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Reducing Animal Use in Hematopoiesis Research - Recent Advances and Future Challenges

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Hematology researchers are committed to replacing the use of laboratory animals with alternative methods, and where this is not possible, reducing the number of animals used, and refining care to minimise animal suffering (the 3Rs, https://nc3rs.org.uk). Reducing animal use addresses ethical concerns, the expectations of the public that fund research, as well as differences between hematopoiesis in humans versus the animals that we use to model them.

As blood is a liquid tissue, it can be acquired from human volunteers for research with minimal invasion. Donated human blood has been used extensively to make significant discoveries in coagulation, transfusion, and other fields of hematology. In contrast, the production of blood cells occurs in the complex and relatively inaccessible tissue of the bone marrow, and it has proven stubbornly difficult to recapitulate this process outside living organisms.

Much research on hematopoiesis has focussed on hematopoietic stem cells (HSCs), which can self-renew and differentiate into all blood cell lineages. The concept of HSCs was established by Till, McCulloch and colleagues based on a series of seminal experiments transplanting mouse bone marrow into irradiated mice, demonstrating that single cells produce myeloerythroid colonies in the spleen, which could subsequently give rise to more colonies. However, no in vitro conditions have been identified that permit long-term HSC self-renewal and multilineage differentiation, so HSCs are still functionally defined by their ability to reconstitute long-term multilineage hematopoiesis following transplantation into a myeloablated host animal. Even research using human hematopoietic cells requires transplantation into immunodeficient mouse hosts to definitively identify HSCs. Studying hematopoiesis and HSCs therefore continues to depend upon the use of animal models.

Recent developments in the *ex vivo* expansion of HSCs, and modelling of hematopoiesis

To reduce and replace animal use for research, other stem cell fields have taken advantage of advanced ex vivo culture systems that recapitulate many aspects of their native physiology, such as organoids and organs-on-chips (Sontheimer-Phelps et al. 2019 PMID: 30647431). 3D culture of pluripotent stem cells (PSCs) can even recapitulate multiple aspects of early embryogenesis, including gastrulation (Steventon et al. 2021 PMID: 34520764, van den Brink and van Oudenaarden 2021 PMID: 34304959, Shahbazi et al. 2019 PMID: 31171690)

Compared to these stem cell systems, HSCs have proven harder to maintain ex vivo. The first HSC culture systems that demonstrated ex vivo expansion of HSCs relied on the use of thrombopoietin-rich cytokine cocktails, variations of which are still used today (Yagi et al. 1999 PMID: 10393959). Other systems have utilised long-term co-culture with bone marrow niche components, including extracellular matrix components, mesenchymal stromal cells and endothelial cells (Butler et al. 2010 PMID: 20207228, Nakahara et al. 2019 PMID: 30988422). Recent optimization of HSC expansion has identified the replacement of bovine serum albumin with polyvinyl-alcohol (PVA) as a key factor, which allowed the longest and largest achieved expansion of murine HSCs to date (Wilkinson et al. 2019 PMID: 31142833). However, the lifespan of HSC maintenance in these ex vivo systems is limited -- HSCs have not yet been expanded ex vivo indefinitely like PSCs.

Other researchers aim to produce HSCs in vitro using a different approach: differentiating PSC cultures into HSCs. These techniques have been used to obtain patient-specific and disease-specific adult HSCs (Sugimura et al. 2017 PMID: 28514439, Doulatov and Papapetrou 2021 PMID: 33264225). However, the complexity, high cost and limited efficiency of PSC-to-HSC differentiation protocols has made them challenging to use for most research applications.

While efforts to expand HSCs ex vivo aim to reduce the use of animals by providing a steady supply of

HSCs, ex vivo modelling of the behaviour of HSCs in the bone marrow will require the development of another suite of techniques. It has been particularly challenging to model HSC differentiation into multiple myeloid and lymphoid lineages using ex vivo culture, although recent methods have begun to address some of these issues (Belluschi et al. 2018 PMID: 30291229). Differentiation media are usually formulated to induce HSCs to cycle and differentiate, and it has proven challenging to permit differentiation while a subset of HSCs concurrently retain their quiescence and dormancy – two of their essential properties in vivo. Researchers have recently shown that it is possible to maintain HSC dormancy in vitro for weeks (Kobayashi et al. 2019 PMID: 33111112, Oedekoven et al. 2021 PMID: 33961793). Balancing differentiation and dormancy within the same culture setting will be essential to faithfully recapitulate in vivo HSC behaviours. Ultimately, researching hematopoiesis outside of animal models will require ex vivo models that recapitulate the entire organ within a dish, together with cell migration into and out of the bone marrow tissue. While epithelial organoids and organs-on-a-chip have flourished in recent decades, developing ex vivo models of the complex bone marrow tissue remains challenging (Bourgine et al. 2018 PMID: 29499148, Sommerkamp et al. 2021 PMID: 33278488, Bai et al. 2019 PMID: 31591594).

Taking these innovations together, two things seem clear. First, there is extensive interest in determining the culture conditions to expand HSCs indefinitely ex vivo and obtain a renewable source that is less dependent on animal use. Second, to model hematopoiesis, methods need to take into consideration all bone marrow cell types and complex physico-chemical properties, creating a complex organ-on-a-chip. Alas, these models are not yet ready for wide implementation. At the current time, advancing our understanding of HSCs and hematopoiesis continues to rely on the ethical use of animal models.

More communication about animal use and its regulation from the scientific community

As scientists, we have a responsibility to communicate our research to the public. This must include the reasons why we currently rely on animals to answer precise biological questions, and what mechanisms are in place to ensure the ethical use of animals. For example, animal research in the UK is strictly regulated by the Home Office (the governmental department responsible for maintaining law and order) at three levels. First, UK scientists can only use animals for research after completing a training process and obtaining a licence. Second, each research project using animals is also licenced after being vetted to ensure that the 3Rs are followed, limiting animal use to specific procedures that are essential for the research, and tailored to minimise animal suffering. Third, the research must be conducted in a designated institution under the supervision of veterinarians and other staff trained to be responsible for animal welfare. Together, these layers of supervision ensure that the 3Rs must be followed by scientists, so they will only use animals if they are essential to answer a biological question and if there are no other alternatives. This type of approach, which is seen in many EU countries, has already contributed to reducing their use of animals in research by 5% between 2017 and 2018 (https://ec.europa.eu/environment/chemicals/lab_animals/pdf/SWD_%20part_A_and_B.pdf). UK-based Institutions also have committees of scientists that will evaluate all experimental plans in an unbiased and

transparent manner to ensure (1) that they follow the 3Rs and (2) that all means are in place in the animal facilities to guarantee the animal wellbeing when an experiment is conducted. This includes monitoring animals multiple times on a daily basis to ensure their wellbeing and providing comfortable facilities with fresh food, water, toys and social interactions. The regulation of animal use for research is extremely strict in the UK, and if a scientist does not comply with the Home Office, they will face severe consequences.

Funding to develop alternatives to animals

Several funding bodies provide grants to support the development of technologies that will reduce the use of animals. Funding is available in the USA (America Fund for Alternative to Animal Research, https://alternativestoanimalresearch.org/), Canada (CCAC, https://ccac.ca/en/three-rs-and-ethics/funding-opportunities.html), UK (FRAME, https://frame.org.uk/), Spain (Spain's national Cancer Research Center, https://www.cnio.es/en/), to name but a few. These funding opportunities will allow further improvements to our current models of in vitro hematopoiesis, and will also assist in the creation of novel technologies that will incorporate the external components involved in hematopoietic cell formation and specification such as the microenvironment.

A role for editors and reviewers in reducing animal use

During the submission of a manuscript to a scientific journal, editors and reviewers can play a role in minimising animal use. Firstly, before requiring an additional experiment using animals, reviewers can ask themselves: Is this experiment essential to support the author's findings? Can I propose an alternative to reduce or eliminate the use of animals? It can be easy to get lost in the science when reviewing a manuscript, but if asking these questions is commonplace during the peer review process, the field can ensure that the progress of research is not impeded, but animals are only used where necessary. Editors can also give authors an opportunity to discuss the necessity of experiments that will require animals, to find out if these experiments will be mandatory for the acceptance of the manuscript. By having more open discussion about these issues inside and outside the scientific community, we can all play our part in reducing the use of animals in research.

This post was contributed by members of the ISEH New Investigators Committee.

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