Bitter Sweet Symphony: the impact of sugars on autoimmunity

Kissel, T.

Addendum

Summary

The studies described in this thesis focus on the chronic, lifelong inflammatory joint disease Rheumatoid Arthritis (RA), which is widespread in our society. RA is an autoimmune disease that occurs when our immune system, which is supposed to protect us from bacterial, viral, and parasitic infections, gets out of control and mistakenly begins to "attack" our own body's tissue. In RA, this causes immune cells such as T cells, B cells, and macrophages to infiltrate joint tissue, which can lead to inflammation and eventually cartilage and bone damage. Despite recent advances in therapy and the identification of the involvement of both, genetic and environmental factors contributing to the disease, the underlying cause of the autoimmune response in RA remains unknown. Up to 80% of RA patients display disease-specific antibodies (soluble B-cell receptors) directed against the body's own proteins (self-antigens). These autoantibodies arise as a result of the loss of the immune system's ability to distinguish between self and non-self (foreign) antigens. Their abundant presence in patients with RA and the effectiveness of targeted B-cell therapies suggest that the underlying autoreactive B-cell response plays an important role in disease development.

The different classes of autoantibodies characteristic of the disease are described in the general introduction (**Chapter 1**). RA is characterized by autoantibodies directed against post-translational modifications (PTMs), i.e. biochemical modifications on individual amino acids of a protein that occur after translation of mRNA into proteins. The most disease-specific autoantibodies are directed against citrulline [anti-citrullinated protein antibodies (ACPAs)], an enzymatic conversion of the amino acid arginine. In addition to ACPAs, autoantibodies against carbamylated [anti-carbamylated protein antibodies (ACarPAs)] or acetylated proteins [anti-acetylated protein antibodies (AAPAs)] have also been observed in patients with RA. Both modifications result from a post-translational modification of the amino acid lysine. In previous studies, the different autoantibody systems were found to occur simultaneously in RA patients, suggesting cross-reactivity of one autoantibody class against several PTMs. In addition to their reactivity against PTMs, ACPA molecules have another important feature: they carry additional sugar structures (glycans) in the region of the antibody molecule responsible for binding to the modified proteins. Glycans consist of individual sugar residues (monosaccharides) that together form sugar chains. The antibodies (immunoglobulins) of class G (IgG) normally carry glycans in the constant domain (Fc) of the antibody molecule. However, ACPA IgG molecules are also abundantly glycosylated in the antigen-binding region (variable domain). With the studies described in this work, we aim to understand how the characteristics of RA-specific autoreactive B cells – their cross-reactivity and the glycosylation of the variable domain – help these cells to survive and escape the strict control mechanisms our body has in place that normally prevent the development of autoimmunity.

To this end, **Chapter 2** reviews the current literature on the checkpoints controlling the induction of autoreactive B cells. In order to be able to ward off pathogens, they are recognized as foreign by our immune system. In contrast, the body's own proteins are tolerated by the immune system (self-tolerance). This self-tolerance develops in the thymus and bone marrow (central tolerance). There are also various control mechanisms in other parts of the body, such as the lymph nodes (peripheral tolerance), which prevent the immune system from getting out of control. In this chapter, we focus specifically on these peripheral control mechanisms which ensure that potentially autoreactive B cells do not cause autoimmunity. We highlighted antigen presentation by follicular dendritic cells (FDCs), somatic hypermutation (SHM) – a mechanism by which the immune system adapts to new antigens through mutations in the variable domains of the B-cell receptor – and cross-reactivity of T-/ B-cell receptors to self- and foreign (microbial/ environmental)-proteins. Furthermore, it is highlighted that autoreactive B cells in RA potentially escape this self-tolerance through their cross-reactivity and variable domain glycans (VDGs).

The data described in **Chapter 3** reinforce the hypothesis that the three autoantibody classes characteristic of RA (ACPA, ACarPA and AAPA) cross-react and thus may belong to one family of antibodies. We therefore introduced the term anti-modified protein antibodies (AMPAs). The finding that AMPAs are cross-reactive was obtained by generating monoclonal antibodies. The B-cell receptor sequences that form the basis for these monoclonal antibodies were isolated from single B cells of RA patients. The binding potential of these antibodies to different antigen candidates (proteins and peptides) carrying citrullinated, carbamylated or acetylated amino acids was analyzed, which revealed high cross-reactivity against at least two different modifications. In this chapter, we also show that B cells originally directed against citrullinated proteins can also be stimulated by acetylated or carbamylated antigens. Taken together, these data suggest that autoreactive B cells in RA can be activated by multiple proteins carrying different modifications. Accordingly, different PTMs may be involved in the initial breach of self-tolerance, such as endogenous acetylated proteins that may have been acetylated during bacterial infection.

In addition to their cross-reactivity to various PTMs, ACPA IgGs themselves carry post-translational modifications, as they are abundantly glycosylated in their variable domains. In the studies described in **Chapter 4** and **Chapter 5**, we were able to elucidate an important function of these glycans in the development of RA. Previous findings already indicated that VDGs confer an advantage to autoreactive B cells producing ACPAs. These results showed that VDGs are selectively introduced into ACPA antigen-binding domains after T-cell help and SHM. It was also already known that the presence of VDGs in ACPAs increases the likelihood that a person who is still symptom-free will later develop RA. The results described in these chapters now show that the presence of VDGs is associated with the most prominent genetic risk factor

for ACPA-positive RA. These are the human leukocyte antigen (HLA) alleles, which encode so-called "shared epitopes" (SE). HLA-SE are involved in the recruitment of T-cell help, which is necessary for the development of a "full-fledged" B-cell response. Accordingly, it is likely that VDGs are introduced into the autoreactive B-cell receptors during SHM after receiving help signals from T cells restricted to the HLA-molecules predisposing to the disease.

This unfavorable "hit" may occur multiple times, which is shown by the increasing frequency of VDGs on autoreactive B cells during the course of disease development as demonstrated by the studies presented in **Chapter 6**. The percentage of variable domain glycosylation was analyzed in 1498 samples from individuals at various clinical stages of disease. The glycosylation of the variable domain of ACPA IgGs increased steadily starting with 56% in healthy individuals, already showing autoantibodies, to 75% in patients with joint pain (arthralgia) and 93% in patients with incipient RA. Once the disease is established, ACPA molecules from RA patients consistently show high expression of VDGs, although there is a slight decrease upon immunosuppression. The data described in this chapter further show that the "quality" of the ACPA immune response, i.e. the frequency of VDGs on ACPA IgGs at disease-onset, may be predictive of the development of chronic persistent disease or the chance of RA patients achieving drug-free remission at a later stage.

The study described in **Chapter 7** focuses on the functional importance of VDGs for autoreactive B cells and their secreted autoantibodies. The data show that the presence of VDGs can reduce the binding of autoreactive B-cell receptors and autoantibodies to citrullinated proteins. The presence of VDGs had no effect on the binding of citrullinated proteins that are recognized with high-affinity. However, it reduced binding to citrullinated proteins that are less well recognized and consequently have low affinity. Structural analyses and modelling of the interactions between autoantibody and antigen confirmed that VDGs interact with the part of the antibody involved in citrulline recognition. In addition, the data showed that VDGs increase the activation status of autoreactive B cells. This may be a consequence of the reduced internalization of glycosylated B-cell receptors after binding to citrullinated proteins. These important functional consequences of VDGs – both the decreased binding to low-affine (potentially self) antigens while retaining binding to high-affine (potentially foreign)-antigens, and a lower activation threshold – may allow autoreactive B cells to overcome important immune checkpoints and thus to breach self-tolerance.

Chapter 8 describes that VDGs, in addition to their functional importance for B-cell receptors, can also influence the effector functions of secreted antibodies. In this study, we observed a lower formation of hexamers of variable domain glycosylated ACPA IgGs. This leads to a reduced binding to the effector molecule C1q and thus a lower activation of the complement cascade, an important pathway within the immune system for the activation and recruitment

of immune cells. Whether this reduced complement activation of glycosylated ACPA IgGs has an impact on the pathogenesis of the disease and possibly makes ACPA less immunogenic, or whether it is just a bystander effect, can only be speculated and requires further investigation. Nevertheless, it can be concluded that IgG VDGs can be exploited to downregulate the antibody-dependent classical complement pathway.

All data are jointly discussed in **Chapter 9** in the context of the current literature. The ability of RA-specific autoantibodies to bind to various modified proteins is demonstrated and explained by the structural analyses of ACPA molecules. ACPA interact specifically with the citrulline modification and not, or only to a minor extent, with the flanking amino acids surrounding this modification. Since the modifications that arise after carbamylation and acetylation (homocitrulline and acetyl-lysine) of proteins are structurally very similar to citrulline, ACPA can also recognize these post-translational modifications "promiscuously". In addition, the selective introduction of glycans into the variable domains of ACPA IgGs and its functional consequences are discussed. The data described in this thesis have led to the hypothesis that VDGs help autoreactive B cells to bypass tolerance checkpoints by influencing binding to modified proteins and B-cell activation. In conclusion, it is discussed that autoreactive B-cell responses in RA most likely result from sequential events ("multiple hits") in which the cross-reactive nature of the response and abundant expression of VDGs play an important role. This new "glycan mechanism" may be useful to improve the diagnosis of RA even before the disease develops. This knowledge is also valuable in understanding how the immune system gets out of control and how autoimmunity develops. However, much more research is needed to understand how and why autoimmune diseases like RA develop (*bitter*) and to unravel the important role of glycan modifications (*sweet*) during disease development.