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The Autoantibody response in rheumatoid arthritis; what makes it unique?

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ABSTRACT

Many autoimmune diseases respond well to therapies targeting B cells, emphasizing the importance of autoreactive B cells in disease induction and progression. In some cases, autoantibodies from plasma cells are the main effectors, while in others, memory B cells are thought to modulate inflammatory responses through antigen presentation or cytokine secretion. Tolerance mechanisms usually prevent the development of autoantibodies, but when these systems fail, autoimmunity and subsequent autoimmune diseases can arise. Understanding the dynamics of autoreactive B cell responses is crucial for delineation the pathogenic pathways underlying disease. Rheumatoid arthritis (RA) is a prototypic autoimmune disease featuring autoreactive B cell responses against post-translationally modified (PTM) proteins. Especially, the Anti-Citrullinated Protein Antibody (ACPA) response, the autoimmune response hallmarking RA, is well characterized over the last two decades. ACPA target citrullinated proteins and their presence is associated with more severe disease progression. In recent years, it has become apparent that ACPA are very promiscuous and able to recognize both human and microbial PTM proteins. Likewise, it is now clear that these antibodies often carry Fab glycans that could, potentially, boost B cell activation and/or be involved in evasion of tolerance mechanisms. Although subjected to tolerance checkpoint, new technologies have shown that the secreted ACPA repertoire is highly polyclonal, unique to each patient, but also dominated by a few ACPA clones.

In this synopsis, these and other findings are discussed. Overall, these shed light on the complexity and evolving nature of the ACPA response in RA, unveiling new insights into autoreactive B cell behavior.

Review

To prevent the development of autoantibodies several tolerance mechanisms are in place. When these tolerance systems fail, it can result in autoimmune diseases. Interestingly, many autoimmune diseases respond well to B cell-targeted therapies indicating a pivotal role of (autoreactive) B cells in disease pathogenesis. Serological studies have shed light on how autoantibodies relate to disease onset, progression, and treatment response. As a result, autoantibodies are commonly used as biomarkers for diagnosis, prognosis, disease classification and treatment decisions. Although their disease specificity and diagnostic potential has been well defined, less information is available on the breach of tolerance of autoreactive B cell responses and the emergence of autoantibodies.

An autoantibody family studied extensively for both its diagnostic potential and the biological/immunological pathways underlying its induction is the Anti-Citrullinated Protein Antibody (ACPA)-response in Rheumatoid Arthritis (RA). ACPA target proteins containing citrulline, a post-translationally modified version of arginine, and are highly specific to RA. ACPA are found in 50–75 % of RA patients and are linked to severe bone erosion, disease progression and poor treatment responses. Interestingly, ACPA can be detected years before an RA diagnosis and several characteristic features likely related to the pathways underlying the induction/propagation of the ACPA response have been described in recent years [1]. For example, it is well known that ACPA can be highly promiscuous and are able to recognized a plethora of citrullinated proteins of human- and microbial origin [2,3]. Likewise, ACPA, as shown on both the polyclonal- and monoclonal level, can also recognize, next to citrulline, other post-translationally modified proteins, such as acetylated- and carbamylated (homo-citrullinated) proteins [3,4]. These findings are relevant to understand the potential induction of ACPA-responses as they underscore the notion that the ACPA B cell response could be induced by microbes expressing modified proteins [5, 6].

A remarkable trait of ACPA is that they express N-linked glycans in their variable regions, also referred to as Fab glycans [7]. The level of

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variable domain glycosylation (VDG) in ACPA-positive healthy individuals has been shown to correlate with the transition to RA, making ACPA not only a key diagnostic and prognostic marker, but also ACPA VDG a potential predictor of RA development [8]. These variable domain glycans are positioned in the vicinity of the antigen-binding pocket and have been shown to enhance B cell activation upon citrullinated antigen encounter [9]. Together, these findings indicate that the presence of variable domain glycans provide a selective advantage to ACPA-expressing B cells that could play a role in the outgrowth of these cells by breaching tolerance checkpoints. Likewise, these observations also show that the ACPA B cell response is, most likely, regulated differently as compared to B cell responses to viral and other microbial antigens that typically do not carry variable domain glycans.

As outlined above, loss of B cell tolerance often leads to the emergence of autoantibodies, a defining feature of many autoimmune diseases. Because B cell autoimmunity is normally controlled by several checkpoints, it is conceivable that the breath of an autoantibody repertoire is limited as compared to e.g. an antibody repertoire to viruses [10]. Next to a potential exclusion of autoreactive clones by tolerance mechanisms, also the distinct context in which autoantigens are recognized through e.g. a single defined post-translational modification, makes that the diversity of repertoires targeting infectious agents may differ considerably from autoreactive antibody repertoires. Therefore, analyzing the full scope of autoantibody repertoires could provide additional understanding into the extent of tolerance failure of the autoreactive B cell response underlying B cell driven autoimmune diseases. Recently, technologies have evolved allowing to determine the diversity of polyclonal antibody mixtures against defined antigens at the molecular level [11]. In these methods, IgGs are purified and then enzymatically cleaved into the constant domain (Fc) and the antigen-binding region (Fab). The released IgG Fab molecules, typically within the 45–53 kDa mass range, are fractionated and analyzed using liquid chromatography coupled with mass spectrometry (LC–MS). As each clone has a unique mass and retention time, a qualitative snapshot of the IgG repertoire to a defined antigen can be obtained. Using these technologies, several studies into the virus-specific IgG antibody repertoires revealed that the virus-specific antibody response is highly polyclonal and unique to each individual [12].

To obtain insight into the diversity of the antibody repertoire to autoantigens, we studied the ACPA response as a prototypic autoantibody response. Our observations show that, similar to antibody repertoires to viruses, also the ACPA repertoire is highly polyclonal consisting of many different unique ACPA clones. These observations indicate that a possible exclusion of autoreactive antibody clones through B cell tolerance mechanisms has not resulted in a restricted set of autoreactive antibody-secreting B cells. Likewise, when comparing the ACPA repertoire obtained from different patients, it became evident that these repertoires hardly overlapped, indicating that the ACPA repertoire is not only diverse, but also unique to each patient [13]. Nonetheless, a quantitative estimation of the frequency of dominant clones indicated that each repertoire was dominated by only a few Fab molecules, with the ten most abundant Fab molecules accounting for up to 50 % of the total repertoire. These observations are interesting as they indicate that individual ACPA-secreting cells with a high production rate and/or the expansion of a few ACPA-secreting cells producing the same ACPA clone underlie the dominance of these unique ACPA molecules. The profiling of the ACPA repertoire also confirmed that ACPA IgG molecules are extensively Fab-glycosylated compared to total plasma IgG. We estimated by the additional resolution provided by the method that, on average, the extent of Fab glycosylation of ACPA is 10 times higher as the level of Fab-glycosylation of the total plasma IgG repertoire, although a portion of ACPA did not carry Fab glycans. Interestingly, approximately 10 % the ACPA IgG repertoire showed a significant increase in Fab molecules containing two or more glycans, an attribute rarely observed in total plasma IgG [13]. The reason for this enrichment is not known, but might be due to the selective expansion/selection of cells secreting ACPA that harbor multiple glycosylation-sites/variable domain glycans.

Collectively, these observations and insights obtained throughout the years have shed light on the complexity of the disease-specific autoimmune response in RA. These insights uncovered that the ACPA response is dynamic and indicated novel mechanisms involved in driving this prominent autoreactive B cell response.

Declaration of competing interest

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