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IgA Responses and Future Development of Rheumatoid Arthritis

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The appearance of serum autoantibodies precedes by many years the development of clinical disease in multiple autoimmune conditions (1). In rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), serum autoantibodies represent the earliest immunologic abnormality, which is followed by the generation of mediators of inflammation in closer temporal proximity to the onset of symptoms that leads to classifiable disease. In both diseases, the complexity of the autoantibody response increases gradually through epitope spreading. In RA in particular, a greater breadth of anti-citrullinated protein antibodies (ACPAs) correlates with joint inflammation in healthy first-degree relatives (2). Recent longitudinal studies indicate that the presence of autoantibodies has a significant positive predictive value for disease development in healthy first-degree relatives of RA patients (3).

These observations indicate a protracted phase of preclinical autoimmunity that provides a unique opportunity to understand the inciting and amplifying immunologic events that underlie disease development. A better understanding of these processes could lead to better risk stratification for disease development and provide an opportunity for early intervention aimed at disease prevention.

To date, essentially all autoantibody studies pertaining to RA development have been performed at the serologic level. This approach determines the antigenic reactivity of polyclonal antibodies of different isotypes (generally IgM, IgG, and IgA) against predetermined antigens. In addition to their convenience, sample availability, and high throughput, serologic assays have the virtue of being highly sensitive, and they provide an important measure of autoantibody complexity. When performed longitudinally, serologic assays are also ideal for measuring epitope spread, as they can determine whether new antigenic specificities are being recruited into the autoimmune compartment.

There are two major drawbacks, however, to serologic approaches. First, at any given point in time, they cannot discriminate between newly produced antibodies and antibodies produced by preexisting long-lived plasma cells that may have been generated years in advance. Second, serologic studies do not provide information about the cellular complexity and cellular dynamics of an antibody response. These features of an antibody response are highly informative, as they reflect whether a given autoimmune response is fixed by the repetitive restimulation of preestablished memory responses or whether instead, it continues

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to evolve by the recruitment of new naive cells and/or by progressive affinity maturation of preexisting autoreactive B cell clones.

Plasmablasts are proliferative antibody-secreting cells produced during the early phases of an active immune response. At steady-state, in the absence of infection or intentional immunization, plasmablasts typically represent <1–2% of all circulating CD191 B cells in healthy subjects. In a normal recall response such as that induced by influenza or tetanus vaccination, plasmablasts circulate in the blood in highly expanded numbers for a few days after immunization. These transient plasmablasts are typically oligoclonal, antigen-specific, and largely derived from preexisting memory cells (4). It has been reported that while plasmablasts induced by systemic immunization largely produce IgG antibodies, steady-state plasmablasts in healthy subjects are predominantly IgA, presumably reflecting ongoing mucosal reactions (5). Thus, plasmablasts represent a window into ongoing immune responses and can separate contemporary events from the historical record; therefore, their study may answer critical questions regarding the origin, complexity, and evolution of an autoimmune response. Owing to their very high content of immunoglobulin messenger RNA, plasmablasts are also an ideal cell source for studying the antigenic reactivity of an antibody response at the single-cell level.

In this issue of *Arthritis & Rheumatology*, Kinslow et al (6) report their analysis of peripheral blood plasmablasts in subjects at high risk of developing RA. This is a tantalizing study built on the strength of a unique patient cohort and experimental design and the utilization of cutting-edge technology for the analysis of plasmablasts at the single-cell level. Thus, the study takes advantage of the Studies of the Etiology of RA (SERA) project to analyze at-risk subjects who, in this case, were selected solely on the basis of their autoantibody status (antibody-positive).

Polychromatic flow cytometry was used to compare the frequency of plasmablasts producing IgM, IgG, or IgA antibodies in antibody-positive at-risk subjects, early RA patients, and healthy control subjects. Barcoded reverse transcription and next-generation sequencing of sorted plasmablasts of different isotypes were used to decode the diversity of the antibody repertoire expressed by these cells and to determine the complexity and morphology of antibody clones and their distribution across the different isotypes. The utilization of barcodes enhances throughput in the pairing of heavy and light chains expressed by single cells and eliminates errors introduced during amplification and sequencing. This elegant approach had previously been used by this group of investigators to demonstrate that circulating plasmablasts from RA patients contain cyclic citrullinated peptide-reactive clones (7).

Of note, both antibody-positive at-risk subjects and early RA patients displayed lower plasmablast frequencies than did the healthy controls. While RA patients typically lack the large plasmablast expansions characteristic of active SLE, previous studies have shown moderate increases relative to healthy controls in patients with longstanding RA, a population that was not included in the Kinslow et al study. Elevated plasmablast frequencies, at least as determined by a genomic signature, have also been shown to correlate with an unfavorable response to B cell depletion (8). Therefore, it will be important

to understand whether the relative dearth of plasmablasts observed in this study is due to the early stages of the disease, methodologic differences between studies, or perhaps the nature of the samples used, as it is well established that plasmablasts are highly sensitive to freezing protocols and even overnight storage.

The central result is the much larger relative frequency of IgA plasmablasts in antibody-positive at-risk subjects compared not only to healthy controls but also to early RA patients. This finding provides an elegant cellular basis for previous observations by the same group regarding the elevated frequencies of serum IgA ACPAs in antibody-positive at-risk subjects and it suggests that these autoantibodies are being actively produced through ongoing immune responses during preclinical autoimmunity. This conclusion is also supported by the coexpression of IgG and IgA isotypes within some of the circulating plasmablast clones.

Combined with the dominant role played by IgA antibodies in mucosal responses, the results support the working model proposed by the investigators in which the preclinical autoimmunity phase of RA would be characterized by mucosal inflammation, presumably systemic in nature given extant evidence for at least lung, gingival, and intestinal abnormalities. Moreover, the dramatic reduction in IgA plasmablast levels observed in early RA patients suggests that the initial phase of mucosal activation would abate by the time clinical disease develops, at which point the immune response might instead be focused on the synovium.

As they discuss, their results contrast with those of previous studies showing that IgA plasmablasts represent the majority of circulating plasmablasts in steady-state healthy controls (5). The source of this discrepancy remains to be clarified. Different approaches to the definition of plasmablasts and to the assessment of IgA expression were used. In particular, Kinslow et al used a negative definition of IgG-expressing plasmablasts, thus raising the question of whether all IgA⁺/IgM⁺ plasmablasts were indeed IgG1. As discussed by the authors, however, the sequencing data support their flow-based classification. As also noted, their studies were performed during influenza vaccination season, a perturbation known to preferentially induce IgG plasmablasts. Nevertheless, the relatively low frequency of plasmablasts detected in healthy controls and the very transient nature of vaccination-induced plasmablasts render this explanation rather unlikely.

In summary, Kinslow's studies open the door to future in-depth investigation of the tissue origin of plasma-blast responses in preclinical RA. Assessing the expression of markers such as CCR10 and $\alpha 4\beta 7$ integrin would be required in order to strengthen the evidence for a mucosal derivation, and evaluating the ability of the circulating plasmablasts to produce polymeric IgA would further support this conclusion. The analysis of monoclonal antibodies representative of expanded plasmablast clones will also be critical in order to determine the antigenic triggers responsible for the expansion of IgA plasmablasts. Finally, longitudinal studies should determine the value of IgA plasmablast expansions for the prediction of the future development of RA. Understanding these issues will contribute greatly to our ability to intervene early to prevent disease development and to do so in a rational way, informed by a detailed knowledge of the underlying immunologic mechanisms.

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