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Interleukin-2, Interleukin-7, T cell mediated autoimmunity and N-glycosylation

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Summary

T cell activation and self-tolerance are tightly regulated to provide effective host defense against foreign pathogens while deflecting inappropriate autoimmune responses. Golgi Asn (N)-linked protein glycosylation co-regulates homeostatic set points for T cell growth, differentiation and self-tolerance to influence risk of autoimmune disorders such as multiple sclerosis (MS). Human autoimmunity is a complex trait that develops from intricate and poorly understood interactions between an individual's genetics and their environmental exposures. Recent evidence from our group suggests that in MS, additive and/or epistatic interactions between multiple genetic and environmental risk factors combine to dysregulate a common biochemical pathway, namely Golgi N-glycosylation. Here, we review the multiple regulatory mechanisms controlling N-glycan branching in T cells and autoimmunity, focusing on recent data implicating a critical role for interleukin-2 (IL-2) and interleukin-7 (IL-7) signaling.

Keywords

Autoimmunity; T cells; N-glycosylation; Interleukin-2 (IL-2); Interleukin-7 (IL-7)

Introduction

T cells are the master regulators of the antigen-specific adaptive immune response and play a central role in the delicate balance between protective immunity and tolerance of self-antigen. T cell activation and self-tolerance are tightly regulated and have evolved to provide effective host defense against foreign antigens while deflecting inappropriate inflammatory and autoimmune responses directed against the host^{1, 2}. The T cell growth cycle is defined by distinct temporal phases comprising 1) basal signaling in the absence of antigen, 2) antigen - major histocompatibility complex (MHC) induced activation signaling via the T cell receptor (TCR), 3) multiple rounds of cell division, 4) growth arrest via cytotoxic T-lymphocyte antigen-4 (CTLA-4) and transforming growth factor- β receptor

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(T β R), 5) differentiation into effector T cells and 6) apoptosis. Dysregulation at any of these phases can lead to aberrant T cell function and promote autoimmune pathogenesis. The common cytokine receptor γ -chain (γ_c) family cytokines have important functions in T cell proliferation, differentiation and survival. Interleukin-2 (IL-2) and interleukin-7 (IL-7) are both potent T cell growth factors and have been widely considered to be key cytokines controlling the effector and regulatory arms of the immune system, yet their underlying mechanisms are not entirely clear^{3, 4}.

Although T cell fate and function have largely been considered to be dictated by changes in gene expression and protein production, alterations in protein glycosylation is increasingly recognized as a major regulatory mechanism. Here, we review evidence suggesting that cytokine signaling by IL-2 and IL-7 regulates T cell function and self-tolerance through changes in protein N-glycosylation. Multiple genetic and environmental factors also influence N-glycosylation, providing an example of the complex genetic and environmental interactions converge on a single pathway to influence autoimmune diseases such as multiple sclerosis (MS).

Regulation of T cells by N-glycosylation

Differential regulation by N-glycan number and branching

Most cell surface receptors and transporters are co- and post-translationally modified with asparagine (N)-linked glycans in the endoplasmic reticulum and Golgi apparatus following sequential but incomplete action of a series of glycohydrolases and glycosyltransferases^{5, 6}. Eukaryotic cell surfaces are heavily glycosylated and the size, abundance and complexity of these glycan structures provide information encoding distinct from the genome⁷. In contrast to proteins and nucleic acids, production of complex carbohydrates is not template driven. Rather, N-glycan biosynthesis depends on the nutrient environment of the cell, metabolic supply of substrates and enzymatic activities of the ER/Golgi enzymes. Cell surface N-glycans serve as ligands for a number of carbohydrate binding protein families, including galectins, siglecs and selectins, all of which play important roles in immunity. Here we focus on the role of N-glycosylation in titrating binding to galectins, interactions that regulate glycoprotein distribution and concentration at the cell surface to affect T cell function and autoimmunity^{8–10}.

Galectins are ubiquitously expressed at the cell surface and extracellular matrix and bind multivalent glycan ligands to form microdomains or molecular “lattices” at the cell surface^{9, 11, 12}. The minimal binding structure for galectins is N-acetyllactosamine (LacNAc: Galactose β 1,4N-acetylglucosamine)¹³ and binding avidity for individual glycoproteins increase in proportion to the degree of LacNAc content within the attached N-glycans. LacNAc density is determined by the degree of N-acetylglucosamine (GlcNAc) branching per N-glycan (nutrient/environment dependent) as well as the number of N-glycans per glycoprotein (gene-encoded; occupied N-X-S/T sites)⁷. N-glycan branching and structural diversity produced in the Golgi is dependent upon the expression and activity of Golgi α -mannosidases and N-acetylglucosaminyltransferases I, II, IV, and V (encoded by *Mgat1*, 2, 4 and 5) and the metabolic supply of the substrate uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) via the hexosamine pathway^{8, 14, 15}.

The multivalent galectin-glycoprotein lattice controls the function of cell surface receptors by regulating membrane localization, lateral mobility, clustering, cell surface retention and endocytosis rates^{8–10, 16–23}. Growth-promoting receptors (e.g. TCR and receptor tyrosine kinases) typically have high numbers of N-glycans ($n > 5$) while growth inhibitory receptors (e.g. T β R and CTLA-4) have few N-glycans ($n \leq 4$). Higher density of N-glycans within a glycoprotein generates significant avidity for galectins at low levels of branching, resulting in significant surface retention under basal Golgi conditions. In contrast, a low density of N-glycans falls below the threshold for stable association under basal Golgi activity and requires high levels of branching activity to incorporate into the galectin lattice and maintenance at the cell surface. For example, with increased Golgi branching activity, surface levels of glycoproteins with high N-glycan density increase with a steady, modest incline, whereas glycoproteins with low N-glycan density increase with a sharp, steep incline.

Golgi branching activity depends on both the activity of Golgi enzymes and metabolic supply of UDP-GlcNAc. As such, the strength of the galectin-glycoprotein lattice reflects both genetic and metabolic conditions to modulate receptor and transporter surface levels and cellular sensitivity to ligands and cytokines. The distinct N-glycan profile of each glycoprotein allows for differential association with the galectin lattice dependent on Golgi branching activity, thereby determining the intricate balance between growth stimulatory and inhibitory receptors at the cell surface. In this manner, the Golgi can globally set growth characteristics of cells, controlling cellular transitions from growth to arrest/differentiation⁸.

Control of TCR signaling and CTLA-4 mediated growth arrest by N-glycans

T cell expansion and inhibition are tightly regulated to ensure effective immune responses. Full T cell activation requires TCR clustering above a certain threshold, determined by engagement of the TCR with a specific MHC-peptide complex and co-signals via CD28. Binding of galectins to the multiple N-glycans of the TCR complex prevents spontaneous TCR oligomerization in the absence of antigen and subsequent filamentous (F)-actin-mediated transfer to lipid microdomains and activation of CD4-lymphocyte protein tyrosine kinase (Lck)¹⁶. Concurrently, galectin binding to the tyrosine phosphatase CD45 blocks F-actin-mediated movement of CD45 out of the lipid microdomain, thus maintaining a negative regulator of TCR-Lck signaling within signaling microdomains. In this manner, N-glycan branching directly controls basal TCR signaling. The same molecular mechanisms serve to set T cell activation thresholds in response to peptide-MHC. Galectin binding to the TCR complex inhibits ligand-dependent TCR clustering while concurrently promoting CD45 at the immune synapse^{9, 16, 22, 24}. For example, mice deficient in the branching enzyme β 1,6-N-acetylglucosaminyltransferase V (Mgat5) or β 1,3-N-acetylglucosaminyltransferase (β 3GnT2), an enzyme that extends branched N-glycans with poly-LacNAc, display lower thresholds to TCR clustering, T cell activation and autoimmune disease^{9, 25}. Moreover, removal of N-glycan sites in the TCR results in increased receptor diffusion, clustering and activation²². Galectin binding to the TCR also inhibits CD8 binding, hindering complex formation¹⁷.

Inhibitory regulators of the T cell response are critical for T cell growth arrest, differentiation and T cell tolerance. CTLA-4 is an inhibitory receptor that competes with CD28 for CD80/CD86 co-stimulatory ligand on antigen presenting cells (APCs) and is induced to the cell surface 4–5 days following T cell activation to initiate growth arrest²⁶. The levels of CTLA-4 transcript and intracellular stores of the protein increase with TCR signaling during the early T cell growth phase. However, endosomal trafficking limits CTLA-4 expression at the cell surface and therefore CTLA-4 is predominantly located in endosomes. Similar to other growth-inhibitory receptors, CTLA-4 has a low number of N-glycans (one - two N-X-S/T sites in humans) and is highly sensitive to UDP-GlcNAc and N-glycan branching by the Golgi to increase binding avidity for galectins. Integration of CTLA-4 into the lattice enhances surface retention by opposing endocytic loss, resulting in sustained and increased growth arrest signaling. TCR signaling increases metabolic flux to N-glycan branching, contributing to the incorporation of CTLA-4 into the galectin lattice to sustain arrest signaling⁸. T cells deficient in Mgat5 or deficient in the galectin lattice (i.e. chemical inhibitors of branching) have decreased levels of CTLA-4 at the cell surface, but high levels inside the cells indicating a deficiency in surface retention. Cell surface levels can be enhanced by metabolic supplementation to UDP-GlcNAc as well as maximal CD28 co-stimulation, which drives metabolism.

Consistent with serving as a critical regulator of both early and late T cell growth, deficiency in N-glycan branching promotes spontaneous autoimmunity. On the 129/Sv background, Mgat5 deficiency induces spontaneous kidney autoimmunity and increased susceptibility to Experimental Autoimmune Encephalomyelitis (EAE), a model of MS⁹. Several mouse strains highly susceptible to EAE (PL/J, SJL and NOD) display intrinsic deficiency in N-glycan branching in T cells when compared with resistant strains (129/Sv, BALB/c, and B10.S)²⁷. The PL/J strain, with the lowest levels of N-glycan branching, contains natural deficiencies in multiple N-glycan branching enzymes (i.e. Mgat1, 2, and 5) as observed by mass spectroscopy and enzyme assays. PL/J mice with targeted deficiency in Mgat5 develop a spontaneous, late-onset clinical MS-like disease manifested by inflammatory demyelination and neurodegeneration²⁷. A much milder form of disease is observed in wild-type PL/J mice, consistent with the defective N-glycan branching inherent to this inbred strain. As discussed below, defective regulation of N-glycan branching in human T cells is also directly associated with MS.

Environmental/Metabolic regulation of N-glycan branching

N-glycan branching in T cells is influenced by metabolism, nutrient status and environmental factors (Figure 1), providing a potential explanation and underlying molecular mechanism for the environmental influence of T-cell mediated autoimmune disease. Golgi Mgat1, 2, 4, and 5 catalyze N-glycan branching using the same sugar-nucleotide donor (i.e. UDP-GlcNAc), but with declining efficiency⁷. Mgat1 and Mgat2 activities are limited by low affinity for N-glycan acceptors, whereas Mgat4 and Mgat5 activities are limited by UDP-GlcNAc concentrations. Therefore, initiation of branching by Mgat1 and 2 depends on rates of bulk protein synthesis while branching by Mgat4 and 5 is sensitive to metabolic production of UDP-GlcNAc. Biosynthesis of UDP-GlcNAc by the hexosamine pathway requires highly regulated intermediates of carbohydrate, nitrogen, and

fatty acid metabolism¹⁴. Indeed, increased supply of glucose, glutamine (a critical nitrogen metabolite), acetyl-CoA (final metabolite of free fatty acids), glucosamine and N-acetylglucosamine (GlcNAc) increases N-glycan branching in T cells in culture, suppressing T cell growth. Oral GlcNAc supplementation in mice also increases N-glycan branching and results in inhibition of Th1 and Th17 responses, EAE and autoimmune diabetes^{14, 28}. Simon Murch and colleagues observed that oral GlcNAc therapy in children with treatment-resistant inflammatory bowel disease inhibited clinical disease in 8 out of 12 cases²⁹. Limited sunlight exposure and associated vitamin D₃ deficiency is a well established environmental risk factor for autoimmune diseases such as MS and Type 1 diabetes. We recently reported that Vitamin D₃ regulates the expression of several Golgi enzymes to enhance N-glycan branching and suppress T cell growth and EAE in mice³⁰. Thus, environmental factors such as sunlight/vitamin d3 and metabolism/nutrient supply can directly influence autoimmune pathogenesis by regulating N-glycan branching and the effector T cell response (Figure 1).

IL-2 and IL-7 regulation of T cell growth via N-glycan branching

Regulation of T-cell dependent immunity by IL-2 and IL-7

The common cytokine receptor γ -chain (γ_c) family cytokines have central roles in the regulation of T cell growth and function, including development, proliferation, survival and peripheral tolerance. The Interleukin-2 receptor (IL-2R) is the prototypical member of this family and consists of three subunits: the α -chain, the β -chain, and the γ_c -chain that is shared by IL-4R, IL-7R, IL-9R, IL-15R and IL-21R. IL-2 is a potent T cell growth factor identified nearly 30 years ago³¹ that is well-known to induce the proliferation and survival of TCR-activated mouse and human T cells^{32, 33} and is required to sustain expansion of T cell populations³⁴. Its crucial role in promoting T cell-dependent immune responses became questionable when it was unexpectedly found that IL-2 deficiency in mice resulted in lymphoproliferation followed by lethal autoimmunity^{35–37}. These findings indicate that a critical function of IL-2 is to regulate peripheral T cell tolerance. Subsequent studies revealed that IL-2/IL-2R signaling is essential for regulatory T cell (T_{reg}) development and peripheral homeostasis^{38–40}. The reduction of T_{reg} cells is thought to be a major factor in the autoimmunity associated with IL-2/IL-2R deficiency as studies show that transfer of T_{reg} cells or their reconstitution with IL-2 in these mice prevents lymphoproliferation and autoimmunity^{41–43}. However, it has also been shown that transgenic overexpression of CTLA-4 can control the lymphoproliferation in IL-2 deficient mice, suggesting an important role for IL-2 in CTLA-4 mediated effector cell growth arrest⁴⁴.

Extensive studies indicate IL-2 is responsible for both generating and subsequently, limiting the T cell-dependent immune response by regulating clonal expansion, effector and T_{reg} cell development and contraction of antigen-specific T cells. The use of IL-2 in the past to boost immune responses and the current status of evaluating its use to inhibit immune responses highlights the need to re-evaluate the current understanding of the biology of IL-2. It is evident that IL-2 has a wide range of actions with multi-faceted roles, promoting growth early in T cell activation but limiting expansion later; yet the underlying molecular mechanisms remain unclear.

The Interleukin-7 (IL-7) receptor (IL-7R) is another member of the γ_c family and is a heterodimer comprised of IL-7R α and the γ_c . IL-7/IL-7R is critical for the development⁴⁵ and survival^{46, 47} of several blood cell types. Mice mutant for IL-7⁴⁶ or either component of its receptor⁴⁵ have major hematopoietic abnormalities and exhibit T-cell-negative (T-), B-cell-negative (B-), natural killer cell-negative (NK-) severe combined immunodeficiency (SCID). In humans, loss of function mutations in the IL-7R α gene have been found to cause T-cell-negative (T-), B-cell-positive (B+), natural killer cell-positive (NK+) SCID⁴⁸. In addition, IL-7 has also been found to be critical for the survival and homeostatic cycling of T-cells in the periphery^{47, 49, 50}, a process that also depends on signaling through the TCR. However, the effector mechanisms underpinning IL-7 signaling and in particular the crosstalk with TCR signaling remain ill-defined.

Our recent data has revealed that IL-2 and IL-7 are major regulators of N-glycan branching in T cells (Figure 1), suggesting that many of the complex and paradoxical actions of these cytokines may be mediated through changes in branching³⁰. IL-2 and IL-7RA deficient *ex vivo* resting mouse T cells display marked increases in N-glycan branching up to ~2.5 fold. In resting human cells, exogenous IL-2 and IL-7 inhibit N-glycan branching. Alterations in N-glycan branching significantly smaller than those observed with IL-2/IL-7R deficiency are sufficient to affect T cell function, growth and autoimmune risk. Therefore, the much larger changes observed with IL-2 and IL-7 deficiencies are highly significant and imply essential regulatory roles for these cytokines in N-glycosylation. Indeed, our data reveals that IL-2 promotes T cell growth early by lowering N-glycan branching through enhanced TCR signaling yet later increases growth arrest of T cell blasts by enhancing branching and CTLA-4 surface retention (Figure 1)³⁰.

IL-2 or IL-7 both modulate mRNA levels of multiple Golgi branching enzymes, but in opposite direction to the changes induced by TCR signaling, the latter an enhancer of N-glycan branching^{30, 51}. However, IL-2 and IL-7 have opposite effects on N-glycan branching in resting and activated T cells, reducing branching in the former while increasing branching in blasting T cells. These opposing effects on resting and blasting T cells appear to result from up-regulation of Mgat1. Due to large differences in Km's, the Mgat1 enzyme sequesters UDP-GlcNAc from the distal Mgat4 and Mgat5 enzymes, thereby reducing branching when UDP-GlcNAc is limiting⁸. IL-2 signaling increases Mgat1 while decreasing Mgat5 and when coupled with low intracellular UDP-GlcNAc levels in resting T cells, results in reduced branching. However, TCR activation signaling increases Mgat5 activity, cell metabolism and UDP-GlcNAc levels, thereby allowing IL-2 induced increases in Mgat1 in T cell blasts to increase N-glycan branching by supplying more glycoprotein substrate to downstream enzymes. In this manner, IL-2 promotes T cell growth early via reduced N-glycan branching and enhanced TCR clustering/signaling while later promoting growth arrest by enhancing branching and CTLA-4 surface retention. Thus, through modulation of Golgi enzymes, IL-2 produces opposing effects on T cell growth early and late in activation, mechanisms sensitive to the metabolic state of the cell. Activation-induced cell death (AICD) is also mediated by receptors low in N-glycan number (i.e. Fas, FasL), which are likely to also rely critically on Golgi N-glycan branching for cell surface retention and are therefore also expected to be promoted later by IL-2 induced increases in branching.

IL-2 also plays an important role in the development and frequency of T_{reg} cells. Spontaneous autoimmunity in Mgat5^{-/-} mice develops despite increases in T_{reg} cells indicating hyperactive effector cell responses and ineffective T_{reg} cell function²⁷. Many human autoimmune disorders including MS, type 1 diabetes and rheumatoid arthritis do not associate with reduced T_{reg} cell numbers, but rather alteration in T_{reg} cell function. As IL-2 controls N-glycan branching in T_{reg} cells, suppressor activity and tolerogenic function may also be affected by changes in N-glycan branching and galectin interactions. CTLA-4 is required for T_{reg} cell function and autoimmune suppression in mice⁵², suggesting that dysregulation of N-glycan branching may alter CTLA-4 surface levels and subsequent T_{reg} cell function.

Maintenance of the peripheral T-cell pool requires a low level proliferative cycling of naïve and memory cells. Under lymphopenic conditions, this proliferative rate is dramatically increased in an effort to reconstitute the T-cell pool, and is referred to as homeostatic peripheral expansion (HPE)^{53, 54}. HPE has been shown to be largely deficient in the absence of IL-7^{47, 49}. Several studies indicate that TCR signal strength is also critical for HPE, with IL-7 neutralization having greatest effect on the slower proliferating pool characteristic of responses to low affinity antigens, but little effect on the most rapidly proliferating pool with high affinity TCR^{50, 55}. This conclusion is consistent with our recent finding that IL-7 decreases N-glycan branching in T-cells. In the absence of IL-7, the resulting increase in N-glycan branching increases the threshold antigen affinity required for TCR clustering, and thus is expected to produce an inhibitory effect that is dominant in low antigen affinity T-cells. These findings suggest that IL-7 enhances TCR signaling and activation of peripheral T-cells by downregulating N-glycan branching. Thus, IL-7 mediated peripheral T-cell survival and homeostatic proliferation may in part be due to promotion of basal TCR signaling through reductions in N-glycan branching.

Although IL-2 and IL-7 have similar effects on N-glycan branching, targeted deficiencies of these pathways in mice paradoxically induce opposing phenotypes, lymphoproliferation/lethal autoimmunity or immunodeficiency, respectively. This is likely due to the expression pattern of their respective receptors, which in turn dictate the dominant effect of deficiency on N-glycan branching. In contrast to IL-2R, whose expression increases with T cell activation and/or cytokine signaling, IL-7R α is expressed by most resting T cells and is down-regulated following T cell activation and/or cytokine signaling^{56, 57}. Thus, the dominant effect of IL-7 signaling *in vivo*, is to reduce branching in resting T-cells and early in activation, thus to promoting TCR clustering/signaling and T cell growth. The non-redundant role of IL-2 is likely later in activation, when it acts to promote growth arrest by upregulating branching and CTLA-4. Continuous IL-7-mediated signaling in naïve T cells induces co-stimulatory and anti-apoptotic responses necessary for survival. IL-7R α expression is low in FOXP3⁺ T_{reg} cells and it is interesting to note that in contrast to non-T_{reg} cell populations, activated FOXP3⁺ T_{reg} cells up-regulate expression of IL-7R⁵⁸. Signaling by IL-2 and IL-7 affect the expression of both their own as well as other cytokine receptors, allowing for intricate cross-regulation and confounding interpretation of studies looking just at cytokine levels. A model that integrates and sums the many inputs affecting

N-glycan branching may illuminate seemingly complex and contradictory regulation by these cytokines.

MS risk factors alter N-glycosylation in T cells

As with other complex trait diseases, multiple genetic and environmental factors combine to determine disease risk in autoimmune disorders such as MS and type 1 diabetes. However, defining how these factors combine at the molecular level to promote disease has been a great challenge. A role for genetics in MS was defined in studies showing first-degree relatives and identical twins display ~20–40 and ~300 fold increases risk over the general population⁵⁹. Candidate gene studies have validated association of MS with genes in the MHC region and more recent genome wide association studies (GWAS) have identified a number of potential genes associated with MS including receptors for IL-7 (*IL7RA*) and IL-2 (*IL2RA*)⁶⁰. *IL7RA* and *IL2RA* also associate with Type 1 diabetes and the IL-2 and IL-7 pathways have previously been demonstrated to regulate autoimmunity and EAE in animal models^{4, 45, 61–63}. Although many potential genetic risk factors for MS have been identified, critical issues remain. Most identified variants confer relatively small increments in disease risk and explain only a small proportion of the genetic variance⁶⁴. Furthermore, monozygotic twins co-develop MS only ~30% of the time⁵⁹, inferring direct environmental impact on genetic risk. Indeed, recent studies by Baranzini *et al* have reported that there is no evidence for genetic, epigenetic, or transcriptome sequence variation to explain disease discordance in monozygotic twins⁶⁵. Despite known genetic and environmental risk factors for MS, there appears to be no obvious shared molecular mechanisms or pathways. These observations may be explained by epistatic and/or additive interactions between multiple seemingly unrelated alleles and environmental factors that converge to dysregulate a critical final common pathway. Consistent with this hypothesis, we recently reported that multiple genetic variants (*IL7RA*, *IL2RA*, *MGAT1* and *CTLA-4*) interact with multiple environmental factors (vitamin D₃ deficiency and metabolism) to dysregulate Golgi N-glycosylation in MS (Figure 1)³⁰.

The *IL2RA**T and *IL7RA**C MS risk alleles are associated with enhanced secretion of soluble receptors that block signaling by cognate cytokines. Consistent with the effects of IL-2 and IL-7 on N-glycan branching, the soluble receptors associated with the *IL2RA**T and *IL7RA**C MS risk alleles down-regulate *MGAT1* mRNA and N-glycan branching in human T cell blasts³⁰. Based on this, we screened the *MGAT1* gene for functional variants. Indeed, we defined an MS-associated gain of function haplotype of *MGAT1* (IV_A and V_{T-T} polymorphisms) that reduces or enhances N-glycan branching depending on metabolism and UDP-GlcNAc supply to the Golgi (Figure 1). The *MGAT1* IV_AV_{T-T} variant enhances mRNA levels and enzyme activity ~2–3 fold, thereby increasing the N-glycan product of Mgat1 but also limiting UDP-GlcNAc supply to downstream Mgat4 and Mgat5. As Mgat4 and Mgat5 activities are limited by UDP-GlcNAc concentrations, increased Mgat1 protein out-competes Mgat4 and Mgat5 for UDP-GlcNAc under basal UDP-GlcNAc levels, thereby reducing N-glycan branching. However, with increasing UDP-GlcNAc and/or Mgat5 levels, enhanced Mgat1 expression is not as effective in limiting UDP-GlcNAc supply to Mgat4 and Mgat5, allowing these enzymes to act upon the increased supply of N-glycan acceptors to increase N-glycan branching. Thus, the phenotypic effect of the *MGAT1* IV_AV_{T-T} variant

depends upon the metabolic status of the cell, providing an example of how the same genetic risk factors may promote or inhibit MS based on environmental variation.

Importantly, the *IL2RA**T and *IL7RA**C MS risk variants lower *MGATI* mRNA while the *MGATI* IV_A V_{T-T} risk haplotype enhances *MGATI* mRNA. Consistent with these effects, up-regulation of Mgat1 by IL-2 and/or IL-7 signaling increases N-glycan branching in *MGATI* IV_A V_{T-T} non-carriers, but decreases N-glycan branching in *MGATI* IV_A V_{T-T} carriers³⁰. In other words, IL-2 and/or IL-7 signaling up-regulates Mgat1 and either enhances or reduces N-glycan branching depending on baseline Mgat1 activity.

The Thr17Ala polymorphism in the human *CTLA-4* gene (49A/G) associates with Type 1 diabetes but not MS, encodes a signal peptide variant with inefficient glycosylation (N-glycan occupancy at the two N-X-S/T sites is reduced from two to one) and results in decreased CTLA-4 surface levels to enhance T cell growth^{66–69}. The *MGATI* IV_A V_{T-T} variant also limits CTLA-4 surface levels when expressed with the common *CTLA-4* allele with two N-glycans, yet combining it with the *CTLA-4* Ala17 with one N-glycan further reduces CTLA-4 surface levels³⁰.

The non-linear biological interactions observed between *IL2RA**T, *IL7RA**C, *MGATI* IV_A V_{T-T} and *CTLA-4* Thr17Ala predict similar genetic interactions in MS. Indeed, the expected genetic interactions observed between these four genetic variants in MS are summarized in Figure 1. It is interesting to note that the active form of Vitamin D₃, 1,25(OH)₂D₃ enhances *MGATI* mRNA levels similar to the *MGATI* IV_A V_{T-T} variant but opposite of the *IL2RA**T and *IL7RA**C risk alleles, providing a molecular mechanism for the association of vitamin D₃ deficiency in MS.

In summary, complex genetic interactions regulate N-glycan branching, T cell activation thresholds, and CTLA-4 surface levels with sensitivity to metabolic conditions, vitamin D₃, Golgi enzyme activity, cytokine signaling (IL2, IL7), and the number of N-glycan sites. Complex trait diseases such as common autoimmune disorders develop from intricate and poorly understood interactions between genetics and the environment. Our data suggests that epistatic and additive interactions between multiple seemingly un-related genetic and environmental risk factors combine to dysregulate a common biochemical pathway, namely Golgi N-glycosylation.

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a)

Factor/SNP	Naïve T cells		T cell Blasts		References
	Branching	Growth [TCR clustering]	Branching	Growth [CTLA-4 endocytosis]	
IL-2	↓	↑	↑	↓	30, 32, 33, 44
<i>IL2RA</i> *T	n.d.	n.d.	↓	↑	30
IL-7	↓	↑	↑	n.d.	30, 49, 50
<i>IL7RA</i> *C	n.d.	n.d.	↓	n.d.	30
<i>CTLA-4</i> Ala17	#	#	#	↑	30, 67, 68,
<i>UDP-GlcNAc</i>					
<i>MGAT1</i> IV _A V _{T-T} low	↓	↑	↓	↑	30
<i>MGAT1</i> IV _A V _{T-T} high	↑	↓*	↑	↓*	
Vitamin D ₃	#	#	↑	↓	30
UDP-GlcNAc	↑	↓	↑	↓	8, 14, 30

n.d. – not determined # receptor not expressed *predicted by computational modeling

b)

Figure 1.

Genetic and environmental factors contribute to varied levels of N-glycan branching. Resulting effects on T cell function then determine risk of autoimmunity.