in the absence of infections, by merely depleting the microbiome in genetically predisposed mice. This has implications for antibiotic usage in early life[8] especially in those infants with a genetic predisposition to pB-ALL (these, including germline PAX5 mutations,[9] are only a very rare subgroup in childhood ALL), or more importantly those born with a preleukemic "first hit."

Although we must bear in mind that the defect in B-cell development in a *Pax5*-deficient mouse model may in itself drive the changes evident in the microbiome rather than *Pax5*$^{+/-}$ being a preleukemic condition, similar changes seen in *ETV6–RUNX1*$^{+}$ mice and in WT mice transplanted with hematopoietic stem cells from *Pax5*$^{+/-}$ mice suggests that an altered microbiome may be present in several preleukemic states. This warrants further studies to investigate the presence of an altered microbiome, and the development of leukemia in other preleukemic mouse models.

The results of this study have exciting epidemiological and public health implications. Is the gut microbiome altered in all children with a preleukemic clone or only in those that will transform to leukemia? If changes in the gut microbiome



The interplay of genetic predisposition, altered gut microbiome, and delayed infection in development of pB-ALL. Schematic representation of results of study by Vicente-Dueñas et al: *Pax5*$^{+/-}$ mice with abnormal B lymphoid development have a genotype-specific gut microbiome in a pathogen-free environment. The microbiome gets further altered when the mice are transferred to conventional facility (CF) housing before the development of pB-ALL. Depletion of the genotype-specific microbiome with antibiotics does not prevent pB-ALL in CF but leads to development of pB-ALL in pathogen-free facilities.

can be detected before the development of overt leukemia, could it potentially be used as a biomarker to identify those children with preleukemic "first hits" at high risk of developing leukemia? The gut microbiome in babies and children is likely to be quite different from that of mice housed in controlled biomedical facilities.[10] The relevance of this study therefore needs to be confirmed in a systematic manner in a human setting first and could involve, for example, prospective screening of newborns to first identify those at risk of leukemia, and then studying their gut microbiome (although such population-based screening comes with its own ethical implications). Perhaps in the future, the human microbiome could even be used to identify children predisposed to ALL and/or become a modifiable therapeutic target for preventing childhood leukemia.

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

REFERENCES

1. Vicente-Dueñas C, Janssen S, Oldenburg M, et al. An intact gut microbiome protects genetically predisposed mice against leukemia. *Blood*. 2020;136(18):2003-2017.
2. Greaves M. In utero origins of childhood leukaemia. *Early Hum Dev*. 2005;81(1):123-129.
3. Mori H, Colman SM, Xiao Z, et al. Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc Natl Acad Sci USA*. 2002;99(12):8242-8247.
4. Schäfer D, Olsen M, Lähnemann D, et al. Five percent of healthy newborns have an *ETV6-RUNX1* fusion as revealed by DNA-based GIPFEL screening. *Blood*. 2018;131(7):821-826.
5. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia [published correction appears in Nat Rev Cancer. 2018;18(8):526]. *Nat Rev Cancer*. 2018;18(8):471-484.
6. Martín-Lorenzo A, Hauer J, Vicente-Dueñas C, et al. Infection exposure is a causal factor in B-cell precursor acute lymphoblastic leukemia as a result of Pax5-inherited susceptibility. *Cancer Discov*. 2015;5(12):1328-1343.
7. Rodríguez-Hernández G, Hauer J, Martín-Lorenzo A, et al. Infection exposure promotes *ETV6-RUNX1* precursor B-cell leukemia via impaired H3K4 demethylases. *Cancer Res*. 2017;77(16):4365-4377.
8. Gradel KO, Kaerlev L. Antibiotic use from conception to diagnosis of child leukaemia as compared to the background population: a nested case-control study. *Pediatr Blood Cancer*. 2015;62(7):1155-1161.
9. Shah S, Schrader KA, Waanders E, et al. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. *Nat Genet*. 2013;45(10):1226-1231.
10. Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*. 2006;118(2):511-521.

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