

## NEWS AND COMMENTARY

### Autoimmunity

# Altered self-*N*-glycans trigger innate-mediated autoimmunity

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The ability to distinguish self from nonself is a central feature of the immune system. Without it, immune cells attack various tissues within the body that are mistakenly recognized as foreign, resulting in the development of autoimmune disorders. The initial events in this process have remained unclear, but disease is often accompanied by autoantibody production and involvement by T and B cells of the adaptive immune system. A new study by Green *et al.*<sup>1</sup> however, reveals that autoimmune tissue destruction can develop in the absence of adaptive immunity, apparently triggered by innate recognition of malformed *N*-glycans produced by cells lacking alpha-mannosidase-II ( $\alpha$ M-II). Intriguingly, the abnormal *N*-glycans mimic those commonly expressed by lower eukaryotes and prokaryotes, thereby providing an initiating signal for innate immune cell activation and subsequent pathologic autoimmunity.

Asparagine (*N*)-linked glycans are broadly categorized into three groups based on structural characteristics coinciding with distinct biosynthetic stages: high mannose, hybrid and complex (Figure 1). The initial events generating high mannose *N*-glycans are remarkably conserved among eukaryotic cells. A mannose-rich oligosaccharide precursor molecule is synthesized in the endoplasmic reticulum (ER), transferred *en bloc* to nascent polypeptides and modified to ensure proper protein folding and sorting.<sup>2,3</sup> Upon transit through the Golgi, cell- and species-specific diversification is introduced as this *N*-linked oligosaccharide is subjected to trimming and extension by the sequential actions of various Golgi-resident glycosyltransferases and glycosidases. In vertebrates, these modi-

fications produce primarily complex-type *N*-glycans that decorate cell surface glycoproteins, in contrast to invertebrates, such as yeast, which express high-mannose and hybrid-type *N*-glycoforms.<sup>1</sup>

The conversion of hybrid- into complex-type *N*-glycan structures in vertebrates requires the trimming of terminal  $\alpha$ 3- and  $\alpha$ 6-linked mannose residues on hybrid-*N*-glycan structures before further branching. *In vivo* mouse genetic studies have established that two isozymes,  $\alpha$ M-II and  $\alpha$ M-IX, perform this critical mannose-trimming step, without which complex-type structures fail to be produced, and hybrid-type *N*-glycans accumulate.<sup>4–7</sup> Deficiencies of both  $\alpha$ M-II and  $\alpha$ M-IX have severe consequences, as double-null mice die shortly after birth, a phenotype which supports observations made in other glycosyltransferase-deficient mice unable to synthesize complex *N*-glycans.<sup>7–10</sup> In contrast, mice deficient in either isozyme alone display less drastic phenotypes, due to apparent compensation in specific cell types:  $\alpha$ M-IX-deficient mice, for instance, appear normal except for a defect in spermatogenesis, whereas  $\alpha$ M-II-deficient mice display a complete absence of complex *N*-glycans on cells of the erythroid lineage (with variable compensation among other cell types) and develop dyserythropoietic anemia accompanied by an age-related autoimmune disease resembling systemic lupus erythematosus (SLE).

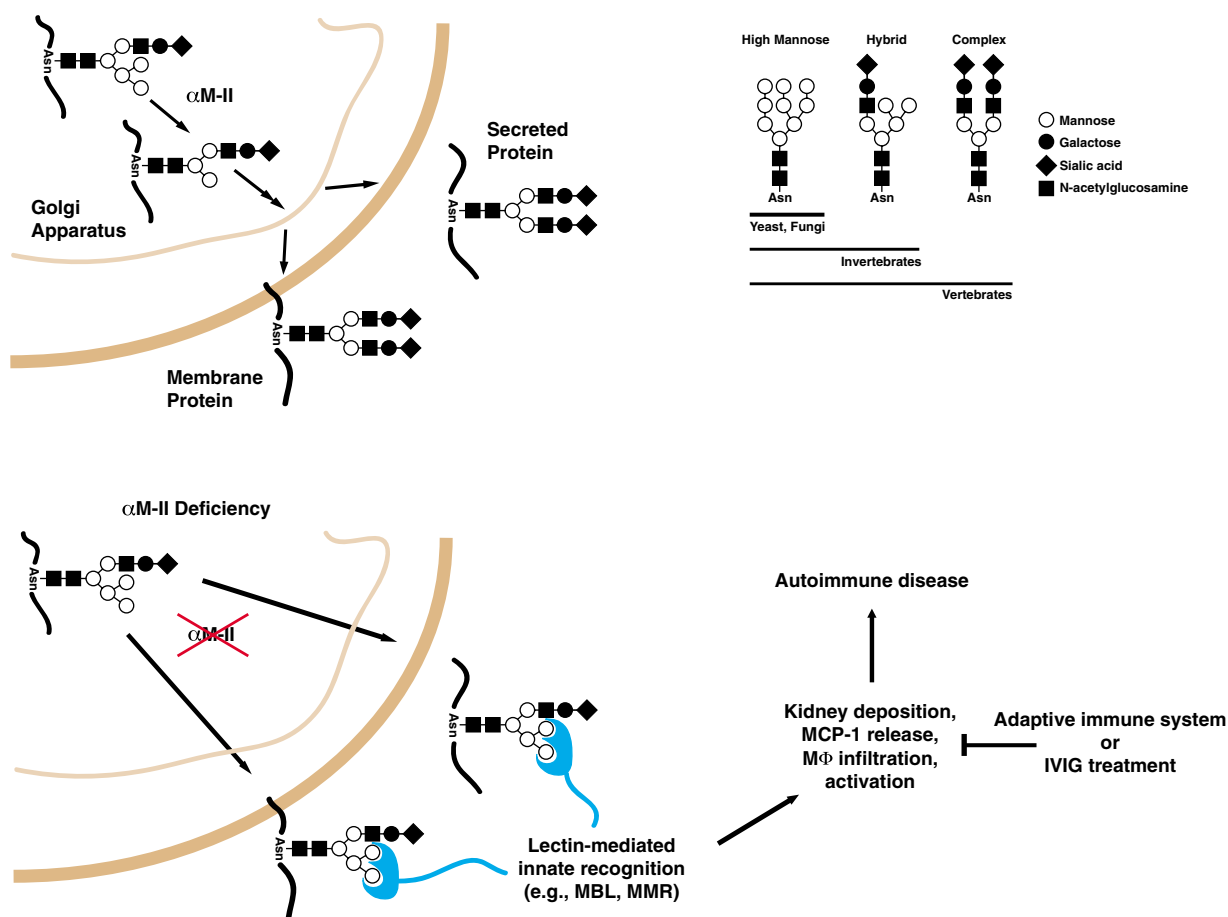
Green *et al.*<sup>1</sup> exploited the cell- and tissue-specificity of  $\alpha$ M-II gene disruption to gain compelling insights about the initiation of autoimmunity. In this study, the authors performed bone marrow transplants among  $\alpha$ M-II null mice and wild-type littermates in an effort to identify the cellular origin of disease observed in the absence of  $\alpha$ M-II. Surprisingly, they found that increased autoantibody titers and kidney dysfunction were

observed when the bone marrow recipient, not the donor, was deficient in  $\alpha$ M-II, establishing that the disease pathogenesis arose from non-hematopoietic cells. The anemia due to  $\alpha$ M-II deficiency was ameliorated by wild-type bone marrow transplantation, however, thereby uncoupling this phenotype from the development of autoimmunity, further highlighting the cell-type specific effects of defective complex *N*-glycan formation.

Given the non-hematopoietic origin of disease in  $\alpha$ M-II deficiency and the well-documented adaptive component of autoimmune diseases, the authors examined the contribution of adaptive immune cells to disease using recombinase-activating gene-1 (RAG-1)-deficient mice, which lack mature lymphocytes and antibodies. In an unexpected twist, mice deficient in both  $\alpha$ M-II and RAG-1 failed to reduce markers of kidney disease, which instead were exacerbated in the double-deficient mice, accompanied by increased numbers of kidney-infiltrating macrophages and mesangial cells expressing activation markers. Suspecting that this attenuation of disease by the adaptive immune system was due to a lack of lymphocyte-derived immunoglobulin G (IgG), a molecule that can bind inhibitory Fc receptors on innate immune cells, Green *et al.*<sup>1</sup> administered intravenous IgG (IVIG) to the  $\alpha$ M-II/RAG-1 double-deficient mice over a period of several months. IVIG treatment led to a reduction in the autoimmune disease markers and improved kidney function, and lessened both glomerular expression of the chemokine MCP-1 and the magnitude of activated macrophage infiltration.<sup>1</sup>

So what do these results imply about the mechanism of autoimmune disease initiation by altered *N*-glycans? Innate immune cells possess a diverse array of lectin recep-

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**Figure 1** In normal cells, most membrane-bound and secreted glycoproteins are modified with complex-type *N*-glycans (simplified glycan structures indicated). In the absence of  $\alpha$ M-II, hybrid *N*-glycans are produced, mimicking structures expressed on lower eukaryotes and leading to engagement by lectin receptors, followed by chronic innate immune activation and autoimmune disease development, which is attenuated by the adaptive immune system.

tors that recognize carbohydrate-containing ligands expressed on pathogenic organisms.<sup>11</sup> These receptors include the macrophage mannose receptor (MMR), mannose binding lectin-A (MBL-A) and mannose binding lectin-C (MBL-C), all of which Green *et al.*<sup>1</sup> found to be present at increased levels in the glomeruli of  $\alpha$ M-II null mice. In addition, endogenous ligands for MMR and MBL-C were detected in the  $\alpha$ M-II null sera, which could stimulate MCP-1 production from isolated glomeruli comparable to that induced by yeast cell wall-derived mannans.<sup>1</sup> This suggested that the hybrid *N*-glycan structures produced on extracellular glycoproteins in the absence of  $\alpha$ M-II are bound by lectin receptors that can initiate innate immune responses in the kidney glomeruli, leading to chronic inflammation and the development of autoimmune disease.

The work by Green *et al.* presents a fascinating new connection between altered self-carbohydrate ligand sensing by the innate immune system and the development of

autoimmune disease. Although complement deficiencies in humans have been associated with SLE, and glomerulonephritis with deficiencies in complement regulatory proteins,<sup>12</sup> the authors found that genetic absence of complement C3 did not reduce autoimmune disease markers in  $\alpha$ M-II-deficient mice.<sup>1</sup> Future experiments aimed at identifying the altered serum *N*-glycoproteins in  $\alpha$ M-II deficiency, as well as their cellular source, are warranted. It might also be possible to target the mammalian mannose-binding lectin receptors or a specific cell type in the control of glomerulonephritis. Further, the presence or distribution of high mannose or hybrid *N*-glycan structures on pathogens and their immunostimulatory properties may lead to receptor identification or opportunities to increase adjuvency in vaccine development. As a precautionary note, this study shows that adaptive immune responses to dysregulated innate inflammation may, in some cases, be protective, suggesting that simply ablating adaptive immunity in all

cases of autoimmunity may be counterproductive.

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