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Glycan-mediated modification of the immune response

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Aberrantly glycosylated tumor antigens represent promising targets for the development of anti-cancer vaccines, yet how glycans influence immune responses is poorly understood. Recent studies have demonstrated that GalNAc-glycosylation enhances antigen uptake by dendritic cells as well as $CD4^+$ T-cell and humoral responses, but prevents $CD8^+$ T-cell activation. Here, we briefly discuss the relevance of glycans as candidate targets for anti-cancer vaccines.

Aberrant glycosylation is a key feature of carcinogenesis. The dense surface distribution of unique glycan structures on malignant cells makes carbohydrates attractive targets for the development of anti-cancer vaccines. In particular, mucin-type O-glycan synthesis is a complex, regulated process involving more than 50 gene products. A consistent feature of cancer cells is the aberrant truncation of mucin-type O-glycans to short monosaccharides and disaccharides, also known as pan-carcinoma antigens, Tn (GalNAcα-O-Ser/Thr) and STn (NeuAcα2,6GalNAcα-O-Ser/Thr). Truncated, immature glycans are recognized by natural IgM antibodies and by lectin receptors on antigen-presenting cells. This may lead to the presentation of aberrantly O-glycosylated proteins to the adaptive immune system.[1] Thus, immune responses may be directed against aberrant carbohydrate structures, peptide epitopes in the protein backbone, as well as against new epitopes generated by an aberrant O-glycan structure combined with the protein backbone. The latter O-glycopeptide epitopes are particularly interesting as they are virtually specific of malignant cells.[2]

Besides their usefulness in developing vaccines that target glycopeptide antigens, glycans may also improve the uptake of tumor antigens by dendritic cells (DCs). This strategy could open several new avenues for the design of anti-cancer vaccines. Antigen-presenting cells are armed with a variety of carbohydrate receptors (**Fig. 1**).[1] When several of these receptors are targeted, antigen uptake increases and several intracellular signaling pathways are activated, resulting in cytokine secretion, cell activation, phagocytosis, and antigen presentation. The latter is pivotal for the differentiation of helper T cells and hence for the elicitation of adaptive immune responses.[1] Targeting an antigen to the DC mannose receptor has been shown to stimulate the activation of $CD4^+$ and, to a lesser extent, $CD8^+$ T cells.[3] Similarly, the incorporation of Lewis structures into protein antigens has been shown to result in the targeting of the DC receptor DC-SIGN.[4] In addition, we and others have demonstrated an important role for GalNAc residues in DC antigen uptake and functional responses.[5–7] In contrast to other methods for targeting antigen-presenting cells, including liposomal delivery systems, the loading of DCs in vitro and Toll-like receptor (TLR)-targeted delivery, glycans are advantageous in that they increase the specificity of elicited humoral responses for cancer cells. The choice of the glycan might also affect MHC presentation, and this might be used to obtain the desired type of immune response from a given vaccine.[1] It is important to note, however, that the glycosylation of peptide antigens may complicate the antigen processing and presentation. This is particularly true for $CD8^+$ T-cell responses, as in some locations GalNAc residues can compromise the function of the immunoproteasome.[8]

We have recently examined the influence of GalNAc on antigen uptake, MHC presentation, as well as on the activation of $CD4^+$ and $CD8^+$ T cells.[5] We chemoenzymatically synthesized an array of model peptides encompassing MHC Class I and II ovalbumin (OVA)-derived epitopes fused to peptides derived from the prototypic tumor-associated antigen mucin 1 (MUC1), with and without GalNAc residues. MUC1 is a surface-associated mucin that carries a high number of O-linked glycans. MUC1 is highly upregulated in a majority of epithelial cancers. Interestingly, the presence of GalNAc residues increased the MHC Class II-dependent activation of $CD4^+$ T-cells but inhibited the processing and presentation of OVA epitopes in association to MHC Class I molecules in a dose dependent manner. In line with these findings, the immunization of mice with GalNAc-glycosylated MUC1 abolished $CD8^+$ T-cell responses. This was originally interpreted as a processing problem, but the $CD8^+$ T-cell response was abrogated regardless of the location at which GalNAc

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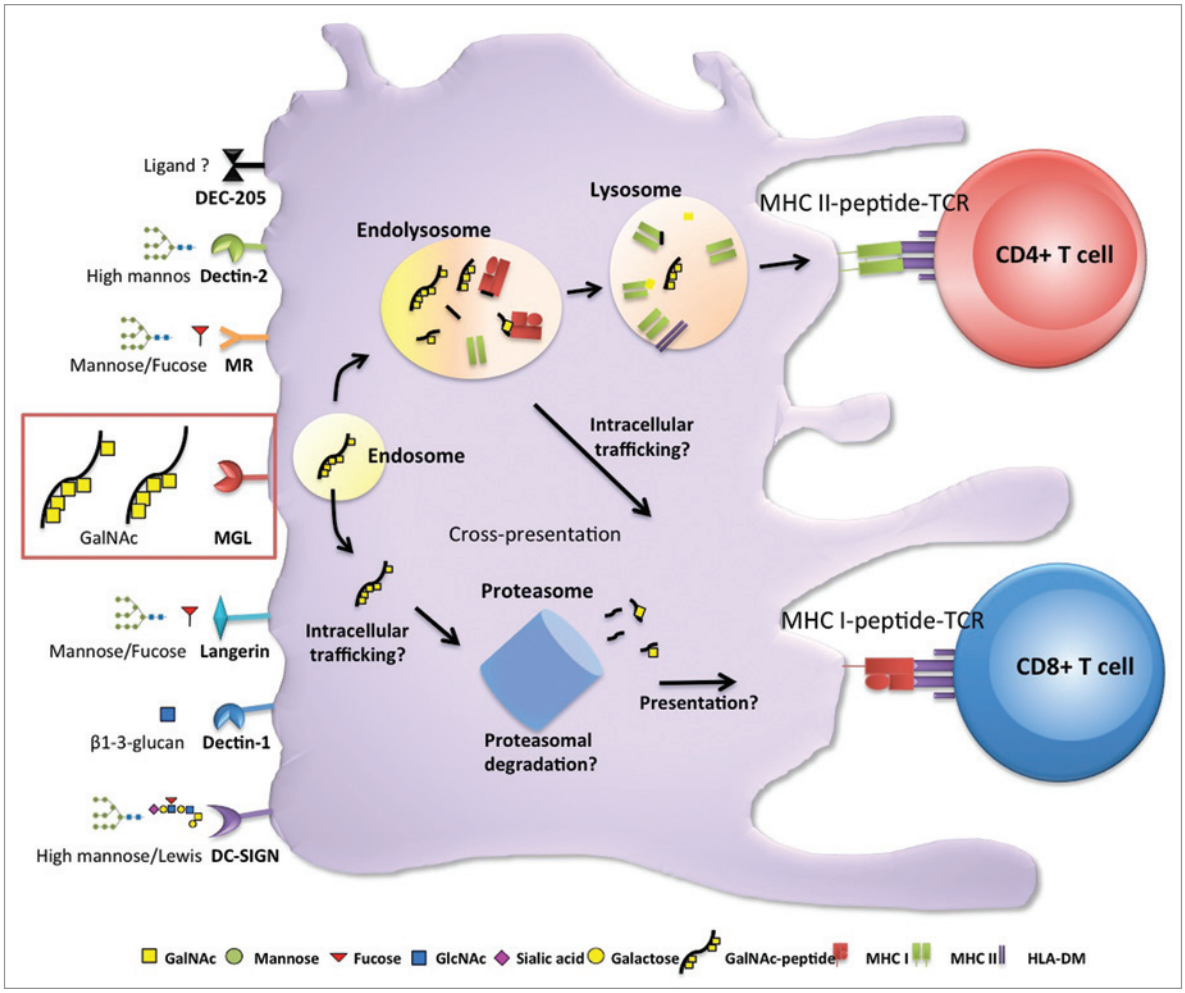

Figure 1. Uptake, processing and presentation of a GalNAc-glycosylated antigen in dendritic cells. Antigen-presenting cells are armed with a variety of receptors that bind specific carbohydrates (ligands), including MGL (GalNAc), DC-SIGN (high mannose/Lewis structures), dectin 1 (β1–3-glucan), dectin 2 (high mannose), mannose receptor (mannose and fucose), Langerin (mannose and fucose) and DEC205 (ligand not yet identified with certainty). The addition of GalNAc moieties to a mucin 1 (MUC1)-targeting vaccine may potentiate dendritic cell (DC) uptake and increase MHC Class II presentation, most likely through the normal antigen presentation pathway. However, it is not clear how GalNAc-modified peptides are cross-presented on MHC Class I molecules. When processed correctly, small glycan moieties have been shown to fit into the MHC Class I peptide-binding groove, eliciting an MHC Class I-restricted, glycopeptide-specific, $CD8^+$ T-cell response. However, the pathways leading from the uptake of GalNAc-glycosylated antigens to their proteasomal processing and cross-presentation on MHC Class I molecules are not well characterized. Antigen processing in the proteasome might also be negatively affected by GalNAc glycosylation, depending on the location of the GalNAc residues. Conjugation and the choice of a linker region can cause steric hindrance, potentially interfering with antigen processing via the MHC Class I pathway. Indeed, GalNAc-modified antigens appear to preferentially generate $CD4^+$ T-cell responses, resulting in increased activation of $CD4^+$ T cells at the expenses of their $CD8^+$ counterparts.

residues were incorporated. Indeed, even when GalNAc residues were placed far from the actual T-cell epitope with appropriate cleavable linkers, very little MHC Class I and $CD8^+$ T-cell responses were generated. Thus, we hypothesized that the lack of $CD8^+$ T-cell responses against GalNAc-glycosylated epitopes might be due to an alternative intracellular routing of these antigens by DCs.[5–8] Alternatively, DC effector functions might be reduced upon the uptake of glycosylated antigens, or the latter may trigger an early acidification of endosomes (discussed in ref. 5). It is also possible that a spatial restriction in the MHC-peptide-TCR interaction might play a role, although this would depend on the size and localization of the glycan structure within the antigen. Glycans can fit into the MHC groove, creating a MHC-restricted glycopeptide binding. Moreover, glycans can protrude away from the MHC groove into the TCR pocket, inducing a glyco-specific immune response.[9] These observations suggest that it may be possible to design a small antigen that contains a single GalNAc-glycosylation coupled to a DC-targeting moiety. This design would avoid the potential localization and processing problems inherent to highly GalNAc-glycosylated antigens, and hence allow for the induction of $CD8^+$ T-cell responses that maintain the specificity for cancer cells dictated by glycopeptide epitopes. Lakshminarayanan et al. have developed such type of vaccine, consisting of a TLR2 agonist (Pam_3CysSK_4), a T_H epitope from poliovirus and a MUC1-derived epitope.

In MUC1-transgenic mice, this vaccine elicited both a glycopeptide-specific cellular response that targeted a known $CD8^+$ T-cell epitope within the MUC1 tandem repeat and a humoral response.[10]

A similar vaccine design, however, harbors several limitations that complicate its application to other tumor-associated antigens. A different and relatively simple solution would be to use a two-component vaccine, consisting of a glycosylated $CD4^+$ T-cell epitope and a separate, single site, O-glycosylated or non-glycosylated $CD8^+$ T-cell epitope coupled to an alternative DC-targeting moiety. Alternatively, a sugar moiety could be used to target a $CD8^+$ T-cell epitope to DCs. This would require an optimal linker that can be cleaved upon cellular uptake to avoid interferences with antigen localization and processing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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