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Review

Host glycans and antigen presentation☆

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Abstract

The cell-mediated adaptive immune response depends upon the activation of T cells via recognition of antigen in the context of a major histocompatibility complex (MHC) molecule. Although studies have shown that alterations in T cell receptor glycosylation reduces the activation threshold, the data on MHC is far less definitive. Here, we discuss the data on MHC glycosylation and the role the glycans might play during the adaptive host response.

Keywords: Glycosylation; MHC class I; MHC class II; HLA; N-glycan; Antigen presentation

1. Introduction

The host response to infection is a multifaceted mechanism involving "innate" and "adaptive" immune activation in combinations that are only now becoming clear. From the adaptive side, the ability to present foreign antigen to T cells for recognition is a singular event that is required for the initiation of the downstream effector pathways, including $CD4^+$ Th2 T cells that promote antibody class switching, proinflammatory $CD4^+$ Th1 cells that promote microbial clearance, cytotoxic $CD8^+$ T cells that are critical for clearing infected host cells, or a number of other variants (*e.g.*, NKT cells). Indeed, the genetic variability of MHC molecules and the control of antigen presentation might be the two most important aspects of the adaptive immune response, and thus for protection against infectious disease.

It is not surprising that essentially every autoimmune disorder has some degree of linkage to at least one MHC allele. A rather famous example dating back to the mid-1970s is Ankylosing spondylitis which shows remarkably strong linkage to HLA-B27, although carrying that allele does not necessarily mean an individual will develop disease (or vice versa)(recently reviewed in Refs. [1,2]). While we will not directly address the linkages of MHC alleles with autoimmunity in this review, it is mentioned here for context in that MHC sits at the crossroads of the self-versus-non-self decision the immune system is faced with constantly. It is a delicate balance and when something alters that decision at the molecular level, trouble nearly always ensues.

2. The MHC glycoprotein family

The major histocompatibility complex (MHC), referred to as the human leukocyte antigen (HLA) in humans, is a family of structurally and genetically related glycoproteins [3] that present antigen from both exogenous (MHC class II; MHCII) and endogenous (MHC class I; MHCI) sources to T cells (recently reviewed in Ref. [4]). These glycoproteins are also key factors in lymphocyte development and assist in maintaining overall homeostasis [5,6]. The MHC family is highly polymorphic in some regions of the glycoprotein, while highly conserved in other domains, and are found as heterodimers at the cell surface [3]. Other members of the broad family include CD1, a MHCI homolog that is adapted to present glycolipids rather than the traditional peptide antigens presented by MHCI [7]. As with essentially every protein that travels the secretory pathway, MHC molecules are highly

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glycosylated. There are two major forms of protein glycosylation, O- and N-linked, but the dominant form found on MHC glycoproteins is N-linked [8]. What is perhaps most remarkable about the Asn-X-Thr/Ser acceptor sites for N-linked glycans in MHC molecules is their near absolute conservation across all animal species with MHC homologs. This suggests that there is strong selective pressure to maintain specific sites of glycosylation on MHC molecules, indicating that these glycans are critical for one or more aspects of MHC structure and/or function.

With respect to function, CD1 presents glycolipids such that the acyl chains are buried in long hydrophobic channels and the carbohydrate is protruding from the top of the molecule [7]. MHCI binds short peptides derived from proteins primarily localized in the cytoplasm of the cell, making MHCI the main pathway for presenting antigens from viruses and other intracellular pathogens [4]. MHCII, on the other hand, binds longer peptides derived from proteins released from lysed microbes in the endocytic pathway [4] as well as zwitterionic polysaccharides found within the capsules of some bacteria (*e.g.*, *Staphylococcus aureus* and *Bacteroides fragilis*) [9–11]. Finally, MHCII is also responsible for superantigen (*e.g.*, *Staphylococcus* enterotoxins) binding and the antigen presenting cell-T cell crosslinking [12] that initiates polyclonal and exaggerated T cell responses.

3. The N-glycosylation pathway

The N-glycosylation/secretory pathway in mammalian cells has been extensively reviewed in the past [13], but it is important for the reader to understand some basics for this review (summarized in Fig. 1). Again, we will focus our discussion of the N-linked glycosylation pathway since it is the dominant glycan form on MHC molecules. Proteins destined to be glycoproteins are synthesized in the endoplasmic reticulum (ER), where the oligosaccharyltransferase (OST) enzyme catalyzes the attachment of the initial $Glc_3Man_9GlcNAc_2$ glycan on target asparagine residues in a co-translational fashion. This core oligosaccharide binds to the ER chaperones calreticulin and calnexin, which assist in folding of the nascent glycoprotein [14,15]. The action of glucosidase enzymes then remove the terminal glucose molecules, thereby releasing the properly folded glycoprotein to proceed down the secretory pathway in the Golgi apparatus. As such, ER glycosylation can serve as a quality control step for the folding of glycoproteins.

Once the glycoprotein moves into the Golgi, the "high mannose" glycan attached to the asparagine site is further trimmed of most of its mannose residues by mannosidases [13]. At this point, the glycan receives its first additional GlcNAc residue by the GlcNAc transferase I enzyme (GlcNAcT-I), which is followed by further mannose trimming by the resident mannosidase. The result is termed a "hybrid" N-glycan, where a single branch of GlcNAc has been added but mannose residues remain at the termini [16]. The first step into the "complex-type" N-glycans is the addition of a second GlcNAc, this time by the GlcNAc transferase II (GlcNAcT-II) [17]. This critical step is then followed by the non-sequential modifications performed by the collective activity of other GlcNAcT enzymes (GlcNAcT-III through V) [18] as well as fucosyl-, sulfo-, galactosyl-, and sialyl-transferases [19].

To date, there are few known rules about how (or if) the nature of the glycan on a mature glycoprotein is regulated, but it is clear that metabolic activity of the cell, expression levels of the various enzymes, and the structure of the underlying protein backbone all contribute to the final result. Indeed, it is critical to understand that glycosylation is not a template-driven synthetic pathway, unlike protein and nucleic acid synthesis, and therefore provides complexity and heterogeneity even within the same molecule with multiple glycosylation sites.

4. Glycosylation of the MHC class I molecules

MHCI molecules are heterodimers comprised of a polymorphic transmembrane heavy chain and the non-glycosylated β2-microglobulin. Within the heavy chain, Asn86 is the single conserved site for N-glycosylation across all known alleles. As with essentially all glycoproteins, glycosylation of MHCI molecules can serve a number of fundamental roles. The ability of glycosylation to serve as a structural and folding check point has already been mentioned [20,21], but glycosylation could also act as a means to direct trafficking to the cell surface [20,21], to serve as a protective coat for the underlying protein against proteolytic cleavage via steric hindrance [22], and act as a mechanism by which optimal surface spacing is achieved within the plasma membrane for T cell recognition possibly through interactions with the galectin family of carbohydrate-binding proteins [23,24].

MHCI molecules serve as a key example of the importance of glycosylation in protein folding in ER [25]. The peptides that associate with MHCI molecules are derived primarily from the cytosol and are transported into the ER via the tapasin/TAP-dependent pathway [26,27]. As a result, peptide loading of MHCI occurs in the ER, prior to the N-glycan processing in the Golgi. In fact, the core $Glc_1Man_9GlcNAc_2$ glycan binding to the calnexin/calreticulin chaperones is critical to promote association of the heavy chain with β2-microglobulin as well as loading of the antigenic peptide into the binding groove [15]. This can be seen when N-glycosylation is inhibited or in cells that express mutant MHCI lacking the conserved asparagine, where the amount of properly folded and loaded MHCI at the cell surface is dramatically reduced [25]. Although it appears that the conserved N-glycan site on MHCI does not directly interact with or impact the nature of the peptide that is presented, the N-glycan is critical for antigen binding to occur by virtue of its importance in recruiting the chaperones to assist in peptide loading.

The importance of N-glycosylation in peptide loading of MHCI in the ER is well established, but the role for the complex N-glycan on MHCI after moving through the secretory pathway and to the cell surface for T cell recognition is much less clear. One possibility is found in a theory born out of the crystal-packing properties of purified immune receptors