Innate-like B cells: local drivers of non-HLA immunity in rejecting kidney allografts?

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Innate-like B Cells: Local Drivers of Non-HLA Immunity in Rejecting Kidney Allografts?

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Detrimental humoral immunity in solid organ transplantation can manifest itself as antibodies directed towards mismatched donor HLA. The process may also exhibit as antibodies targeting autoantigens or allogeneic non-HLA targets on the graft, referred to as non-HLA antibodies.[1,2] Although pathways driving non-HLA antibody formation remain poorly understood, the intragraft inflammatory milieu subsequent to (alloimmune) injury might be causing a breakdown of B-cell tolerance, creating targets for non-HLA (auto)antibodies.[2-5] Several studies have demonstrated associations of pretransplant and posttransplant non-HLA antibodies with acute and chronic allograft injury in the absence or copresence of donor-specific anti-HLA antibodies (DSA). In recent years, a growing demand for utilization of bead-based Luminex non-HLA antibody detection tests has arisen. Although such multiplex assays enable extensive characterization of non-HLA antibodies in serum owing to their high sensitivity, the results obtained are frequently inconclusive due to the low specificity of the assays. Therefore, studies aimed at understanding the pathogenicity, antigenic targets, as well as the mechanisms of induction and cell subsets producing these non-HLA antibodies are required to define the contribution of non-HLA antibodies to allograft injury and possible therapeutic interventions.

Over the past years, several studies have shown the presence of B-cell infiltrates in heart and kidney allografts during acute and chronic rejection. Interestingly, in a majority of patients, those infiltrates were present in the absence of circulating DSA.[5-9] Although several roles have been attributed to graft infiltrating B cells, such as contribution to lymphoid angiogenesis of tertiary lymphoid organs and antigen presentation to T cells in addition to their role in (local) antibody production, their exact role remains to be elucidated.

In a recent publication in *Nature Communications*, Asano et al[10] provided an in-depth characterization of kidney-graft-infiltrating B cells by investigating their transcriptional phenotype, B-cell receptor/immunoglobulin repertoire, as well as the antigenic targets of recombinant antibodies generated from these B cells and plasma cells.[10] The authors sorted activated B cells from clinical biopsy samples of kidney transplant recipients showing chronic or active antibody-mediated rejection and using single-cell RNA sequencing compared their gene expression profiles to B cells sorted from tonsillectomy samples. Both intrarenal and tonsillar B cells had a similar composition of unswitched (70%, IgM or IgD expressing) and switched status (30%, IgG or IgA or IgE). When zooming in to the class-switched B cells, an enrichment for innate immune response genes was observed exclusively in intrarenal B cells. This signature was marked by elevated expression of the *AHNAK* gene, accompanied by AHNAK protein expression, raising the question of which type of B cells had been identified. In the absence of data on human B-cell subsets characterized by *AHNAK* expression, the authors mapped *AHNAK* and its covariant gene enrichments to murine peritoneal cavity B1 cells, thereby identifying a human innate B-cell (Bin) subset for the first time. This innate-like transcriptional phenotype was further supported by intrarenal B-cell expression of interleukin (IL)-15. Interestingly, although these isotype-switched Bin cells had intragraft retention mechanisms, contrary to that of germinal center B cells, tertiary lymphoid organ formation was not observed. Noteworthy, the innate-like transcriptional phenotype did not differ between DSA-positive and-negative patients.

B-cell receptor/immunoglobulin gene repertoire analysis revealed that many intragraft plasma cells were clonally related to Bin cells, suggesting intragraft differentiation of Bin cells to antibody-producing plasma cells, driven by the local antigenic milieu. The authors produced 105 recombinant monoclonal antibodies (mAbs) generated from Bin and plasma cells and tested for HLA reactivity. Although 15% of mAbs showed multiple HLA reactivity with HLA-C being the most frequent, none were DSAs, and no epitope sharing could explain the broad HLA reactivity. Interestingly, when mAbs were diluted in negative control serum, HLA reactivity was abrogated. This observation is in line with the description of polyreactive mAbs cross-reacting with HLA or autoantigens.[3] Mass spectrometry analysis of peptides recognized by mAbs generated from selected plasma cell clones revealed Ki67 as the top hit antigen, with mAbs also binding to Ki67 on tonsil tissue. Further examination of 28 highly mutated antibodies expressed by intrarenal B cells revealed 6 antibodies binding to inflamed kidney tissue, while 3 of these also bound to native kidney tissue, suggesting selection of B cells by

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antigens expressed in the rejecting graft because of breakdown in self-tolerance occurring at the B-cell level.

Asano et al[10] present an exquisite approach to identify kidney graft infiltrating Bin cells, their selection by locally expressed antigens, and their differentiation into highly mutated class-switched antibody-producing cells, partially targeting inflammation-restricted kidney-specific antigens. The cause of posttransplant intragraft de novo self-reactive antibody formation is presented as a breakdown of B-cell tolerance, as patients had no history of auto immune disease. Although this work builds on existing knowledge of graft infiltrating B-cell clonality and the presence of polyreactive antibodies from B-cell clones in transplant patients,[9] it raises the question of whether Bin cells are kidney-specific or can also be detected in other types of allografts. Moreover, future studies should clarify whether Bin cells contribute to rejection, and if so, underlying mechanisms will need to be identified. In this context, the interaction of Bin cells with cells of the innate immune system as well as with intragraft T cells may provide insight into the interplay between innate and adaptive immune responses leading to graft injury.

It would also be interesting to know whether antibodies produced by intragraft plasma cells can also be detected systemically. With the currently available bead-based kits which contain >60 antigenic targets, patients appear to have a broad and variable spectrum of non-HLA antibodies. The incorporation of organ-specific, clinically relevant antigenic targets to these kits may refine our understanding of the trigger and progression of posttransplant de novo non-HLA antibody formation and may feed international collaborative studies, such as the non-HLA component of the 18th International HLA and Immunogenetics Workshop (https://www.ihiw18.org/project-testing-the-clinical-utility-of-commercial-non-hla-antibody-kits/). In addition, identifying pathways that lead to autoantibody formation in the transplant setting will be critical for developing effective treatments. Further studies in line with the game-changing work from Asano et al[10] may aid in an in-depth understanding of the development of non-HLA immunity in solid organ transplantation.

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