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EDITORIAL

The Road Traveled: Genomics and Biomarkers in Antineutrophil Cytoplasmic Antibody–Associated Vasculitis

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The search for biomarkers of disease activity and response to therapy is a continuing quest, and the increasing sophistication of genomic data, including transcriptomics, and of capacities for high-dimensional data analytics point to encouraging opportunities on the horizon for many diseases. In this issue of *Arthritis & Rheumatology*, Grayson et al (1) leveraged biologic specimens gathered during the Rituximab in ANCA-Associated Vasculitis (RAVE) trial and applied RNA sequencing to samples of whole blood and separated leukocytes to search for gene expression patterns that might serve as biomarkers that are potentially involved in pathophysiology and response to therapy.

Similar to several other autoimmune diseases with prominent neutrophil-related gene expression signatures (2–5), AAV patients in the RAVE trial had a discernable neutrophil-related profile in the transcriptomes of their circulating leukocytes. A lower intensity of the granulocyte multigene composite score, which was created for this study, was related to a higher likelihood of meeting the primary end point in the trial. Taken in the context of other clinical studies demonstrating neutrophil-related gene expression signatures in AAV, the data reported by Grayson et al further support a potential role of neutrophils and, likely, low-density granulocytes (LDGs) in the pathophysiology of AAV and a role of neutrophil markers in informing therapeutic strategies.

As with all studies at the leading edge of clinical research, this study prompts consideration of a series of opportunities for methodologic refinement and for replication in independent data sets. The authors acknowledge that the use of whole blood, even when depleted of globin transcripts, limits the ability to detect less-common transcripts and may introduce confounding due to variations across participants in the proportion and number of different circulating cell types, each with characteristic gene expression signatures. Nonetheless, it would be interesting to use unsupervised hierarchical clustering as in this study to generate additional granulocyte multigene composite scores derived from other AAV data sets and then compare the performance of such scoring systems in relation to clinical outcomes for the RAVE participants. Agreement on a composite score derived from multiple different training data sets and applied successfully to several different independent sets of outcome data, as is being pursued in other diseases (6), would enhance the utility of a transcriptomic approach to practicable biomarkers. Furthermore, in future longitudinal studies, the performance of granulocyte-related gene expression profiles as predictors of therapeutic responsiveness could be compared with absolute neutrophil counts and flow cytometric measurements of circulating low-density granulocytes (frequency and absolute numbers) as defined by size, granularity, and CD15 and CD16 expression (7). Such an approach would provide further insights into both pathophysiology and real-time clinical feasibility.

An AAV cohort study of neutrophil properties, including membrane proteins and apoptosis, is currently under way in Europe at the Hôpital Cochin (Assistance Publique-Hôpitaux de Paris; ClinicalTrials.gov identifier: NCT01862068) and might provide additional such data. Similar future studies might also include other variables, such as ANCA titer and immunoglobulin class (8), as well as genetic variants that have been implicated in both the pathogenesis and clinical course of AAV (8–14). Since such high-dimensionality data will require large numbers of participants for sufficient statistical power, a cooperative effort across patients, health care professionals, academic institutions, and sponsoring agencies is likely to be necessary. Nonetheless, the opportunity for more individualized evaluation of our patients and more precise use of our therapeutic armamentarium—the

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right therapy at the right time—is both exciting and compelling in the advancement of clinical care.

Insights into pathogenesis that prompted this study are provocative. The ability of ANCA to activate neutrophils with surface proteinase 3 (PR3) for further degranulation and formation of neutrophil extracellular traps (NETs) (8,9,15) may synergize with the spontaneous ability of low-density granulocytes (LDGs), which have now been formally demonstrated in AAV patients to form NETs. The process of perivascular NETosis may enhance vascular injury characteristic of AAV (5,7). While the genesis of circulating LDGs may involve premature release of an immature granulocyte subset from the marrow (7), it may also be that some LDGs represent stimulated, partially degranulated, more-mature neutrophils. Development of a neutrophil-oriented therapeutic strategy might depend on the genesis of, and the role played by, LDGs in AAV, and investigations could approach the hypothesis that LDGs represent stimulated, partially degranulated, more-mature neutrophils by looking for surface PR3 and CD177 on LDGs from AAV patients, as these are the surface constituents that facilitate ANCA-mediated stimulation of neutrophils (16,17).

It might also be insightful to compare the gene expression profiles of LDGs obtained from patients with different diseases with the question of whether differences in these profiles could provide important clues to pathogenesis. Interestingly, 41 of the 281 genes that defined an LDG signature in a lupus cohort (5) overlapped with genes differentially expressed in the RAVE nonresponders. While the 41 overlapping genes support some commonality in the biology of LDGs in different diseases, perhaps the 240 nonoverlapping genes have another story to tell. They may provide insight into the development of LDGs, the heterogeneity of LDGs, and perhaps the different stimuli encountered in the *milieu interieur* of each disease.

The role of neutrophils—and potentially of LDGs—in the pathogenesis of AAV raises several therapeutic challenges. Effective targeting of neutrophils in animal models, especially with depletion strategies, has been in large part problematic because of an impaired host defense when a critical "first responder" cell type is unavailable. The increased infection risk in leukocyte adhesion deficiency syndromes in humans (18) suggests that targeting myeloid cell margination and adhesion, at least chronically, may also have problematic safety profiles. However, an alternative might be to consider compounds such as glyburide, a K_{ATP}-channel blocker and broad-spectrum ATP-binding cassette transporter inhibitor used to treat type 2 diabetes mellitus. Some evidence indicates that glyburide is anti-inflammatory and may down-regulate the expression of neutrophil-related genes related to AAV pathogenesis, including CD177, CD89, and CD64 (see Supplementary Table 3A in ref. 19).

Whether such a repurposing strategy with glyburide or similar compounds would be effective with LDGs, as opposed to mature neutrophils, and effective in reducing neutrophil-mediated tissue injury in AAV are questions for future investigation. Nonetheless, the clear presence of a neutrophil gene expression signature, the role of ANCA and ANCA target on the neutrophil surface, and the efficacy of rituximab suggest a complexity in AAV pathogenesis that will likely require a multifaceted approach.

The work by the RAVE–Immune Tolerance Network (RAVE-ITN) Research Group encourages us to consider genomics, in addition to genetics and the many parameters of an activated immune system, in both the pathogenesis and the management of AAV. Increasingly, genetics is assuming a role in precision medicine, and we can anticipate that, as functional genomics provides additional insight into pathophysiology, it will also contribute to the portfolio of biomarkers that inform our therapeutic decisions. There is much work to be done, but the road to be traveled with our patients will take us to exciting insights into the mechanisms and better treatments for the diseases that challenge us each day.

AUTHOR CONTRIBUTIONS

Dr. Kimberly drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

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