

The Impact beyond Recombinant Antibody: Recent Advances in Understanding the Disease Associated Glycan Alterations in Endogenous Antibodies

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Received: June 20, 2016

Revised: July 5, 2016

Accepted: July 7, 2016

Published online: September 14, 2016

ABSTRACT

The changes in antibody *N*-glycosylation in patients with rheumatoid arthritis and osteoarthritis have been known for more than three decades. With the advances in analytical technologies, glycosylation alterations in endogenous antibody were recently reported to be associated with many diseases, especially autoimmune diseases. Since glycosylation is also related to antibody function and many carbohydrate receptors are present in immune cells, these alterations may play a role in modulating disease progression, resulting in a shift of the immune system toward a pro-inflammatory status. Recent work also suggested the contribution of certain environmental and genetic factors to glycosylation alterations in antibodies.

Key words: Antibodies; Endogenous antibodies; Disease associated autoantibodies; *N*-glycosylation; N297 glycans; Glycans; Disease associated glycan alterations; Autoimmune diseases; Pro-inflammatory status; Antibody-dependent cellular cytotoxicity; Complement-dependent cytotoxicity; Anti-inflammatory activity; Genome-wide association study

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Zhou Q. The Impact beyond Recombinant Antibody: Recent Advances in Understanding the Disease Associated Glycan Alterations in

Endogenous Antibodies. *Journal of Biochemistry and Molecular Biology Research* 2016; 2(3): 170-172 Available from: URL: <http://www.ghrnet.org/index.php/jbmr/article/view/1763>

EDITORIAL

Antibodies, or immunoglobulins, play a critical role in humoral immune response. They have been used as important therapeutics for treatment of cancer and autoimmune diseases. There are glycans which are attached to N297 at a well conserved *N*-glycosylation site in C_H2 domain of IgG Fc region. Unlike those present in other glycoproteins, the N297 glycans are mainly truncated fucosylated biantennary complex-type structures with minimal amounts of sialic acid.

These N297 glycans have a significant impact on antibody function^[1]. It was first demonstrated ~17 years ago that there was a 50 to 100-fold higher antibody-dependent cellular cytotoxicity (ADCC) with glycoengineered non-fucosylated bisecting GlcNAc containing recombinant IgG than with fucosylated ones^[2]. The ADCC enhancement was shown to be related to increased binding affinity of the non-fucosylated antibody to an Fcγ receptor, FcγRIIIA.

Besides this effector function, the intravenous immunoglobulin (or IVIG) with highly sialylated N297 glycans was shown to have a 10-fold increased anti-inflammatory activity in multiple autoimmune disease models in mice, such as K/BxN serum induced arthritis^[3]. When it was sialylated, the IVIG switched its specificity from FcγRIIIA to a C-type lectin-like receptor. The C-type lectin-like receptor is specific intracellular adhesion molecule-grabbing nonintegrin R1 (SIGN-R1) in mice, which is expressed on macrophages in splenic marginal zone, or a human homolog protein named dendritic-cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), which is present on dendritic cells and macrophages.

In addition to the effects demonstrated with recombinant antibodies, the altered *N*-glycosylation of endogenous polyclonal antibody IgG was also observed in many autoimmune diseases. It

was first described ~31 years ago that relatively low galactosylation of IgG was associated with rheumatoid arthritis and osteoarthritis^[4]. This phenomenon was confirmed thereafter in many studies with more patients.

With advances in technologies used for glycan characterization, there have been increased reports recently, which showed altered *N*-glycosylation of IgG from patients with other autoimmune diseases, including systemic lupus erythematosus^[5], inflammatory bowel disease (ulcerative colitis and Crohn's disease)^[6], granulomatosis with polyangiitis (GPA)^[7], multiple sclerosis^[8] and Guillain-Barre syndrome^[9]. In most cases, the galactosylation and sialylation were reduced in IgG from patients as compared to those from a healthy population or from patients with disease remission (Figure 1). The changes in *N*-glycosylation were not only for galactosylation and sialylation, but also for core fucosylation or bisecting GlcNAc in certain autoimmune diseases^[5,6,8]. However, they varied with either increased or reduced levels depending on the individual disorder.

Until most recently, it was unknown whether the changes in *N*-glycosylation of endogenous antibodies reflect the alteration in disease associated autoantibodies, which could potentially explain the disease related mechanism.

In GPA, the *N*-glycosylation of the disease associated antibody, anti-proteinase 3 specific anti-neutrophil cytoplasm antibodies (ANCA), was changed^[7]. It showed reduced galactosylation, sialylation and bisecting GlcNAc compared to that in total IgG. The galactosylation of anti-proteinase 3 ANCA correlated with the amount of inflammatory cytokines in serum and time to remission in patients. Interestingly, it was also demonstrated by Ohmi *et al* that the reduced sialylation was a common feature of rheumatoid arthritis-associated IgG, such as anti-citrullinated protein antibodies, in human and mouse models of arthritis^[10].

Indeed, the altered *N*-glycosylation in the antibody is related to disease progression in humans. Harre *et al* reported the impact of IgG glycosylation on osteoclast differentiation and bone loss^[11]. They found enhanced osteoclastogenesis of non-sialylated IgG immune complexes *in vitro* and *in vivo*. While Fc sialylation of random IgG and specific IgG autoantibodies seemed to determine bone architecture in rheumatoid arthritis patients, increased sialylation resulted in less susceptibility to inflammatory bone loss. The amount of sialylation, but not galactosylation, in N297 glycans of IgG decreased in collagen-induced arthritis model^[10]. Loss of antibody sialylation in mice deficient in a sialyltransferase, ST6GalI, resulted in exacerbation of joint inflammation. When the sialylated anti-type II collagen antibodies, including anti-citrullinated protein antibodies, were injected into mice, they not only attenuated arthritogenic activity, but they also suppressed the development of collagen-induced arthritis. This data suggests that sialylated autoantibodies, but not other IgGs, have certain regulatory activity in modulating autoimmune disease. Further, the sialylation of N297 glycans in recombinant monoclonal antibodies impaired their complement-dependent cytotoxicity (CDC)^[12]. The fully sialylated antibodies were largely devoid of complement C1q binding with decreased levels of complement C3b deposition to the cell surface. When patients with chronic inflammatory demyelinating polyneuropathy (the most common chronic autoimmune neuropathy) were treated with IVIG, their disease remission was significantly associated with an induction of total IgG Fc sialylation. Those with high levels of IgG Fc sialylation showed significantly lower levels of complement activation. Further, galactosylation of IgG1 was demonstrated to modulate FcγRIIB-mediated as well as complement-mediated

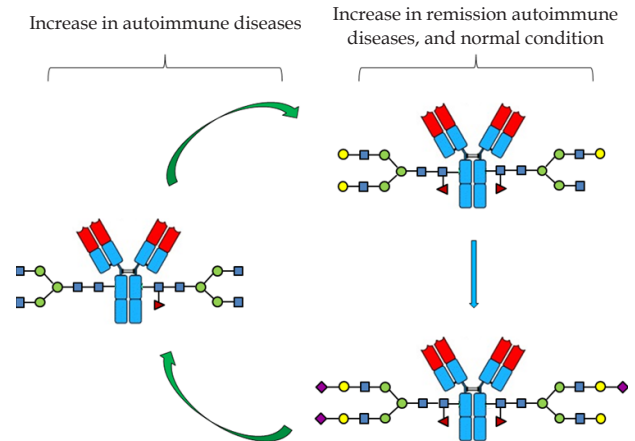


Figure 1 Alterations in N297 glycans of IgG are associated with many autoimmune diseases. The antibody galactosylation is reduced with proinflammatory activity, while galactosylation and sialylation results in less inflammation or more anti-inflammation.

immune suppression^[13,14].

Thus, the change of N297 glycans in disease associated autoantibodies can have an impact on their effector function as well as immune modulatory activity through a DC-SIGN or FcγRIIB related mechanism, which may have a direct effect on disease progression. On the other hand, the alteration in N297 glycans in total antibodies may also have an indirect immune regulation. The alteration in *N*-glycosylation in autoimmune diseases could result in a shift of the immune system toward a pro-inflammatory status.

The reduced level of galactosylation or sialylation of IgG in many autoimmune diseases may be due to the down-regulation of glycosyltransferases, including β1,4 galactosyltransferase and α2,6 sialyltransferase, expressed in human B cells through pro-inflammatory cytokines induced by environmental factors. However, recent studies also suggest that genetic factors also contribute to the alteration of *N*-glycosylation of IgG. A genome-wide association study of thousands of individuals identified 16 genetic loci associated with variations in IgG glycans^[15]. Among these loci, several were previously shown to be associated with many autoimmune diseases. Thus, changes in *N*-glycosylation of IgG could partly be a genetically predetermined predisposition and may be one of the molecular mechanisms related to the association between these genetic polymorphisms and autoimmunity.

The alterations in antibody N297 glycans can potentially be applied to disease risk assessment, prognosis, and follow-up of treatment efficacy. They may also have value for designing and engineering of future therapeutic antibodies to improve therapeutic index.

CONFLICTS OF INTEREST

The author has no conflicts of interest to declare.

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Peer reviewer: Yuriy Kit, Ph.D, D. Sci., Head of Laboratory of Molecular Mechanisms of of Intercellular Interactions at Department of Regulation of Cell Proliferation and Apoptosis of Institute of Cell Biology of National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005, Lviv, UKRAINE.