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REVIEW

Pathogenesis of multiple sclerosis via environmental and genetic dysregulation of *N*-glycosylation

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Abstract Autoimmune diseases such as multiple sclerosis (MS) result from complex and poorly understood interactions of genetic and environmental factors. A central role for T cells in MS is supported by mouse models, association of the major histocompatibility complex region, and association of critical T cell growth regulator genes such as interleukin-2 receptor (*IL-2RA*) and interleukin-7 receptor (*IL-7RA*). Multiple environmental factors (vitamin D_3 deficiency and metabolism) converge with multiple genetic variants (*IL-7RA*, *IL-2RA*, *MGAT1*, and *CTLA-4*) to dysregulate Golgi *N*-glycosylation in MS, resulting in T cell hyperactivity, loss of self-tolerance and in mice, a spontaneous MS-like disease with neurodegeneration. Here, we review the genetic and biological interactions that regulate MS pathogenesis through dysregulation of *N*-glycosylation and how this may enable individualized therapeutic approaches.

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Introduction

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disorder of the central nervous system (CNS) characterized by inflammatory demyelination, axonal degeneration, and neuron loss [1–3]. Although mouse models of MS, such as experimental autoimmune encephalomyelitis (EAE), provide pathogenic insights, their relevance to MS is indirect. For example, MOG-induced EAE in C57BL/6 mice is monophasic and may more closely mimic the non-relapsing demyelinating disease acute disseminated encephalomyelitis rather than MS. Even "relapsing models" such as PLP-induced EAE in SJL mice do not closely recapitulate relapsing MS. Relapses in MS are separated by months and afflict new areas of the CNS, whereas SJL EAE relapses are recurring episodes of motor weakness separated by days. A direct approach to define pathogenic mechanisms in MS would delineate how known disease risk factors function and interact at the molecular level, utilizing mouse models such as EAE to confirm pathogenic mechanisms.

As with other complex trait diseases, multiple genetic and environmental factors combine to influence disease risk in MS and many other human autoimmune disorders, including systemic lupus erythematosus and type 1 diabetes (T1D) [4, 5]. Epidemiological studies indicate that MS risk is influenced by gender, sex hormones, ethnic origin, continental location/latitude/distance from the equator, smoking, viral exposure (e.g., Epstein–Barr virus), and vitamin D_3 status [6–9]. As vitamin D_3 is synthesized from 7-dehydrocholesterol in the skin following ultraviolet light exposure, a link between latitude, hours of sunshine, and

vitamin D_3 status has been made. This connection is supported by the observation that migration from high-risk areas with limited hours of sunlight to low-risk areas with greater hours of sunshine prior to puberty affords some protection against MS [10]. The latent viral or infectious hypothesis has been proposed, but all attempts at isolating and proving a causal role for a pathogen have failed, suggesting that pathogens act through molecular mimicry to drive pathogenic auto-reactive T cells.

A definitive role for genetics in MS was first demonstrated in elegant family studies by George Ebers and colleagues, where it was observed that first-degree relatives and identical twins display ~20−40- and ~300-fold increased risk over the general population, respectively [4]. Candidate gene studies have validated association of MS with genes in the major histocompatibility complex (MHC) region [11]. In African American patients, it was determined that the primary association was with the DRB1 gene, which was subsequently confirmed in large cohorts of patients of European descent [12, 13]. More recently, genome-wide association studies (GWAS) have identified approximately 50 potential genes associated with MS [14, 15]. A number of these genes also associate with other autoimmune diseases, such as *IL7RA* and *IL2RA* in T1D [16–18]. While the IL-7 and IL-2 pathways have previously been demonstrated to regulate autoimmunity and EAE in animal models [19, 20], such data are lacking for many of the other MS-associated variants. Validation as true MS risk factors requires much more than statistical association; rather, functional characterization of the changes induced by the polymorphism and evidence for pathogenicity of the same molecular pathway in animal models is necessary.

Although GWAS has identified approximately 50 genetic loci associated with MS, many critical issues remain. First, whether the detected polymorphism alters the biology of the nearest gene, as often assumed and labeled as such, or a more distant unrelated gene is left unresolved. Similarly, whether the detected variant is causal in disease or simply in linkage disequilibrium (LD) with a distant undetected causal polymorphism is also not addressed. The critical importance of these issues was best demonstrated by a GWAS of sickle cell anemia [21], a disease where the single genetic variant that induces disease has been established for many years. Despite this, the GWAS identified 179 non-causal polymorphisms with genome-wide significance that encompassed a 2.5-Mb region harboring multiple LD blocks and dozens of non-disease-related genes. Thus, many irrelevant variants and genes may be identified by GWAS analysis. A second critical issue of GWAS studies is the missing heritability. Except for the human leukocyte antigen (HLA), the identified variants in MS confer relatively small increments in disease risk and explain only ~20 % of the genetic variance that we know exists [15, 22], questioning the source of this missing heritability. Many explanations have been suggested including additional common variants with smaller effects or rare and highly penetrant variants that are overlooked in the current genome-wide arrays that are restricted to variants with allele frequencies of ≥5 % [21–24]. However, rare and highly penetrant variants often underlie disorders with Mendelian-type inheritance that have little or no environmental influences (e.g., cystic fibrosis). The fact that comprehensive loci analyses to date have not accounted for the predicted genetic variation in complex trait diseases suggests that the resolution of this dilemma lies in the complexity of the underlying genetics.

Susceptibility to complex trait diseases is multifactorial and results from the interactions of multiple contributing genes and environmental factors, each with potential to interact in nonlinear ways. Epistatic interactions, where two or more independent variants promote disease only when combined [25], are likely to go undetected by genetic screens such as GWAS that examine for point association. Evidence consistent with epistatic interactions in autoimmune disease has been reported in both humans and mice [26–28]. Moreover, MS concordance rates in monozygotic twins are only ~30 % [4], implying direct environmental impact on genetic risk. Indeed, Baranzini et al. have recently reported that there is no evidence for genetic, epigenetic, or transcriptome sequence differences that explain disease discordance in monozygotic twins discordant for MS [29]. It is interesting to note that all twin pairs studied had identical genotypes within the HLA loci and only one of the three twin pairs had DRB1*1501, a genetic variant with the strongest association with MS.

Despite the identification of multiple environmental and genetic risk factors for MS, there appears to be no obvious shared molecular mechanisms, although most appear immune related [15]. Single-gene disorders displaying Mendelian inheritance disrupt molecular pathways at a single step. However, a similar degree of pathway disruption may also be obtained through small defects in multiple genes within a single pathway. Thus, complex trait diseases like MS may arise from epistatic and/or additive interactions between multiple seemingly unrelated alleles and environmental factors that converge to dysregulate a critical final common pathway. Indeed, we recently reported that multiple environmental factors (vitamin D_3 deficiency and metabolism) converge with multiple genetic variants (*IL-7RA*, *IL-2RA*, *MGAT1*, and *CTLA-4*) to dysregulate Golgi *N*-glycosylation in MS. Defective *N*-glycosylation of the T cell receptor (TCR) and cytotoxic T lymphocyte antigen 4 (CTLA-4) induces T cell hyperactivity, promotes loss of self-tolerance and in mice, induces a spontaneous MS-like disease [30–33]. Here, we review the genetic and biological interactions that differentially regulate MS risk through dysregulation of *N*-glycosylation, how this may promote pathogenesis, and the

potential for individualized approaches to diagnostics and treatment (Fig. 1).

N-glycosylation and regulatory mechanisms of growth and differentiation

The majority of cell surface receptors and transporters are modified by co-translational addition of asparagine (*N*)-linked glycans in the endoplasmic reticulum, with further modifications in the Golgi secretory pathway [34, 35]. Cell surfaces and the extracellular matrix with which they interact are heavily glycosylated, and the size, abundance, and complexity of these glycan structures provide information encoding distinct from the genome [36]. In contrast to proteins and nucleic acids, production of complex carbohydrates is not template driven, but rather depends on enzymatic activities and metabolic supply of substrates. Glycoprotein concentrations at the cell surface can be differentially regulated according to their affinities for the galectin family of endogenous lectins [30, 31, 37]. Galectins are ubiquitously expressed at the cell surface and extracellular matrix and interact with multivalent glycan ligands to form a molecular "lattice" at the cell surface [31, 38, 39]. The minimal binding structure for galectins is *N*-acetyllactosamine (Galactose β1,4*N*-acetylglucosamine) [40], with binding avidity to glycoproteins increasing in proportion to the number of *N*-glycans per protein (gene-encoded) and the degree of branching/structural modifications per *N*-glycan (context/environment dependent) [36]. *N*-glycan branching produced in the Golgi is dependent upon the sequential yet incomplete action of the Golgi α-mannosidases and *N*-acetylglucosaminyltransferases I, II, IV, and V (encoded by *Mgat*1, 2, 4, and 5), along with hexosamine pathway production of the substrate uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) [30, 41, 42]. Growth-promoting receptors frequently have high numbers of *N*-glycans ($n>5$), while growth inhibitory receptors frequently have few *N*-glycans ($n\leq 4$). This allows differential association with the galectin lattice dependent on Golgi branching activity, thereby regulating cellular transitions from growth to arrest [30]. This paradigm has been demonstrated for TCR/CTLA-4 in T cells and receptor tyrosine kinases/transforming growth factor-β receptor (TβR) in epithelial cells [30].

The intricate interplay between growth stimulatory and inhibitory signals shapes the T cell immune response and is critical for T cell tolerance. Golgi-mediated changes in *N*-glycan branching differentially control cell surface retention and endocytosis rates of glycoproteins, and in this manner, the galectin–glycoprotein lattice appears to incorporate both genetic and metabolic cues to control cellular function and cell fate decisions.

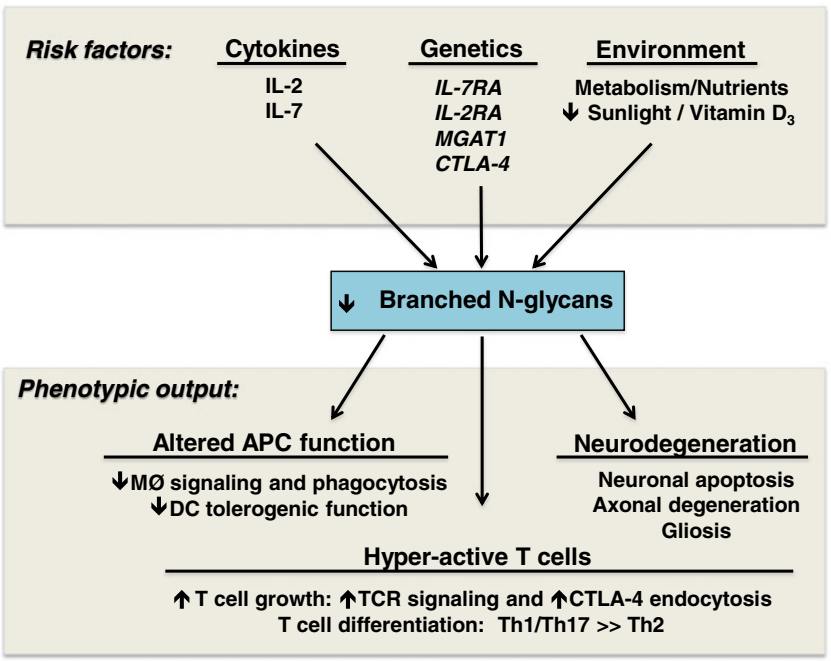

Fig. 1 Multiple risk factors decrease *N*-glycan branching to promote diverse pathogenic mechanisms in multiple sclerosis. Human and mouse data indicate that genetic factors, the environment, and cytokines combine to decrease *N*-glycan branching. This in turn leads to multiple pathogenic mechanisms in multiple sclerosis (MS), including T cell hyperactivity, altered antigen-presenting cell (APC) function, and enhanced susceptibility to neurodegeneration. Recent mouse data also support a potential negative role for *N*-glycan branching in Treg suppressor function and re-myelination by oligodendrocyte precursor cells. Thus, defective *N*-glycan branching in MS results from multiple inputs, which in turn results in multiple phenotypic outputs that likely drive MS pathogenesis

N-glycosylation and T cell-mediated autoimmunity in mice

In mice, targeted deficiencies of factors that inhibit growth of naïve T cells, such as CTLA-4, TβR, and regulatory T cells (Treg), result in spontaneous autoimmunity. Similarly, human autoimmunity is often associated with risk factors that control T cell growth, including the MHC region, *CTLA-4* Thr17Ala (rs231775), *IL2RA**T (rs2104286), *IL7RA**C (rs6897932), and vitamin D_3 deficiency. *N*-glycan branching is also a critical negative regulator of T cell growth and when genetically disrupted in mice, results in spontaneous autoimmunity [43]. Antigen independent and antigen-induced TCR clustering and signaling are both suppressed by galectin interactions with the TCR via *N*-glycans, thereby suppressing both basal and activation signaling [32]. IL-2 and IL-7, two well-described enhancers of T cell growth, regulate mRNA expression of multiple Golgi genes to suppress *N*-glycan branching and thereby enhance ligand-induced TCR clustering and signaling [44]. After cell division, *N*-glycan branching increases in T cell blasts, promoting cell surface retention of CTLA-4 to induce growth arrest [30].

After growth arrest, T cells differentiate into pro-inflammatory T helper 1 (Th1)/T helper 17 (Th17) cells, anti-inflammatory T helper 2 (Th2) cells, and/or induced T regulatory cells (iTreg). Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 and provide host defense against extracellular pathogens, assist B cells and humoral immunity, and are generally anti-inflammatory. Th1 and Th17 cells are pro-inflammatory effector cells that secrete IFN-γ and IL-17, respectively, and have been shown to independently promote autoimmunity [45]. iTregs strongly inhibit growth of other T cells and are crucial in downregulation of T cell responses. The relative balance of these different cell types dictates inflammatory, allergic, and autoimmune responses. Deficiencies in *N*-glycan branching promote Th1 and Th17 responses over Th2 responses [46, 47] (Araujo and Demetriou, unpublished data).

In summary, *N*-glycan branching is a critical negative regulator of T cell growth, is directly downregulated by cytokines (IL-2 and IL-7) that enhance growth, and inhibits pro-inflammatory Th1/Th17 responses. Not surprisingly, genetic deficiencies in *N*-glycan branching in mice promote spontaneous autoimmunity. For example, mice deficient in Mgat5 develop spontaneous autoimmune kidney disease and display increased sensitivity to EAE [31]. Furthermore, significant differences in *N*-glycan branching and Golgi enzyme activity are observed among inbred mouse strains, with strains susceptible to EAE displaying defective *N*-glycan branching in T cells [33]. 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 demonstrated 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 neurodegeneration [33]. 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.

Autoimmunity and defective *N*-glycosylation in non-T cells

Data in mice suggest that defective *N*-glycosylation may also promote autoimmunity through dysfunction of non-T cells (Fig. 1). For example, deficiencies in the galectin–glycoprotein lattice also alter antigen-presenting cell (APC) function. Defective *N*-glycan branching and blockade of polylactosamine synthesis, which both weaken the galectin lattice, increase sensitivity to cytokine signaling and lower antigen-presenting cell activation thresholds [37, 48], consistent with a regulatory role for *N*-glycosylation in tolerogenic signaling in APCs. Indeed, galectin-1, through binding to cell surface glycans and strengthening the galectin lattice, induce tolerogenic dendritic cells that secrete IL-27 to promote IL-10-mediated T cell tolerance and suppress EAE [49].

Defective *N*-glycosylation may also promote autoimmunity through molecular mechanisms distinct from the galectin–glycoprotein lattice. Spontaneous autoimmunity in mice deficient in Golgi alpha-mannosidase-II (αM-II) is associated with minimal reductions in *N*-glycan branching in T cells but marked deficiencies in other tissues such as the kidney and red blood cells [50]. αM-II deficiency induces a systemic lupus erythematosus-like syndrome in mice characterized by elevated systemic anti-nuclear antibody titers, dyserythropoietic anemia, glomerular deposition of immunoglobulins and complement component C3, and glomerulonephritis leading to sclerosis, renal dysfunction, and kidney failure [51]. Jamey Marth and colleagues have proposed that αM-II deficiency induced increases in cell surface mannose exposed *N*-glycans hyperactivates an innate immune response through binding to mannose-binding lectin receptors [51]. Mannose exposed *N*-glycans are normally only seen at high density in pathogens [52], with increased levels from αM-II deficiency potentially resulting in a defect in self-tolerance by innate immune cells and chronic activation.

Organ-specific autoimmune diseases such as MS may also be influenced by increased sensitivity of target cells to death. For example, in addition to inflammatory demyelination, MS is characterized by neuron loss and axonal damage even in the absence of inflammation. Consistent with this, Mgat5 deficiency in PL/J mice results not only in spontaneous inflammatory demyelination but also neurodegeneration,

characterized by neuronal loss and axonal damage in both inflamed and non-inflamed CNS tissue [33]. Moreover, targeted deficiency of Mgat1 in neurons induces their apoptosis in vivo, confirming that *N*-glycan branching directly regulates neuronal viability [53]. These data suggest that *N*-glycan branching independently promotes both T cell-mediated autoimmunity and neurodegeneration, two hallmarks of MS.

Environmental regulation of autoimmunity via *N*-glycosylation

N-glycan branching in T cells is directly influenced by metabolism and vitamin D_3, thereby providing a molecular mechanism for environmental regulation of T cell-mediated autoimmunity. The *N*-glycan branching enzymes (*Mgat*1, 2, 4, and 5) all utilize the same sugar-nucleotide donor, namely UDP-GlcNAc, but do so with declining efficiency [36]. The Km of Mgat4 and Mgat5 for UDP-GlcNAc is ~5 and ~11 mM, respectively, whereas the Golgi concentration of UDP-GlcNAc is only ~1.5 mM. Thus, these enzymes are under-saturated for UDP-GlcNAc, and small changes in UDP-GlcNAc concentration can lead to significant changes in *N*-glycan branching, T cell growth/differentiation, and autoimmunity [30, 41].

De novo synthesis of UDP-GlcNAc by the hexosamine pathway requires highly regulated intermediates of carbohydrate, nitrogen, and fatty acid metabolism [41], and in this manner, *N*-glycan branching is sensitive to metabolic status and the nutrient environment of the cell. Indeed, increased supply of glucose, glutamine (a critical nitrogen metabolite), or acetyl-CoA (the final metabolite of free fatty acids) enhances *N*-glycan branching in T cells in vitro. UDP-GlcNAc may also be synthesized through salvage of the monosaccharides glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc). However, unlike GlcNAc, GlcN may also be shunted into glycolysis and ATP production. Indeed, when titrated in culture, GlcN first increases then decreases *N*-glycan branching in T cells [41]. In contrast, GlcNAc cannot enter glycolysis, is not metabolized, and is observed to only enhance *N*-glycan branching [41, 54]. Indeed, GlcNAc supplementation in vitro and/or in vivo suppresses T cell growth by limiting TCR signaling and enhancing CTLA-4 surface retention, inhibits Th1 and Th17 responses, and suppresses EAE as well as autoimmune diabetes [41, 47]. Moreover, Murch et al. observed that oral GlcNAc therapy inhibited clinical disease in 8 of 12 children with treatment-resistant inflammatory bowel disease [55]. Thus, metabolism regulates *N*-glycan branching and thereby influences susceptibility to T cell-mediated autoimmunity in mice.

Vitamin D_3 deficiency is a well-described environmental risk factor associated with MS that we have recently shown to regulate *N*-glycan branching in T cells to suppress growth and EAE. Previous epidemiological investigations revealed that MS risk increases with distance from the equator and the corresponding decline in ultraviolet exposure [56, 57]. Vitamin D_3 is synthesized from 7-dehydrocholesterol in the skin upon ultraviolet sun exposure, and its deficiency strongly associates with MS [8, 58, 59]. 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D_3, inhibits T cell activation, Th1 differentiation, and suppresses EAE in mice by acting on T cells [60–62], yet molecular mechanisms have been unclear.

$1,25(OH)_2D_3$ increases *N*-glycan branching in activated ex vivo T cells to suppress their growth [44]. Reducing dietary supply of vitamin D_3 in mice decreased *N*-glycan branching in T cells, whereas intraperitoneal injection of $1,25(OH)_2D_3$ increased *N*-glycan branching. Myelin basic protein-induced EAE was inhibited by intraperitoneal injection of $1,25(OH)_2D_3$ in the absence but not presence of swainsonine, an inhibitor of *N*-glycan branching. Combined, these data suggest that vitamin D_3 suppresses T cell growth and EAE by enhancing *N*-glycan branching in T cells.

In summary, two independent environmental factors, namely metabolism/nutrient supply and sunshine/vitamin D_3, influence T cell-mediated autoimmunity by regulating *N*-glycan branching (Fig. 1). Metabolic homeostasis consists of multiple feedback mechanisms, yet small changes in homeostatic set points with age and environmental cues can be clinically important in complex trait diseases such as MS. Therapeutic intervention with oral GlcNAc and/or vitamin D_3 may provide a simple treatment to enhance *N*-glycan branching and suppress MS.

Genetic and environmental dysregulation of *N*-glycosylation in multiple sclerosis

Multiple genetic and environmental risk factors have been linked to MS; however, defining how these combine at the molecular level to promote disease has been a great challenge. The data described above define a critical role for environmental and genetic dysregulation of *N*-glycan branching in mouse T cells and autoimmunity, suggesting similar mechanisms may be relevant to human T cells and MS. Indeed, our group recently reported that multiple environmental factors (sunlight/vitamin D_3 and metabolism) converge with multiple genetic variants (*IL-7RA*, *IL-2RA*, *MGAT1*, and *CTLA-4*) to dysregulate *N*-glycosylation in MS [44].

The *IL2RA**T (rs2104286) and *IL7RA**C (rs6897932) MS risk alleles are the common alleles in Caucasian populations

(frequency ~75 %) and are associated with enhanced secretion of soluble receptors that block signaling by cognate cytokines [7, 16, 44, 63–66]. We observed that IL-2 and IL-7 are critical regulators of *N*-glycan branching, thereby controlling T cell growth [44, 67]. Consistent with this, soluble receptors associated with the *IL2RA**T and *IL7RA**C MS risk variants downregulate *MGAT1* mRNA and *N*-glycan branching in human T cell blasts (Fig. 1). As these two MS risk variants directly regulated *MGAT1*, targeted sequencing of the human *MGAT1* gene was undertaken. An MS-associated haplotype of *MGAT1* (IV_A and $V_{T\text{-}T}$ polymorphisms; rs7726005, rs2070924, and rs2070925) was identified that reduced or enhanced *N*-glycan branching depending on metabolism and UDP-GlcNAc supply to the Golgi. The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype enhances mRNA levels and enzyme activity ~2−3-fold, thereby increasing the *N*-glycan product of Mgat1 while also limiting UDP-GlcNAc supply to downstream Mgat4 and 5. Mgat1, 2, 4, and 5 act in a sequential manner but with declining efficiency as enzyme levels and catalytic efficiencies of UDP-GlcNAc utilization decrease in the same order. The Km of Mgat4 and 5 for UDP-GlcNAc is significantly worse than Mgat1 (~5 and ~11 mM versus ~0.04 mM, respectively); allowing increased Mgat1 protein to outcompete Mgat4 and 5 for UDP-GlcNAc in the medial Golgi [30]. Thus, under basal UDP-GlcNAc levels (~1.5 mM), the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype functions dominantly to reduce *N*-glycan branching. However, with increasing UDP-GlcNAc and/or Mgat5 levels, enhanced Mgat1 expression is not as effective in limiting supply of UDP-GlcNAc to Mgat4 and 5, allowing Mgat4 and 5 to act upon the increased supply of *N*-glycan acceptors from *MGAT1* $IV_AV_{T\text{-}T}$, resulting in enhanced *N*-glycan branching. Thus, the phenotypic effect of the MS-associated *MGAT1* $IV_AV_{T\text{-}T}$ haplotype directly depends on metabolic status of the cell and production of UDP-GlcNAc; albeit basal UDP-GlcNAc conditions and reduced *N*-glycan branching are expected to predominate. Monozygotic twins are discordant for MS ~70 % of the time. The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype provides an example of how the same genetic risk factor may both promote and inhibit MS conditional on the environment.

The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype and the *IL2RA**T and *IL7RA**C MS risk alleles influence *N*-glycan branching by having opposing effects on Mgat1 expression. Consistent with this, upregulation of Mgat1 by IL-2 and/or IL-7 signaling enhances *N*-glycan branching when Mgat1 is suppressed by *IL2RA**T and *IL7RA**C but further decreases *N*-glycan branching when Mgat1 is already increased by the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype [44]. In other words, upregulation of Mgat1 by IL-2 and/or IL-7 enhances or reduces branching depending on baseline Mgat1 activity, which differs based on the presence of the different *MGAT1* variants. This provides a second conditional mechanism that controls *N*-glycan branching in MS.

Genetically induced downregulation of *N*-glycan branching in human T cell blasts is expected to reduce CTLA-4 surface retention and thereby promotes T cell growth [30]. The Thr17Ala polymorphism in the human *CTLA-4* gene (49A/G, rs231775) encodes a signal peptide variant with inefficient glycosylation [68, 69]. This non-synonymous polymorphism associates with type 1 diabetes but not MS [70, 71], reduces average *N*-glycan occupancy at the two N-X-S/T sites from two to one, and decreases the number of branched *N*-glycans and CTLA-4 surface levels to enhance T cell growth (Fig. 1). The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype also limits CTLA-4 surface levels when expressed with the common *CTLA-4* allele (*CTLA-4* Thr17; two *N*-glycans), whereas combining the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype with the *CTLA-4* Ala17 variant (one *N*-glycan) further reduces CTLA-4 surface levels [44]. CTLA-4 surface expression is restored by increasing UDP-GlcNAc levels with GlcNAc supplementation in all genotype combinations, confirming an additional mechanism regulated by metabolism.

In summary, the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype lowers *N*-glycan branching, T cell activation thresholds, and CTLA-4 cell surface expression in a manner that is sensitive to metabolic conditions (i.e., UDP-GlcNAc), activity of other Golgi enzymes (e.g., Mgat5), the number of *N*-glycans attached to CTLA-4, and IL2/IL-7 signaling (Fig. 1).

These biological interactions predict specific genetic interactions in MS. Indeed, epistatic and additive interactions were observed between the four variants as expected [44]. The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype increases MS risk when there are less than four copies of the *IL2RA*T* and *IL7RA*C* risk alleles, whereas no association is observed in the presence of four copies of the *IL2RA*T* and *IL7RA*C* variants, the latter consistent with opposing effects on Mgat1 expression optimizing Mgat1 activity and enhancing *N*-glycan branching. The *MGAT1* $IV_AV_{T\text{-}T}$ haplotype also significantly associated with MS in *CTLA-4* Ala17 carriers (one *N*-glycan), but not *CTLA-4* Thr17 homozygotes (two *N*-glycans). Moreover, the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype promotes MS when there are less than six alleles of *CTLA-4* Thr17, *IL2RA*T*, and *IL7RA*C*, whereas a marginally significant protective effect was observed with six alleles of *CTLA-4* Thr17, *IL2RA*T*, and *IL7RA*C* [44]. The latter combination is expected to be protective as Mgat1 activity, *N*-glycan branching, and *N*-glycan number on CTLA-4 are optimized. Importantly, these genetic interactions are observed despite lack of point association and marginal effects of *CTLA-4* Thr17Ala, indicative of epistatic interactions.

Vitamin D_3 enhances *N*-glycan branching to suppress T cell growth and EAE in mice, while deficiency of vitamin D_3 is associated with MS. To investigate possible interactions with genetic variants, we examined the effects of 1,25 $(OH)_2D_3$ on human T cell blasts [44]. Remarkably, 1,25

$(OH)_2D_3$ enhanced *MGAT1* mRNA levels, similar to the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype but opposite of the *IL2RA*T* and *IL7RA*C* risk alleles. Consistent with this effect on Mgat1, $1,25(OH)_2D_3$ enhanced *N*-glycan branching in T cells with two or more copies of the *IL2RA*T*+*IL7RA*C* risk alleles (where Mgat1 expression is reduced). In contrast, *N*-glycan branching in T cells homozygous for the *IL2RA*C* and *IL7RA*T* protective alleles, where Mgat1 expression is not suppressed, was unchanged or reduced [44]. As a very small minority of Caucasians is homozygous for both the *IL2RA*C*+*IL7RA*T* protective alleles (~0.5 %), vitamin D_3 deficiency is expected to reduce *N*-glycan branching in the majority of the Caucasian population.

*IL2RA**T, *IL7RA**C, *CTLA-4* Ala17, and vitamin D_3 deficiency also associate with T1D [16–18, 72]. The non-obese diabetic mouse is deficient in *N*-glycan branching in T cells, while oral GlcNAc is able to suppress development of autoimmune diabetes in these mice [33, 41]. Another independent variant of *IL2RA* (rs11594656) also associates with both MS and T1D, but paradoxically in opposite directions [16]. These data suggest that defective *N*-glycosylation also contributes to T1D risk.

Conclusions

Complex trait diseases such as MS develop from multifaceted and poorly understood interactions between genetics and the environment. While genetic and epidemiological studies have identified a number of genetic and environmental risk factors in MS, most appear to only marginally increase risk, do not account for all heritability, and display no obvious common molecular mechanism. Epistatic interactions, where two or more factors promote disease only when combined, are likely to go undetected in approaches assessing only point association such as GWAS. Here, we reviewed evidence suggesting that in MS, epistatic interactions between multiple independent genetic variants and environmental factors combine in a nonlinear fashion to dysregulate a common biochemical pathway, namely Golgi *N*-glycosylation. Each factor may only have a minor genetic or biological effect on risk and *N*-glycosylation, but specific combinations lead to more dramatic changes in *N*-glycan branching. Moreover, the same variant may either increase or decrease risk depending on co-inheritance of other variants and/or environmental factors. This paradigm suggests that future studies only examining point association, such as GWAS, are unlikely to adequately define heritability. Rather, molecular mechanistic studies of human variants enlightened by mouse data are likely required to intelligently and selectively examine for epistatic interactions and define disease mechanisms. For example, there are at least ~30 genes that alter *N*-glycan branching and may be screened for functional variants and epistatic interactions.

Defective *N*-glycosylation in MS results from multiple inputs, both environmental and genetic, but importantly also results in multiple phenotypic outputs (Fig. 1). Human and mouse data suggest that defective *N*-glycosylation contributes to MS by affecting multiple cell types and molecular mechanisms. In addition to defects in T cell growth and self-tolerance, defective *N*-glycosylation may also promote disease via hyperactive innate immune responses and increased sensitivity of neurons to death [37, 48, 49, 51, 53]. While the effects on T cells are defined in both mouse and humans, additional work is required to determine whether the genetic (e.g., *MGAT1* $IV_AV_{T\text{-}T}$, *IL2RA**T, and *IL7RA**C) and/or environmental (e.g., vitamin D_3 and UDP-GlcNAc metabolism) factors also directly alter innate immune activity and neurodegeneration in human cells via defective *N*-glycosylation. For example, the *MGAT1* $IV_AV_{T\text{-}T}$ haplotype increases the amount of mannose exposed *N*-glycans in peripheral blood monocytes. If this phenotype was also prominent in oligodendrocytes, exposure of these cryptic mannose residues may hyperactivate innate immune responses to promote demyelination.

Current treatment strategies for MS are predominated by injectable therapies with modest efficacy, high cost, and significant side effects, which can affect tolerability and compliance. The limitations of current medications warrant investigations into alternative therapeutic strategies, particularly those that directly target an underlying molecular mechanism promoting disease, rather than nonspecific immunomodulation and/or immunosuppression. Therapeutic supplementation of the Golgi to increase *N*-glycan biosynthesis may provide such a therapy. Both vitamin D_3 and GlcNAc are orally active, reverse deficiencies in *N*-glycan branching in mice and humans, and inhibit EAE and spontaneous autoimmune diabetes in mice [41, 61, 73]. More recent data from our lab have shown that oral GlcNAc also inhibits Th1 and Th17 responses and disease progression in EAE when administered after disease onset [47]. A pilot study of oral GlcNAc in pediatric treatment-resistant inflammatory bowel disease reported that 8 out of 12 children with severe disease went into clinical remission with evidence of histological improvement [55]. Three of the responders relapsed within ~1 month following disruption of GlcNAc therapy, but improved again once therapy was reinitiated [55]. These data suggest that a human clinical trial of GlcNAc in MS is warranted.

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