Worms on stage

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The eloquent quote by pencillin-discoverer Alexander Fleming stating that '*the unprepapred mind cannot see the outstretched hand of opportunity*' resonates with a paper published in the current issue of *Haematologica* in which the authors clearly saw the opportunity to report on their serendipitous discovery stemming from an initially frustrating lack of reproducibility.[1] In 2013, Scott Kogan's group assessed the interplay between ETV6::Runx1 (formerly TEL-AML1) inducible transgene expressed in hematopoietic cells (*E6R1*$^{+}$) and Cdkn2a loss (whole body knockout, *Cdkn2a*$^{-/-}$) in the context of B-cell lymphoblastic lymphoma/leukemia development. A clear oncogenic cooperation was observed between Cdkn2a null mice that expressed ETV6::Runx1 (*E6R1*$^{+}$ *Cdkn2a*$^{-/-}$) compared to *Cdkn2a*$^{-/-}$ controls, an effect that was further exacerbated by irradiation.[2] However, following the 2013 publication, attempts to recapitulate the main phenotype of the *E6R1*$^{+}$ *Cdkn2a*$^{-/-}$ mice (development of B-cell neoplasms faster and with a higher incidence compared to *Cdkn2a*$^{-/-}$ controls) failed. A review of the Specific-Pathogen-Free (SPF) facility records revealed that there was an outbreak of the pinworm *Aspicularis* during the time of the 2013 study. While such an infection is certainly not uncommon in animal facilities, what is commendable is that the authors then sought to systematically unroot the cause of the discrepancy in the reproducibility of their research and subsequently report on the new discovery that the pinworm infection was likely responsible for the initially observed differences from the 2013 publication. Surprisingly, after medicinal eradication of the pinworm outbreak, the differences in leukemia latency and incidence between the double hit *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ model and the single hit *Cdkn2a*$^{-/-}$ model disappeared. The authors concluded that pinworm infection was protective in the *Cdkn2a*$^{-/-}$ model but not in the *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ one.

Indeed, as described in the current issue of *Haematologica*,[1] when *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ and control *Cdkn2a*$^{-/-}$ mice were prospectively transferred at 4 weeks of age from the SPF facility to a conventional facility (CF) infected with *Aspicularis*, the disease phenotype was restored between *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ and control *Cdkn2a*$^{-/-}$ mice (*Cdkn2a*$^{-/-}$-pinworm *vs.* *E6R1*$^{+}$*Cdkn2a*$^{-/-}$-pinworm). As before in the 2013 publication, pinworm infection significantly delayed leukemia/lymphoma development in the single leukemogenic hit *Cdkn2a*$^{-/-}$ model compared to the two-hit *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ model (Figure 1A). Interestingly, when the authors combined their cohorts to assess genotype-environmental (*Cdkn2a*$^{-/-}$-SPF *vs.* *Cdkn2a*$^{-/-}$-pinworm, *E6R1*$^{+}$*Cdkn2a*$^{-/-}$-SPF *vs.* *E6R1*$^{+}$*Cdkn2a*$^{-/-}$-pinworm) comparisons, there were no lymphoma/leukemia-associated survival advantages in the pinworm-CF. Rather, the median latency in pinworm-infected *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ mice was slightly shorter (231 *vs.* 253 days) than in SPF-housed controls. It might be expected that the normally silent ETV6::Runx1 predisposition becomes detrimental when combined with another cancer-inducing genetic alternation and that the leukemogenic effects of this 'double hit' model were too potent to overcome even when combined with the "protective pinworm intervention". However, the lack of pinworm protective effects in the *Cdkn2a*$^{-/-}$-SPF *vs.* *Cdkn2a*$^{-/-}$-pinworm comparison is surprising. However, this cohort was aggregated from two different experimental settings. In one cohort (2009-2011), the mice were likely continuously infected with pinworm from the developmental, *in utero* stage to adulthood, while in the second cohort, mice were transferred 4 weeks after birth (when immune competence has already been largely established) into a pinworm-prevalent CF.

These intriguing observations raise several important questions about delayed infection in an untrained immune system and its contribution to the development of B-cell precursor acute lymphoblastic leukemia.[3,4] Infection of mice with other helminths has been shown to affect anti-viral and bacterial immunity and to protect mice from inflammation.[5] Therefore, this kind of bystander infection has not only experimental consequences but potential disease-specific translational relevance. Indeed, in adults, helminth infections have been retrospectively demonstrated to protect against several inflammatory disease states and to be prospectively effective as vaccines in inflammatory bowel diseases.[6] Immunologically, this has been linked to their induction of Th2-like regulatory responses. In children, helminth exposure has been proposed to have protective effects in unique isolated settings[7] and intestinal nematodes led to decreases in

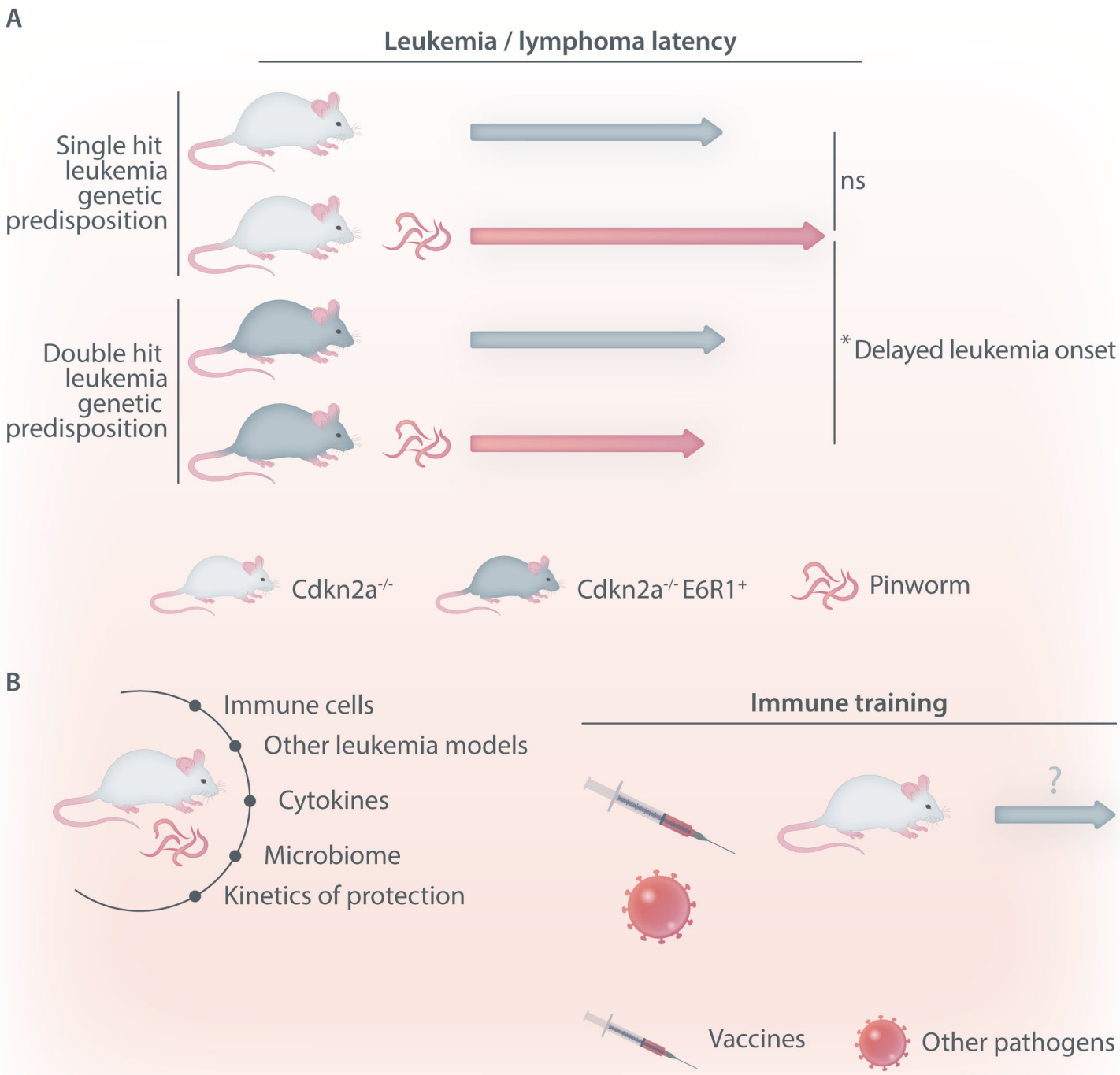

Figure 1. Summary of significant findings and future directions regarding pinworm infection and leukemia models. (A) Pinworm infection is protective against leukemia/lymphoma in the single hit *Cdkn2a*$^{-/-}$ model when compared to the double hit *E6R1*$^{+}$*Cdkn2a*$^{-/-}$ model. (B) Potential future avenues of investigation to further delineate the mechanisms governing protective effects of pinworm infection in leukemia models. ns: difference not statistically significant.

markers of intestinal inflammation in some studies.[8] However, due to the risk of heavier parasite burdens and other associated comorbidities, disease-specific preventative strategies in children will likely favor probiotic interventions rather than helminth infection by intention.

Given the strong disease penetrance of the above models, bulk differences could be directly relevant to the observed phenotype. Serial sample stool and blood collection in potential future investigations would enable a thorough characterization of multiplexed cytokine, microbiome and immunological profiles including immunophenotyping of key immune populations (T cells, monocytes, B cells, NK cells) for exhaustion/activation/immunosuppressive markers (Figure 1B). Another important question is whether Th2-like responses could contribute to early immune surveillance and eradication of the pre-leukemic clone.

Helminth infections induce central trained immunity responses,[9] which are known to delay tumor progression.[4,10] Therefore, the mechanisms governing trained immunity responses using the above leukemia predisposition models following infection with pinworms and other trained immunity inducers should be further explored. Taken together, the authors' serendipitous discovery provides support for further exploring the hypothesis that immune training influences the development of B-cell acute lymphoblastic leukemia.

Disclosures

No conflicts of interest to disclose.

Contributions

AB and AP contributed equally to this work.

References

1. Fitch BA, Situ J, Wiemels JL, Kogan SC, Zhou M. Impact of pinworm infection on the development of murine B-cell leukemia/lymphoma in the presence and absence of ETV6::RUNX1. Haematologica. 2023;108(12):3480-3484.
2. Li M, Jones L, Gaillard C, et al. Initially disadvantaged, TEL-AML1 cells expand and initiate leukemia in response to irradiation and cooperating mutations. Leukemia. 2013;27(7):1570-1573.
3. Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leukemia. 1988;2(2):120-125.
4. Hauer J, Fischer U, Borkhardt A. Toward prevention of childhood ALL by early-life immune training. Blood. 2021; 138(16):1412-1428.
5. Tao L, Reese TA. Making mouse models that reflect human immune responses. Trends Immunol. 2017;38(3):181-193.
6. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis therapy in Crohn's disease. Gut. 2005;54(1):87-90.
7. Soloski MJ, Poulain M, Pes GM. Does the trained immune system play an important role in the extreme longevity that is seen in the Sardinian blue zone? Front Aging. 2022;3:1069415.
8. Gildner TE, Schrock J, et al. Soil-transmitted helminth infection and intestinal inflammation among the Shuar of Amazonian Ecuador. Am J Phys Anthropol. 2019;170(1):65-74.
9. Cunningham KT, Finlay CM, Mills KHG. Helminth imprinting of hematopoietic stem cells sustains anti-inflammatory trained innate immunity that attenuates autoimmune disease. J Immunol. 2021;206(7):1618-1630.
10. Kalafati L, Kourtzelis I, Schulte-Schrepping J, et al. Innate immune training of granulopoiesis promotes anti-tumor activity. Cell. 2020;183(3):771-785.e12.