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Worms on stage

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The eloquent quote by penicillin-discoverer Alexander Fleming that ‘the unprepared mind cannot see the outstretched hand of opportunity’ resonates with the current issue of *Haematologica* where the authors clearly saw the opportunity to report on their serendipitous discovery stemming from an initial frustrating lack of reproducibility. In 2013, the group of Scott Kogan 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 express ETV6::Runx1 (*E6R1+Cdkn2a*−/−) compared to *Cdkn2a*−/− controls, an effect that was further exacerbated by irradiation. Following the 2013 publication, attempts to recapitulate the main phenotype of the *E6R1+Cdkn2a*−/− mice developing of B-cell neoplasms faster and with a higher incidence compared to the *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 the 2013 study. While the latter is certainly not uncommon in animal facilities, what is commendable is that the authors then sought to systematically unroot the cause of this discrepancy and subsequently report on the new discovery that the pinworm infection was likely responsible for the initially observed differences of 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 pinworm to be protective in *Cdkn2a*−/− but not in the *E6R1+Cdkn2a*−/− model.

Indeed, in the current issue of *Haematologica*, 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*−/− when 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−/− 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 post birth (when immune competence is already 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 BCP-ALL development. Infection of mice with other helminths has been shown to affect anti-viral and bacterial immunity and to protect mice from inflammation. 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 state and to be prospectively effective as vaccines in inflammatory bowel diseases. 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 and intestinal nematodes led to decreases in intestinal inflammation markers in some studies. However, due to the risk of heavier parasite burdens and other associated comorbidities, disease-specific preventative strategies will likely favour probiotic interventions rather than helminth infection by intention.

Given the high 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 (T cells, monocytes, B cells, NK cells) populations for exhaustion/activation/immunosuppressive markers (Figure 1B).
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\(^9\) and the latter is 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 impacts B-ALL development.

References:


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**Figure Legends**

**Figure 1:** Summary of significant findings and future directions. (A) Pinworm infection is protective against leukemia/lymphoma in the single hit Cdkn2a<sup>−/−</sup> model when compared to the double hit E6R1<sup>−/−</sup>Cdkn2a<sup>−/−</sup> model (B) Potential future avenues of investigation to further delineate the mechanisms governing pinworm protective effects in leukemia models.