Editorial Commentary

Lessons learned from RAG1-deficient mice in hypertension.

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Mice deficient in recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) are considered a robust model to study the immune response in whole organisms. This is based on the immunologic concept that Rag1 and Rag2 are essential regulators of genes that encode immunoglobulin and T-cell receptor (TCR). Rag1 and Rag2 are expressed exclusively in lymphocytes and are essential in the development and maturation of T and B cells, key components of the adaptive immune system. In the absence of Rag1 or Rag2, lymphocyte development is arrested resulting in T and B cell deficiency. Rag1 knockout (Rag1 KO) mice have been extremely useful to study the role of the immune system in cancer, immunodeficiency disorders, inflammatory conditions, autoimmune diseases, lymphoid tissue pathologies and graft versus host disease. Exploiting adoptive transfer has provided a useful experimental approach to investigate the influence of different cell populations in adaptive immunity as well as the importance of the interaction between adaptive and innate immune responses in pathophysiological processes.

Rag1 KO mice have also been employed to study immune responses in cardiovascular disease, including atherosclerosis, aneurysms and hypertension. Double knockout ApoE/Rag1 and LDLR/Rag1 mice are resistant to atherosclerosis indicating a role for immune cells in atherogenesis (1). On the other hand, Rag1 deficiency had no effect on Ang II-induced aortic abdominal aneurysm in ApoE-/- mice suggesting that lymphocytes are not involved in aortic aneurysm formation (2). In their pivotal studies, the Harrison group was the first to show that blood pressure elevating responses to Ang II and DOCA-salt are blunted in Rag1 KO and that adoptive transfer of T cells, but not B cells, reestablished the hypertensive response to Ang II (3,4). It was thus concluded that the immune system, and especially T lymphocytes, have an important causal role in the development of hypertension, and that T cells may be an attractive target in the treatment of hypertension. This notion has gained increasing acceptance over the past 15 years.

However, not all evidence from Rag1-/- mice supports a role for adaptive immunity in the pathophysiology of hypertension as highlighted by Seniuk et al in the current issue (5). In Rag1-/-

mice, identical to those studied by the Harrison group (3,4), Ang II and high salt diet unexpectedly induced a significant increase in blood pressure with associated vascular remodeling and target organ damage, responses that were unaffected by adoptive T-cell transfer (5). Based on these findings, the authors negated a role for B and T cells in the development of hypertension and target organ damage. Ji et al also failed to show that Rag1 KO mice are protected from Ang II-induced hypertension (6) and Uchida et al demonstrated that total lymphocyte deficiency in Rag1-/- mice had no effect on systolic blood pressure prior to and during Ang II infusion (2). Moreover, in a model of arterial injury, immune deficiency in Rag1 KO mice was associated with augmented, rather than reduced, neointima formation, effects that were attenuated by CD8 T cells, but not by CD4 T cells (7).

These provocative findings (2,5-7), which challenge those that initially demonstrated blunted hypertension responses in Rag1-deficient mice (3), raise a number of important questions. Firstly, why does the same Rag1 immunodeficient mouse model yield diametrically opposed results? Secondly is the Rag1 KO animal the most appropriate pre-clinical model to study immune responses in hypertension? Thirdly, could it be possible that the adaptive immune system is not critically involved in the pathophysiology of vascular injury and hypertension?

Reasons for the lack of reproducibility by Seniuk (5), and others (2,6,7), of the earlier hypertension studies in Rag1 KO mice (3) are unclear but are likely complex and multifactorial. Ji et al attributed the unexpected Ang II prohypertensive response in Rag1-/- mice to a spontaneous mutation (6), a notion not supported by Seniuk et al (5). Factors that may contribute to variation in responses include genetic drift over multiple generations, especially in C57BL6 mice, the background strain for Rag1-/- mice. Not all C57BL/6 strains are genetically equivalent as highlighted by the International Mouse Phenotyping Consortium (8). Another factor includes the habitat of the mice where husbandry conditions, such as pathogen status, bedding, water, room temperature, noise, light/dark cycles, and housing conditions can impact the phenotype (8). Moreover, the microbiome can have a significant effect on the mouse phenotype (8). This is especially relevant when studying

the immune system in hypertension because both the immune response and development of hypertension have been causally and independently linked to the microbiome. Finally, an important factor to consider when studying Rag1-/- mice (or other immunodeficient models) is the possibility that 'basal' levels of T and B cells differ between mice, since genetic efficiency varies from from mouse to mouse. Accordingly, it may be prudent to establish immune cell criteria before experimentation, for example inclusion of models only if <2% of circulating CD45 cells are CD3 or CD20.

Another consideration often overlooked in Rag1-/- mice is that immune cells besides B- and T-lymphocytes are present and functionally active and may contribute variably to immune and inflammatory responses. Natural killer (NK) cells are derived from the same common lymphoid precursor (CLP) as lymphocytes and are present in Rag1 KO animals. Innate lymphoid cells (ILC) are also from the lymphoid lineage and similar to NK cells, they do not have Rag1-mediated recombined antigen receptor. They are ubiquitously expressed in Rag1 KO mice and play an important role in cell-cell interaction of the immune system. This cell population is sub-grouped according to the expression profile of transcription factors [ILC1 (T-bet+), produces the Th1 cytokine IFNγ; ILC2 (GATA3+), produces Th2 cytokines, IL-5 and IL-13; ILC3 (RoRγT+), produces IL-17, IFNγ and GM-CSF; and ILCreg (Sox4+), produces IL-10 and TGFβ]. Therefore, even though RAG1-/- animals exhibit severe immunodeficiency with B and T cell deficiency, the presence of functionally active NK and ILCs suggests that these mice are still able to mount an immune response.

Although Rag1-deficient mice are well established models to study the immune system in pathological processes, the suitability to recapitulate human disease has been questioned (8). This may be particularly pertinent in the context of human hypertension where there is still little conclusive clinical evidence that immune activation and inflammation cause hypertension. While numerous studies have demonstrated associations between chronic inflammatory and immune

diseases (eg psoriasis, rheumatoid arthritis, SLE and hypertension (4), causality has not been established. On the contrary, some observations from clinical studies actually suggest the opposite. Human immunodeficiency virus is associated with hypertension and cardiovascular disease (9) and some immunosuppressive drugs, such as calcineurin inhibitors, can cause hypertension. Patients with immunotherapy-induced cytokine release syndrome (CRS), a highly inflammatory condition, is associated with hypotension (not hypertension) (10). Additionally, treatment of patients with psoriasis and spondyloarthritis with IL-17 antibodies, secukinumab and ixekizumab, caused an increase in blood pressure (11), even though Ang II-induced hypertension is blunted in mice with IL-17-deficiency (12). In the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), IL-1β inhibition reduced cardiovascular event rates but did not significantly influence blood pressure (13). Furthermore, anti-cancer treatment with ibrutinib and rituximab, which target B cells, is associated with a significantly increased risk of hypertension (14), while no blood pressure effects were observed in Rag1 KO animals transplanted with B cells (3).

While extensive experimental evidence indicates that immune cells and inflammation are involved in hypertension-associated vascular injury and target organ damage (5), immune activation as a cause of hypertension still remains unclear. Irreproducibility of hypertension resistance in Rag1-/- mice and the lack of clinical evidence linking immune activation causally to hypertension, highlight the importance of further interrogating the exact role of the immune system in the pathophysiology of blood pressure elevation. The study in this issue (5) is also a call to action to revisit the mouse models used to study involvement of T- and B lymphocytes in hypertension and the relevance in human disease.

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Figure legend

Schematic summarizing different hypertension phenotypes in Rag1-/- mice and possible mechanisms responsible for this. Using the same immunodeficient mouse model, B6.Rag1-/-, Jackson Laboratories. Guzik et al (4) demonstrated that development of hypertension in response to pro-hypertensive factors is blunted while Senuik et al (6) showed that Ang II-infused Rag1-/- mice develop hypertension similar to wildtype mice.

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