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An intrinsic mechanism of secreted protein aging and turnover

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The composition and functions of the secreted proteome are controlled by the life spans of different proteins. However, unlike intracellular protein fate, intrinsic factors determining secreted protein aging and turnover have not been identified and characterized. Almost all secreted proteins are posttranslationally modified with the covalent attachment of N-glycans. We have discovered an intrinsic mechanism of secreted protein aging and turnover linked to the stepwise elimination of saccharides attached to the termini of N-glycans. Endogenous glycosidases, including neuraminidase 1 (Neu1), neuraminidase 3 (Neu3), beta-galactosidase 1 (Glb1), and hexosaminidase B (HexB), possess hydrolytic activities that temporally remodel N-glycan structures, progressively exposing different saccharides with increased protein age. Subsequently, endocytic lectins with distinct binding specificities, including the Ashwell–Morell receptor, integrin αM, and macrophage mannose receptor, are engaged in N-glycan ligand recognition and the turnover of secreted proteins. Glycosidase inhibition and lectin deficiencies increased protein life spans and abundance, and the basal rate of N-glycan remodeling varied among distinct proteins, accounting for differences in their life spans. This intrinsic multifactorial mechanism of secreted protein aging and turnover contributes to health and the outcomes of disease.

glycosylation | protein | homeostasis | glycosidase | lectin

The secreted proteome is a diverse repertoire of proteins, each of which is maintained at concentrations appropriate to control various physiological processes. Although an intrinsic mechanism that determines the life span and turnover of secreted proteins has not been identified, its existence would explain how the composition of the secreted proteome is regulated and how it might differ in health and disease. Almost all secreted proteins are posttranslationally modified during their biosynthesis and transit through the secretory pathway with one or more N-glycans (1, 2). The N-glycans of mammalian proteins are multiantennary structures consisting of two or more oligosaccharide branches that typically include a characteristic terminal sequence of saccharides. This sequence includes sialic acid (Sia) as the distal linkage attached to underlying galactose (Gal), followed by *N*-acetylglucosamine (GlcNAc) and mannose (Man). Each is present in a multivalent context due to N-glycan branching emanating from the core structure. Distinct secreted proteins are therefore modified with similar N-glycans, each of which includes cryptic ligands of lectins that bind Gal, GlcNAc, or Man linkages.

N-glycans have been primarily perceived as static posttranslational modifications that are eventually degraded along with the attached protein in the lysosome of the cell. Alterations of N-glycan structures thus far identified during a protein's life span reflect the activity of exogenous glycosidases or targeted genetic mutations in N-glycan biosynthesis. For example, viral and bacterial neuraminidases cleave Sia linkages from the host glycans, thereby contributing to infection and modulating the severity of disease (3–6). Similarly, exogenous neuraminidase treatment or a genetic deficiency of the ST3Gal4 sialyltransferase was found to unmask Gal ligands of lectins known as asialoglycoprotein receptors in reducing the life spans of some secreted proteins (7, 8). It has been noted that blood plasma contains multiple unidentified glycosidases of unknown functions with activity levels that change in disease (9). We investigated whether the normal activities of circulating glycosidases may hydrolyze N-glycan linkages attached to secreted proteins, thereby generating multivalent ligands of endocytic lectin receptors in contributing to a mechanism of secreted protein aging and turnover.

Results

N-Glycan Remodeling in the Aging of Secreted Proteins. Most secreted proteins are modified with N-glycans before entry into circulatory systems. We isolated secreted proteins among platelet-poor plasma following i.v. biotinylation and examined them 24, 48, and 72 h later. Biotinylated secreted proteins were isolated using nondenaturing conditions by affinity chromatography, first with avidin and subsequently with analytical lectins of binding specificities for Gal, GlcNAc, or Man linkages. Proteomic analyses of plasma identified 603 noncytosolic proteins bearing lectin ligands consisting of Gal linkages, which constituted 99% of the protein mass isolated (Table S1). This diversity further represents the majority of the plasma proteome identified (10, 11). At steady state in healthy WT mice, the fraction of secreted proteins in plasma bearing Gal ligands averaged 2%. We further detected N-glycan remodeling during secreted protein aging with the unmasking of Gal, GlcNAc, and Man linkages (Fig. 1 and Fig. S1). The partial reduction in Sia linkages measured in this time frame indicated that not all N-glycan branches were remodeled. In contrast, Core 1 O-glycans of secreted proteins were not appreciably altered. These data were consistent with the isolation of different glycoforms of secreted proteins bearing increasingly remodeled N-glycans with advanced age.

The unmasking of Gal linkages implied the presence of endogenous neuraminidase activity that hydrolyzed Sia linkages. Using antibodies to the four mammalian neuraminidases (Neu1–Neu4)

Significance

In the blood, secreted proteins have different life spans that determine their abundance and function. Measurements of plasma protein composition and biological activities remain important for many clinical diagnoses. However the molecular mechanisms by which secreted proteins age and turnover have remained unidentified. The findings of this research have established an intrinsic and constitutive mechanism of secreted protein aging and turnover. This mechanism involves multiple factors including circulating glycosidases that progressively remodel the N-glycan linkages attached to most secreted proteins. N-glycan remodeling with time exposes glycan ligands of various endocytic lectin receptors that then eliminate these aged secreted proteins. This mechanism thereby determines the life spans and abundance of secreted proteins, and modulates the pathogenesis and outcomes of disease.

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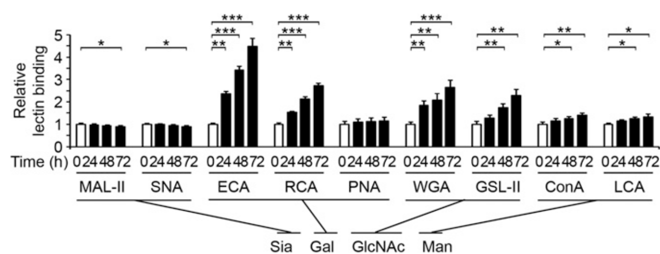

Fig. 1. N-glycan remodeling of aging secreted proteins. Blood plasma proteins from WT mice were isolated at indicated times following i.v. in vivo biotinylation, first by avidin affinity chromatography and next by lectin chromatography. Equivalent amounts of plasma proteins isolated after both chromatographic steps were compared by SDS/PAGE and lectin blotting. Measurements of lectin binding were calculated relative to time 0 (60 min following i.v. biotinylation). Lectin binding specificities for glycan linkages are denoted and were confirmed in parallel studies with competitive inhibitors (Fig. S1). Data are representative of results from six separate littermate cohort comparisons, and are presented as means ± SEM (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). ECA, *Erythrina cristagalli*; GSL-II, *Griffonia simplicifolia*-II; LCA, *Lens culinaris*; MAL-II, *Maackia amurensis*-II; PNA, peanut agglutinin; RCA, *Ricinus communis* agglutinin-I, SNA, *Sambucus nigra*, WGA, wheat germ agglutinin.

(12), we identified Neu isozymes present in the plasma of mice and humans. Both Neu1 and Neu3 were detected in mice of multiple strains, including CD57BL6/J, A/J, and SM/J (Fig. 2*A*). The SM/J strain is homozygous for a point mutation in the *Neu1* allele that reduces Neu1 activity by 50% (13). In the SM/J strain, an increase in Neu3 abundance was detected with the retention of normal Neu activity (Fig. 2*B*). Expression of secreted Neu1 and Neu3 was also detected in plasma samples of healthy humans with consistent basal levels of Neu activity (Fig. 2 *C* and *D*).

Identification of Neuraminidases That Remodel Aging N-Glycans. The contributions of Neu1 and Neu3 were investigated by pharmacological inhibition. The compound 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (DANA) is a broad-spectrum neuraminidase inhibitor of all four Neu isozymes, whereas zanamivir inhibits Neu2, Neu3, and Neu4 but does not significantly inhibit Neu1 (14, 15). The i.v. administration of DANA and zanamivir at multiple dosages and times was studied to achieve and maintain maximal inhibition (Fig. S2). DANA and zanamivir administration (250 mg/kg) every 6 h was required to maintain maximal inhibition. Using DANA, reductions in plasma Neu activity of up to 80% were obtained. Zanamivir administration reduced Neu activity to 35% and 20% of normal in plasma of C57BL/6J and SM/J mice, respectively (Fig. 2*E*). These results indicated that the majority of plasma Neu activity measured in this assay was due to Neu3.

Inhibition of Neu activity was further investigated in the context of N-glycan remodeling during secreted protein aging. Administration of DANA markedly inhibited the exposure of Gal linkages during secreted protein aging (Fig. 2 *F* and *G*). Results with zanamivir treatment were similar, consistent with the involvement of Neu3 (Fig. S3). Remarkably, unmasking of GlcNAc and Man linkages was also inhibited by DANA and zanamivir. This finding implied that N-glycan remodeling during secreted protein aging involves the sequential hydrolysis of exposed glycan linkages unmasked by multiple exoglycosidases.

The identification and study of two secreted proteins with distinct $t_{1/2}$s were undertaken to compare the effects of N-glycan remodeling on individual proteins. We observed that Neu inhibition by DANA or zanamivir resulted in an increase in circulating alkaline phosphatase activity (Fig. 3*A*). Measurements of plasma alkaline phosphatase isozymes indicated that tissue nonspecific alkaline phosphatase (TNAP) and intestinal alkaline phosphatase (IAP) were increased in abundance (Fig. 3 *B* and *C*). This increase was coincident with elevated $t_{1/2}$s of TNAP and IAP (Fig. 3 *D* and *E* and Fig. S4). Moreover, the N-glycans of TNAP and IAP were subject to progressive remodeling with increased age, which was significantly reduced by Neu inhibition (Fig. 3 *F* and *G* and Fig. S4). These findings demonstrated a connection between N-glycan remodeling in secreted protein aging and the rate of protein turnover in circulation, implying the presence of one or more endocytic lectin receptors that participate in determining the $t_{1/2}$s and abundance of secreted proteins.

Asialoglycoprotein Receptors in the Turnover of Aged Secreted Proteins. The Ashwell–Morell receptor (AMR) of hepatocytes is an endocytic lectin discovered by its rapid ability to eliminate from the circulation i.v.-administered glycoproteins that have been desialylated in vitro by Neu activity (7). The AMR binds exposed Gal linkages with increased avidity in multivalent contexts, as exist among the multiple branches of N-glycans (7, 16, 17). AMR function has been found to eliminate desialylated platelets produced during pneumococcal sepsis and following platelet exposure to reduced temperatures (6, 18, 19).

Among mice lacking either the Asgr1 or Asgr2 protein subunit of the AMR, an accumulation of asialoglycoproteins was detected among secreted proteins by lectin affinity chromatography using nondenaturing conditions (Fig. 4 *A* and *B*). This accumulation was accompanied by a two- to threefold increase in the amount of plasma protein isolated. In addition, proteins bearing exposed GlcNAc and Man linkages were increased in abundance to a lesser extent. Neu expression and activity were unaltered in AMR deficiency (Fig. 4 *C* and *D*). It was evident that the accumulation of asialoglycoproteins in AMR deficiency sought in previous studies had been obscured by the appearance of lectin ligands by lectin blotting following denaturing electrophoresis (20–22). This result was directly demonstrated by lectin blotting of secreted proteins including those that failed to bind by affinity chromatography (Fig. S5).

Proteomic analyses identified 291 secreted proteins elevated in Asgr1 and Asgr2 deficiency, with many increased 10-fold or more (Table S2). A gene ontology analysis revealed that these proteins are linked to proteolysis, coagulation, inflammation, and immunity (Fig. S6). Essentially the same processes were indicated in an identical analysis of asialoglycoproteins accumulating in Asgr2 deficiency. Proteomic analyses confirmed the elevation of TNAP and IAP levels in AMR-deficient mice, consistent with results obtained following Neu inhibition (Fig. 3).

Increased abundance of TNAP and IAP in AMR deficiency occurred with a corresponding elevation of alkaline phosphatase activity (Fig. 4 *E* and *F*). No changes were observed in RNA expression encoding TNAP and IAP in multiple tissues, including the liver, bone, kidney, and small intestine (Fig. S7). Circulating TNAP and IAP $t_{1/2}$s were increased in the absence of Asgr1 or Asgr2 with the presence of TNAP and IAP glycoforms bearing increased Gal exposure (Fig. 4 *G*–*J*). These findings provided a mechanistic linkage between Neu activity, N-glycan remodeling, and AMR function in the aging and turnover of secreted proteins, including the individual secreted proteins TNAP and IAP.

Sequential Functions of Multiple Glycosidases and Endocytic Lectin Receptors. Unmasking of GlcNAc and Man linkages subsequent to Neu function implied the possibility that additional endocytic lectin receptors with different glycan binding specificities may also control secreted protein $t_{1/2}$s and abundance. Multiple lectins capable of binding multivalent GlcNAc and Man linkages have been identified. For example, integrin αM encoded by the *Itgam* gene can bind and endocytose proteins bearing exposed GlcNAc linkages such as occurs at the surface of chilled platelets (23). We observed the accumulation of secreted proteins with unmasked GlcNAc by lectin chromatography in plasma of mice lacking integrin αM (Fig. 5 *A* and *B*). This finding was coincident with an approximate 75% increase in the amount of plasma protein isolated and further implied the presence of β-gal activity.

The β-gal Glb1 was identified in the plasma of healthy mice (Fig. 5*C*). Additional studies also identified beta-galactosidase 1 (Glb1) in the plasma of healthy humans. The i.v. administration of the galactosidase inhibitor *N*-nonyl-deoxygalactonojirimycin (NN-DGJ) at optimized doses and times reduced the level of

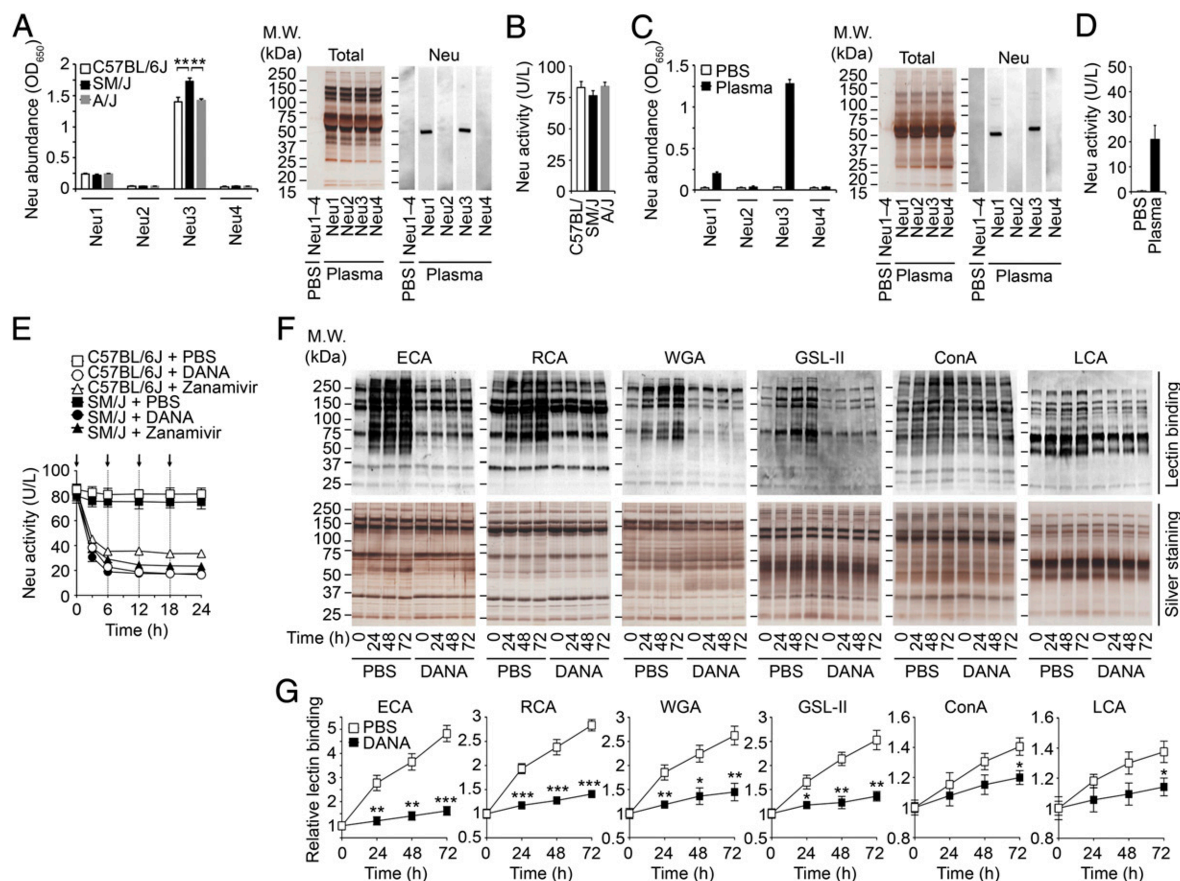

Fig. 2. Identification of circulating neuraminidases and linkage of Neu activity to N-glycan remodeling of aged secreted proteins. (*A*) Antibodies specific to Neu1, Neu2, Neu3, or Neu4 were used to detect Neu protein abundance in the plasma of mice of indicated strains by ELISA and SDS/PAGE, followed by immunoblotting. M.W., molecular weight. (*B*) Neu activity measured in mouse plasma. U/L, units per liter. (*C*) Neu protein abundance in the plasma of healthy human adults by ELISA and SDS/PAGE followed by immunoblotting. (*D*) Human Neu activity in plasma. (*E*) Inhibition of Neu activity in plasma following i.v. injection of DANA or zanamivir (250 mg/kg), compared with saline (PBS), administered at time 0 and every 6 h (arrows) over a 24-h period. (*F*) Biotinylated plasma proteins from WT C57BL/6J mice were isolated at indicated times by lectin affinity chromatography in the presence of PBS or DANA administered every 6 h. Identical amounts of protein (20 μg) were analyzed by SDS/PAGE and lectin blotting. (*G*) Quantification of lectin ligands detected among plasma proteins in *F* was normalized to time = 0. Data are representative of results from four to eight separate mouse littermate cohort comparisons and seven human volunteers, and are presented as means ± SEM (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$).

plasma β-gal activity to 40% of normal (Fig. 5*D* and Fig. S8*A*). Administration of NN-DGJ further resulted in a reduction in the unmasking of both GlcNAc and Man linkages (Fig. 5*E* and Fig. S8*B*). These findings were consistent with the stepwise remodeling of N-glycans during secreted protein aging and implied that circulating β-galactosidase activity produced ligands of integrin αM and possibly other endocytic lectins with similar binding specificity.

Endogenous lectins that bind Man linkages of N-glycans are also expressed in mammals. One example is the macrophage mannose receptor encoded by the *Mmr* gene (24). Previous studies of Mmr deficiency identified elevated serum proteins bearing exposed Man (25). We also detected their accumulation in Mmr deficiency (Fig. 5 *F* and *G*). An approximate 50% increase in plasma protein was isolated by mannose-binding lectin chromatography in Mmr deficiency. Both β-glucosaminidase activity and the β-N-glucosaminidase termed hexosaminidase B (HexB) were identified in the plasma of healthy mice (Fig. 5 *H* and *I*). HexB was also detected in the plasma of healthy humans. Administration of the glucosaminidase inhibitor 2-acetoamido-1,2-dideoxynojirimycin reduced the level of glucosaminidase activity to 33% of normal in mouse plasma (Fig. 5*I* and Fig. S8*C*). This inhibition resulted in a reduction of Man linkages normally unmasked during the aging of secreted proteins (Fig. 5*J* and Fig. S8*D*). Together, these findings demonstrate an integrated molecular mechanism that regulates the proteostasis of secreted proteins (Fig. 6).

Discussion

An intrinsic multifactorial mechanism controls the composition of the secreted proteome by integrating the activities of glycosidases and endocytic lectin receptors with secreted protein aging and turnover. Over 600 plasma proteins were found with lectin ligands detected using nondenaturing experimental conditions, whereas different rates of N-glycan remodeling accounted for different life spans among individual proteins studied. Inhibition of glycosidase activities impaired N-glycan remodeling consistent with the sequential action of multiple exoglycosidases, whereas lectin receptor deficiencies resulted in the accumulation of aged proteins bearing corresponding glycan ligands. In both cases, secreted protein life span and abundance were elevated. This accumulation correspondingly augmented the activities of those enzymes examined, indicating that N-glycan remodeling does not necessarily alter function. This possibility nevertheless exists among some fraction of secreted proteins because desialylation of coagulation factor X, for example, has been demonstrated to diminish its activity (18).

Neu1 and Neu3 operate at the first step in N-glycan remodeling and accounted for the majority of secreted protein turnover. Most glycosidases identified in circulation, including Neu1, Glb1, and HexB, have been localized to lysosomes, where they function in intracellular glycan catabolism, whereas Neu3, in contrast, is expressed at the plasma membrane (26, 27). Their presence in the blood may originate from multiple cell types and likely reflects lysosomal secretion, proteolytic processing, or possibly differential

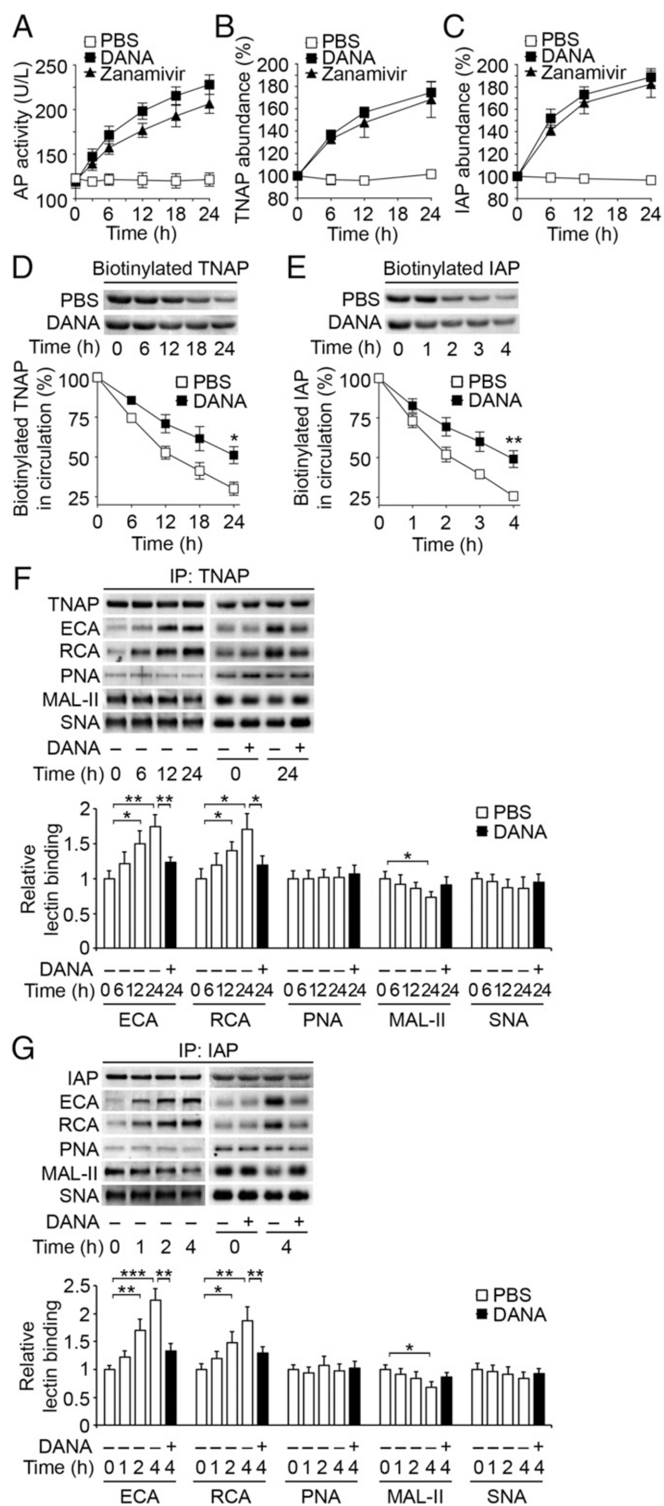

Fig. 3. Alkaline phosphatase $t_{1/2}$s linked rates of N-glycan remodeling. (*A–C*) Plasma alkaline phosphatase activity, TNAP abundance, and IAP abundance among WT C57BL/6J mice following administration of PBS, DANA, or zanamivir every 6 h. Circulating $t_{1/2}$ analyses of TNAP (*D*) and IAP (*E*) biotinylated in vivo in the presence of either DANA or PBS administered every 6 h and quantified following immunoprecipitation relative to time 0. Biotinylated TNAP (*F*) and IAP (*G*) were isolated from WT C57BL/6J mice at indicated times by lectin affinity chromatography in the presence of PBS or DANA administered every 6 h. Identical amounts of TNAP and IAP were then analyzed by SDS/PAGE, and lectin blotting was quantified normalized to time = 0. Data are representative of results from six to eight separate littermate cohort comparisons, and are presented as means ± SEM (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). IP, immunoprecipitation.

RNA splicing. Neu1 appears to account for a relatively small fraction of total Neu activity using a synthetic substrate in the Neu assay; however, Neu1 has a higher specific activity for protein substrates compared with Neu3 (28). The possibility that plasma mannosidase activity may also contribute to N-glycan remodeling with increased protein age was not addressed in this study. Further studies of model systems in the complete absence of specific glycosidases should resolve their relative contributions.

The AMR is expressed on the mammalian hepatocyte surface and is one of multiple mammalian asialoglycoprotein receptors of the C-type lectin family (3, 29). Secreted protein turnover controlled by the AMR comprised ~50% of the plasma proteome identified bearing Gal ligands of the related *Erythrina cristagalli* lectin. Although it is unlikely that the analytical lectins used are precise indicators of AMR ligands, overlap in proteins modulated in AMR deficiency was noted. The functions of proteins modulated by the AMR span proteolysis, coagulation, inflammation, and immunity. Comparative proteomic studies of Asgr1 and Asgr2 deficiency indicated a similar compilation of secreted proteins accumulated. This finding implied that both Asgr1 and Asgr2 are required in the AMR-dependent turnover of secreted proteins, which may reflect the presence of various heteromeric AMR complexes that operate in determining protein binding and endocytic turnover (30). The existence of other asialoglycoprotein receptors, including the Kupffer cell receptor, indicates the possibility of functional compensation moderating the effects of AMR deficiency. The further though lesser accumulation of secreted proteins bearing exposed GlcNAc and Man in AMR deficiency is consistent with reduced turnover with extended life spans that render them susceptible to further remodeling by galactosidase and glucosaminidase activities.

The unmasking of GlcNAc and Man linkages subsequent to Neu activity requires additional time, suggesting that secreted proteins with extended life spans may be cleared by lectins that bind GlcNAc and Man, such as integrin αM and the Mmr. These endocytic lectins may also be involved in the turnover of secreted proteins that escape clearance by the AMR and other asialoglycoprotein receptors. Mammalian lectins that bind GlcNAc and Man linkages may further represent phylogenic vestiges of a primordial mechanism of secreted protein aging and turnover because avian species mostly lack Sia linkages. In this regard, it has been demonstrated that the avian AMR homolog specifically binds GlcNAc, which is exposed following hydrolysis of terminal Gal linkages typical of nascent mature avian N-glycans (31).

Glycosidase substrate recognition and binding represent control points determining the life spans of secreted proteins. Multiple mechanisms may regulate this process. N-glycan remodeling may occur by random interactions of glycosidases with secreted proteins determined by their relative concentrations. Nevertheless, the shorter life span of IAP was associated with an increased rate of N-glycan remodeling compared with TNAP, even though IAP is present at a 10-fold lower concentration than TNAP. The number of N-glycan consensus sites also does not correlate with secreted protein life spans. For example, TNAP and IAP have five and three N-glycan consensus sites conserved in mouse and human homologs, respectively, yet their normal life spans are 12 h and 2 h, respectively. Also relevant are variations in N-glycan site occupancy and the variable presence of bi-, tri-, or tetraantennary N-glycan structures. It is further likely that protein conformation influences lectin binding by the juxtaposition of N-glycans among native folded proteins in forming multivalent lectin ligands (32).

In the regulation of secreted protein homeostasis by biosynthesis and turnover, the most rapid way to achieve changes in protein abundance is by modulating turnover. We have identified an intrinsic multistep mechanism of secreted protein aging and turnover consisting of the sequential glycosidic remodeling of N-glycans thereby unmasking ligands of endogenous endocytic lectin receptors. The synthesis and phylogenic conservation of multiantennary N-glycan structures lends itself to the progressive formation of multivalent ligands with increased binding affinities for lectins. This mechanism may further extend to cell surface proteins as N-glycan number and branching can influence cell surface protein residency

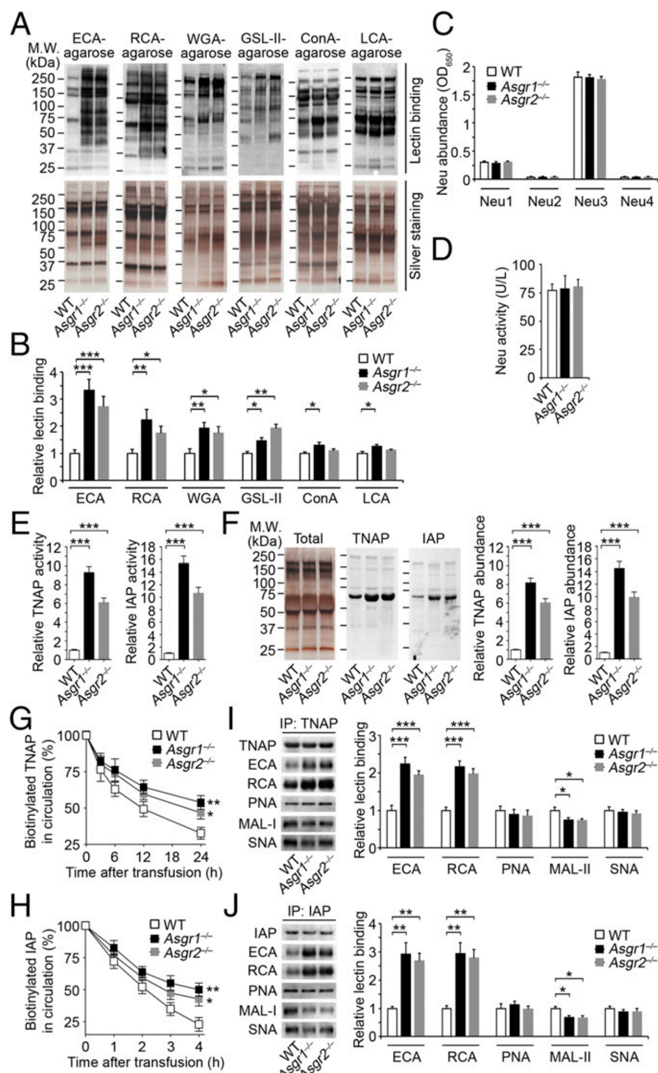

Fig. 4. AMRs in the turnover of secreted proteins. (*A*) Plasma proteins from mice lacking Asgr1 or Asgr2 were isolated by lectin affinity chromatography and analyzed by SDS/PAGE and lectin blotting (*Top*) among amounts of protein indicated by silver staining (*Bottom*). (*B*) Quantification of lectin ligands detected among plasma proteins in *A* normalized to results from WT littermates. (*C*) Abundance of Neu1, Neu2, Neu3, or Neu4 proteins in plasma. (*D*) Plasma Neu activity. (*E*) Plasma TNAP and IAP activities measured in immune precipitates normalized to WT littermates. (*F*) Abundance of TNAP and IAP proteins in plasma measured by SDS/PAGE and immunoblotting. (*G* and *H*) Biotinylated in vivo $t_{1/2}$ analyses of TNAP and IAP. (*I* and *J*) Lectin binding to equivalent amounts of immunoprecipitated TNAP and IAP. Data are representative of six to eight separate littermate cohorts of the indicated genotypes, and are presented as means ± SEM (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$).

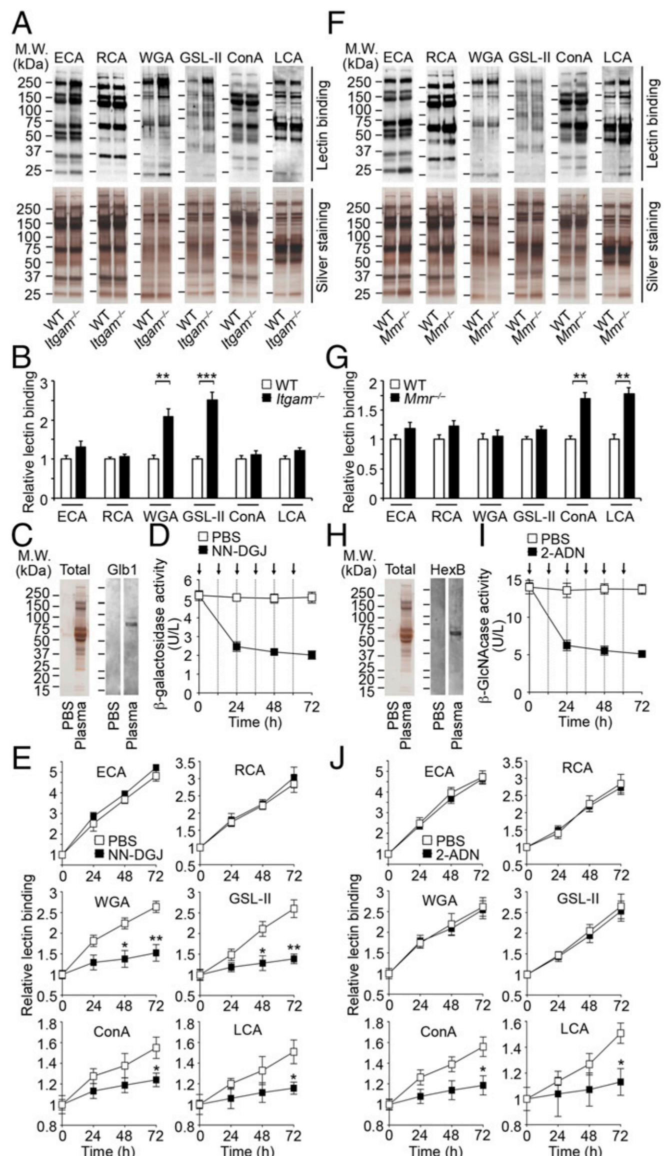

Fig. 5. Integrin αM and Mmr in the turnover of secreted proteins remodeled by β-gal and β-*N*-acetylglucosaminidase activity. (*A*) Plasma proteins from mice lacking integrin αM (*Itgam*) were isolated by lectin affinity chromatography and analyzed by SDS/PAGE and lectin blotting. (*B*) Quantification of lectin ligands detected among plasma proteins in *A* normalized to WT littermates. (*C*) β-Gal Glb1 detected in C57BL/6J mouse plasma by immunoblotting. (*D*) Plasma β-gal activity measured in the absence (PBS) or presence of β-gal inhibitor NN-DGJ (50 mg/kg) administered at time 0 and every 12 h (arrows) over a 72-h period. (*E*) Quantification of lectin ligands detected among plasma proteins in the presence of PBS or NN-DGJ normalized to time 0. (*F*) Plasma proteins from mice lacking *Mmr* were isolated by lectin affinity chromatography and analyzed by SDS/PAGE and lectin blotting. (*G*) Quantification of lectin ligands detected among plasma proteins in *F* normalized to WT littermates. (*H*) β-*N*-acetylglucosaminidase (β-GlcNAcase) HexB detected in C57BL/6J mouse plasma by immunoblotting. (*I*) Plasma β-*N*-acetylglucosaminidase activity measured in the absence (PBS) or presence of β-*N*-acetylglucosaminidase inhibitor 2-acetoamido-1,2-dideoxynojirimycin (2-ADN; 25 mg/kg) administered at time 0 and every 12 h (arrows) over a 72-h period. (*J*) Quantification of lectin ligands detected among plasma proteins in the presence of PBS or 2-ADN normalized to time 0. Data are representative of six to eight separate littermate cohorts of the indicated genotypes, and are presented as means ± SEM (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$).

(33). Moreover, the aging and turnover of blood cells and platelets may also be similarly controlled. The reported presence of asialoplatelets in AMR deficiency (34) may represent platelets of increased age. Because altered levels of secreted glycosidases have been associated with various diseases, including diabetes, pancreatitis, cancer, myocardial infarction, renal deficiency, cirrhosis, and viral hepatitis (9), it is reasonable to anticipate that the targeted modulation of secreted protein aging and turnover is involved in these diseases. In this regard, microbial targeting of neuraminidase activity to host platelets and specific coagulation factors, which determines the outcome of infection during pneumococcal sepsis (6, 18), can be reinterpreted as a focused modulation and acceleration of secreted protein aging and turnover.

Materials and Methods

Detailed methods, including descriptions of reagents, are included in *SI Materials and Methods*. All methods are routine and have been previously

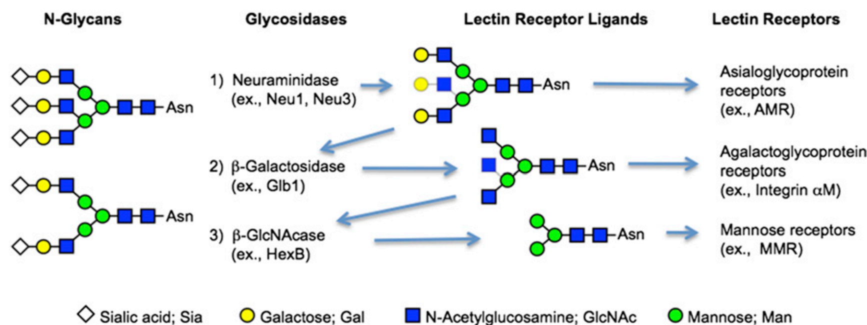

Fig. 6. An intrinsic mechanism of secreted protein aging and turnover. Multiantennary N-glycans of secreted proteins are progressively hydrolyzed by exoglycosidases, including neuraminidase (also known as sialidase), galactosidase, and glucosaminidase, during protein aging, generating remodeled N-glycans with unmasked multivalent Gal, GlcNAc, and Man linkages. These remodeled N-glycans are ligands of various endocytic lectin receptors. Multiple glycosidases and lectin receptors together determine secreted protein life span and abundance.

published multiple times in the referenced literature, including affinity chromatography, lectin blotting, Western blotting, and enzyme activity assays, with further details provided in the figure legends. Data were analyzed and presented as means ± SEM unless otherwise stated. ANOVA on ranks or the Student *t* test was used to determine statistical significance in some experiments. *P* values of less than 0.05 were considered significant. The Institutional Animal Care and Use Committees of the University of California, Santa Barbara and the Sanford–Burnham Medical Research Institute approved studies undertaken herein. Informed consent was obtained for blood collected from human subjects.

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