Extrinsic sialylation is dynamically regulated by systemic triggers *in vivo*

Received for publication, May 8, 2017, and in revised form, July 12, 2017 Published, Papers in Press, July 17, 2017, DOI 10.1074/jbc.C117.795138

Charles T. Manhardt[‡], Patrick R. Punch[‡], Christopher W. L. Dougher[§], and Joseph T. Y. Lau[‡1]

From the Departments of [‡]Molecular and Cellular Biology and [§]Immunology, Roswell Park Cancer Institute, Buffalo, New York 14263

Edited by Luke O'Neill

Recent reports have documented that extracellular sialyltransferases can remodel both cell-surface and secreted glycans by a process other than the canonical cell-autonomous glycosylation that occurs within the intracellular secretory apparatus. Despite association of the abundance of these extracellular sialyltransferases, particularly ST6Gal-1, with disease states such as cancer and a variety of inflammatory conditions, the prevalence of this extrinsic glycosylation pathway *in vivo* remains unknown. Here we observed no significant extrinsic sialylation in resting mice, suggesting that extrinsic sialylation is not a constitutive process. However, extrinsic sialylation in the periphery could be triggered by inflammatory challenges, such as exposure to ionizing radiation or to bacterial lipopolysaccharides. Sialic acids from circulating platelets were used *in vivo* to remodel target cell surfaces. Platelet activation was minimally sufficient to elicit extrinsic sialylation, as demonstrated with the $FeCl_3$ model of mesenteric artery thrombosis. Although extracellular ST6Gal-1 supports extrinsic sialylation, other sialyltransferases are present in systemic circulation. We also observed *in vivo* extrinsic sialylation in animals deficient in ST6Gal-1, demonstrating that extrinsic sialylation is not mediated exclusively by ST6Gal-1. Together, these observations form an emerging picture of glycans biosynthesized by the canonical cell-autonomous glycosylation pathway, but subjected to remodeling by extracellular glycan-modifying enzymes.

The presence of extracellular glycosyltransferases in the blood has been known for decades and systematically evaluated more recently (1). Although the theoretical ability of these enzymes to catalyze extracellular glycosylation was never in question, whether or not "extrinsic glycosylation" is a significant mode of glycan modification *in vivo* remained unknown. The conundrum stemmed, at least in part, from the perception that there is no sustainable extracellular source of sugar donor substrates for the extensive pool of blood-borne glycosyltransferases to function. Hence, the prevalence of extrinsic glycosylation *in vivo* remained an open question.

Two key recent findings rejuvenated the notion that extrinsic glycosylation can take place and that it is a physiologically important process. First, the data show that the circulatory pool of the sialyltransferase, ST6Gal-1, long regarded as a component of the hepatic acute phase response (2), is a systemic factor regulating new inflammatory cell production by imposing a checkpoint in blood cell development (3, 4). α2,6-Sialylation of marrow hematopoietic progenitor cells is dependent on the presence of extracellular ST6Gal-1, and does not require endogenous expression of the enzyme by the progenitor cells (5). Animals deficient in the circulatory pool of ST6Gal-1 had exaggerated responses to T helper cell inflammatory stimuli attributed to overly robust production of granulocytes and eosinophils (4). There is also accumulating evidence implicating extrinsic ST6Gal-1 in generating the sialyl-epitope on the Fc region of the IgG critical in immunosuppressive IgG activity (6, 7). The second key set of findings, pioneered by Wandall *et al.* (8), implicated circulating platelets as carriers of activated sugar donor substrates. Lee *et al.* (9) demonstrated that the cargo of sialic acid substrate, which is releasable upon platelet activation, is sufficient to drive extrinsic ST6Gal-1 sialylation *in vitro*.

Because ST6Gal-1 and other sialyltransferases are freely available in systemic circulation (1), we had expected extrinsic sialylation to be a significant contributor in the biosynthesis of cell-surface sialyl-glycans. Here we provide evidence that extrinsic sialylation does take place to a significant degree *in vivo*, but the data also show that extrinsic sialylation is not a constitutive process. The data point to a process tightly regulated by platelet function. Platelet activation, occurring during inflammation (9–11), triggers extrinsic sialylation putatively by making donor sialic acids available. Thrombosis is minimally sufficient to trigger extrinsic sialylation, and platelet sialic acids are utilized to decorate target cell-surface glycans *in vivo*. These observations highlight the potential of extrinsic sialylation to impact a diverse plethora of physiologically important events in inflammation, maintenance of blood cell numbers, and cell survival.

Results

Extrinsic sialylation is not a constitutive process

To recapitulate our earlier observation that *St6gal1*-null hematopoietic cells repopulating in a wild-type C57BL/6 host

This work was supported by NHLBI Program of Excellence in Glycosciences Grant P01HL107146 and NIAID Grant R01AI056082 from the National Institutes of Health (to J. T. Y. L.). The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

This article contains supplemental Figs. 1 and 2.

[1] To whom correspondence should be addressed: Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263. Tel.: 716-845-8914; Fax: 716-845-5908; E-mail: joseph.lau@roswellpark.org.

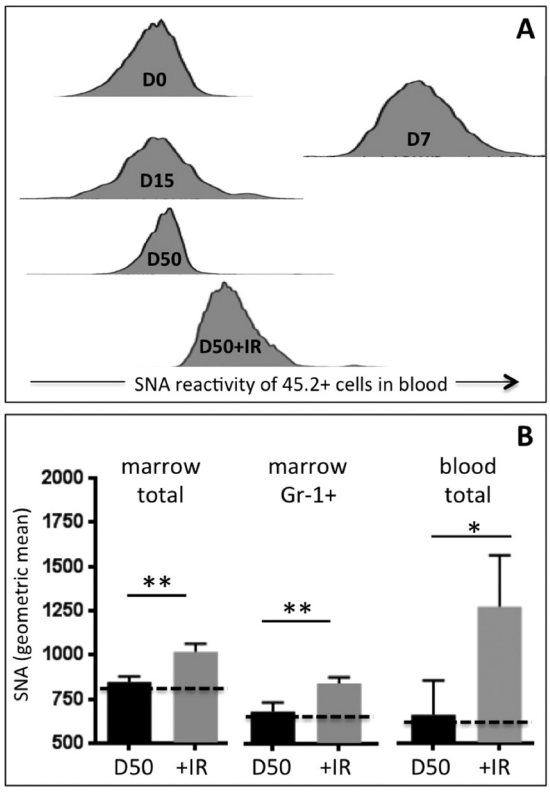

Figure 1. Irradiation triggers extrinsic sialylation *in vivo*. *St6gal1*-null marrow cells (CD45.2$^+$) were transplanted into irradiated C57BL/6 (CD45.1$^+$) recipients as described previously (12). Donor-derived CD45.2$^+$ cells in the blood were assessed for SNA reactivity to determine cell-surface α2,6-sialylation. *A*, *D0* represents *St6gal1*-null blood cells harvested from the native *St6gal1*-null mouse as baseline negative α2,6-sialylation profile. *D7*, *D15*, and *D50* represent CD45.2$^+$ cells recovered from recipients at 7, 15, and 50 days after transplantation, respectively. *D50+IR* represents samples from animals that received 12 grays of irradiation 24 h prior to blood collection at D50. *B*, comparison of geometric mean of SNA signal in CD45.2$^+$ cells collected at D50 and D50+IR. Shown are total marrow nucleated cells (*Total Marrow*), marrow GR-1$^+$ cells (*Marrow Gr-1*$^+$), or total circulating cells (*Total Blood*). *Dashed lines* signify the geometric mean of equivalent cell fractions from the native *St6gal1*-null animal (*D0*) as baseline negative α2,6-sialylation. *n* of 3 animals were used for each data point. Statistical significance for the difference is indicated by * ($p <= 0.05$) or ** ($p <= 0.005$).

were modified by a non-cell-autonomous mechanism but by systemic, *e.g.* host-derived, ST6Gal-1 (12), *St6gal1*-null marrow cells (CD45.2$^+$), genetically unable to mediate attachment of α2,6-linked sialic acids onto Type-II lactosaminyl-glycans, were used to repopulate the hematopoietic compartment of wild-type (CD45.1$^+$) recipients. α2,6-Sialylation of the *St6gal1*-null hematopoietic cells was monitored by their acquisition of cell-surface SNA reactivity. Earlier, we had also verified that SNA reactivity is an accurate indication of the degree of α2,6-sialylation by ST6Gal-1, as supported by identical results obtained using another α2,6-sialic acid-specific lectin from *Polyporus squamosus* (PSL) and definitively confirmed by MALDI-TOF MS^n fragmentation analysis (12). At day 7 after transplantation, which was immediately upon re-establishment of circulating blood counts, the circulating *St6gal1*-null hematopoietic cells were intensely SNA-reactive (Fig. 1*A*), reproducing those earlier observations.

We now observed that over the next several weeks, surface SNA reactivity on the circulating *St6gal1*-null cells diminished. By day 15 and day 50, the *St6gal1*-null cells reverted to an SNA-negative state consistent with their genotype (Fig. 1*A*). We hypothesized that the transient presence of cell-surface α2,6-sialyl-glycans was due to the inflammation resulting from full-body ionizing radiation. Radiation was used to prepare the animals for adoptive transfer, and extrinsic sialylation ceased upon resolution of the inflammatory response. To test this idea, the chimeric animals at day 50 were again irradiated, and we observed the reacquisition of cell-surface SNA reactivity on the *St6gal1*-null cells (Fig. 1*A*). Shown in Fig. 1*B* are the geometric means of the SNA signal, comparing the *St6gal1*-null cells in the chimeric animals at day 50 with and without irradiation. This shows irradiation-dependent acquisition of cell-surface SNA reactivity of *St6gal1*-null cells in the marrow and in peripheral circulation. Without irradiation, the geometric means of SNA of marrow and circulating *St6gal1*-null cells were similar to the values observed in the native *St6gal1*-null mouse. The data indicate that extrinsic ST6Gal-1 remodeling is not a constitutive process. There was extensive remodeling of the adopted hematopoietic cells immediately following transplantation. Extrinsic remodeling diminished as the animals recovered from the procedure, and the cells reverted to a negative α2,6-sialylation status consistent with their ST6Gal-1–null genotype. Extrinsic ST6Gal-1 remodeling was again triggered by exposure to radiation, resulting in α2,6-sialylation of the ST6Gal-1–null cells. We hypothesize that the severe systemic inflammation caused by irradiation was the trigger for the extrinsic remodeling.

We observed that extensive α2,6-sialylation in the newly reconstituted hematopoietic compartment at day 7 could be reproduced only partially upon irradiation of the re-established marrow at day 50. A possible explanation is that the day 7 point was a healthy, actively repopulating hematopoietic compartment from cells that had never been exposed to radiation. On the other hand, all the cells at the day 50 point were irradiated, and these day 50 cells, if not already apoptotic, would enter cell death within the next few days. Therefore, the health status of the populations as well as the composition of the hematopoietic subsets may contribute to differences in frequencies of the observed extrinsic sialylation. Health differences may contribute to altered levels of exposed Type-II lactosaminyl termini on the cell surfaces available to be extrinsically sialylated, or availability of sialic acid donor substrates. Although this treatise needs to be explored in detail, likely necessitating the use of mass spectrometric analysis, it is beyond the scope of the present report.

Involvement of platelets in extrinsic sialylation

To further test the idea that inflammation drives extrinsic sialylation, intratracheal delivery of LPS was used to elicit localized, acute airway inflammation (13). However, the intense endogenous SNA reactivity in the wild-type lung hampered the ability to detect extrinsic sialylation by SNA. Therefore, we leveraged the ability of platelets to release sialic acid donor substrate to support extrinsic sialylation (9) by tracking platelet-derived sialic acids. To accomplish this, wild-type (C57BL/6) platelets with the Neu5Gc form of sialic acid were transfused into the CMAH-null mouse, which does not natively express Neu5Gc due to inactivation of the cytidine monophosphate-*N*-

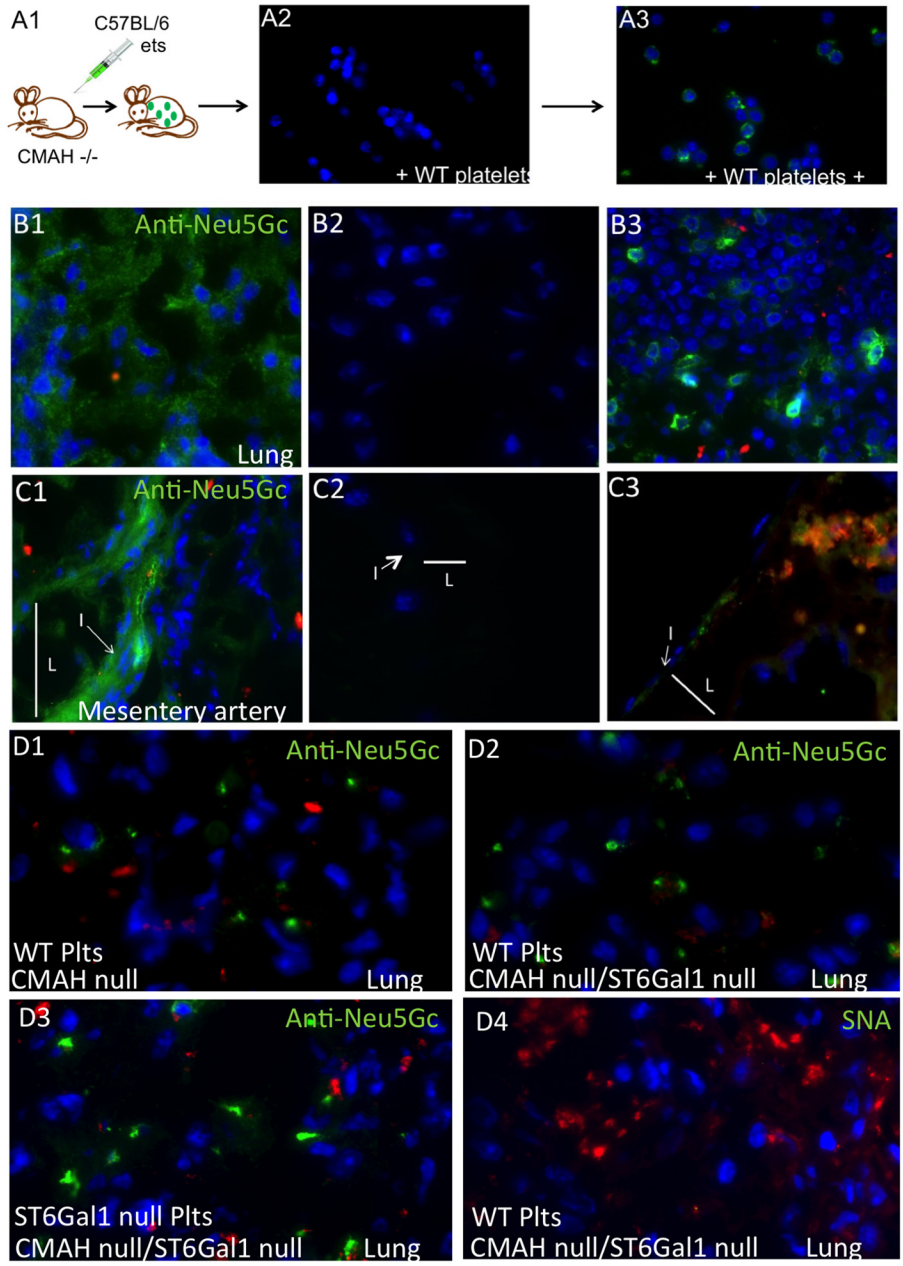

Figure 2. Inflammation and platelet activation triggers extrinsic sialylation. *A*, wild-type platelets from C57/BL6 animals, which contain the Neu5Gc form of sialic acid, were transfused into CMAH-null animals natively lacking Neu5Gc and expressing only Neu5Ac (*A1*), circulating white cells were probed for Neu5Gc either not subjected to or 3 h after being subjected to intratracheal inoculation of LPS to elicit a localized bout of acute airway inflammation (*A2* and *A3*, respectively), as described under "Experimental Procedures." *B*, Neu5Gc staining of frozen lung sections. *B1*, uniform staining of a C57BL/6 lung. *B2* and *B3*, respectively, Neu5Gc staining of CMAH-null recipients transfused with C57BL/6 platelets either not subjected to or 3 h after being subjected to intratracheal inoculation of LPS. *C*, mesentery arteries stained for Neu5Gc. *C1*, C57BL/6 at baseline. *C2* and *C3*, respectively, CMAH-null recipients transfused with C57BL/6 platelets either without or with a 10-min application of FeCl3 to induce localized thrombosis as described under "Experimental Procedures." *I* and *L* denote intima and lumen of the artery, respectively. *D*, comparison of CMAH-null and CMAH-null/*St6gal1*-null recipients receiving Neu5Gc$^+$ wild-type platelets and 3 h after being subjected to intratracheal inoculations of LPS (*D1* and *D2*, respectively), demonstrating the ability to extrinsically transfer platelet Neu5Gc despite the absence of systemic ST6Gal-1, which still occurs using platelets from ST6Gal-1–deficient animals (*D3*). *D4* shows the absence of SNA reactivity in the CMAH-null/*St6gal1*-null recipients despite receiving SNA$^+$ wild-type platelets. For all panels, CD41-phycoerythrin (CD41-PE) (platelet marker) is visualized in *red*.

acetylneuraminic acid hydroxylase (CMAH)[2] critical for the formation of Neu5Gc from Neu5Ac (14, 15). Thus, platelet-released Neu5Gc can be tracked in otherwise exclusively Neu5Ac-negative CMAH-null recipients. This is diagrammed in Fig. 2*A1* using an antibody specific for Neu5Gc. The presence of Neu5Gc platelets, by itself, did not generate significant levels of Neu5Gc in the host cells observable in the peripheral blood, in the lung, or in the mesenteric artery (Fig. 2, *A2*, *B2*, and *C2*, respectively). However, within 3 h of LPS instillation to elicit an acute bout of airway inflammation, Neu5Gc-positive (*green channel*) cells are clearly visible in peripheral circulation and in the lung (Fig. 2, *A3* and *B3*, respectively), and these cells are clearly distinguished from the CD41-positive (*red channel*)

[2] The abbreviation used is: CMAH, cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase.

platelets. Neu5Gc reactivity presented as punctate patterns visualized on circulating cells that have not been permeabilized prior to exposure to the antibody, consistent with the interpretation of the cell-surface localization of the transferred Neu5Gc. Within the lung, the intense Neu5Gc staining present in the wild-type animal (Fig. 2*B1*) was notably absent in the CMAH-null lung at baseline (Fig. 2*B2*), despite being transfused with wild-type platelets. The segmented lobular nuclei of many of the NeuGc-positive cells are consistent with infiltrating granulocytes. Statistics from flow cytometry are included in supplemental Fig. 1. When compared with B cell ($B220^+$) and T cell ($CD3^+$), neutrophils ($CD11b^+$ and $Ly6G^+$) were more extensively modified by platelet Neu5Gc. The short time course, within 3 h of LPS insult, minimized the possibility of cell-autonomous phagocytosis and recycling of platelet glycans onto recipient cell surfaces.

The data so far implicate the necessity of inflammation in extrinsic sialylation and demonstrate that activated platelets are a significant physiologic source of sialic acids. We now ask whether platelet activation alone, in the absence of inflammation, can be minimally sufficient to drive extrinsic sialylation. The well-established $FeCl_3$ model to induce arterial thrombosis without inflammation was used (16, 17). Blood clots formed within 10 min of $FeCl_3$ application to an exposed mesenteric artery. The wild-type C57BL/6 artery is shown as reference, where the ubiquitous Neu5Gc signal outlines the mesenteric arterial wall (Fig. 2*C1*). In the CMAH-null recipients transfused with $Neu5Gc^+$ platelets, there was no anti-Neu5Gc signal observable in the mesenteric artery at baseline (Fig. 2*C2*). However, 10 min following $FeCl_3$ application, Neu5Gc was distinctly present on the arterial lumen wall adjacent to the newly formed platelet clot (Fig. 2*C3*), supporting the idea that platelet activation can be minimally sufficient to initiate extrinsic sialylation. The extremely short time course, within 15 min, also minimized cell-autonomous phagocytosis and recycling of platelet glycans onto recipient cell surfaces as a potential mechanism.

The ability of extracellular ST6Gal-1 to mediate extrinsic sialylation is well-documented. However, the transfer of platelet Neu5Gc can result by other sialyltransferase activities known to be present in systemic circulation (1). To demonstrate that enzymes other than ST6Gal-1 can mediate extrinsic sialylation, the CMAH-null/*St6gal1*-null animal, natively deficient in Neu5Gc and functional ST6Gal-1, received a transfusion of wild-type Neu5Gc-positive platelets. Three hours after LPS elicited airway inflammation, the Neu5Gc-positive cells, clearly segregated from the CD41-positive platelets, were visible in frozen lung histological sections (Fig. 2*D2*). The ability to transfer platelet Neu5Gc in the complete absence of functional ST6Gal-1 confirms that extrinsic sialylation is not catalyzed exclusively by ST6Gal-1. The corresponding Neu5Gc signal in lung of a CMAH-null but ST6Gal-1–intact animal is shown for comparison (Fig. 2*D1*), and the roughly equal Neu5Gc signal intensity observed between the ST6Gal-1–intact and ST6Gal-1–null recipients strongly suggests that systemic ST6Gal-1 may not even be the dominant enzyme driving extrinsic sialylation. Neu5Gc staining frequencies between the CMAH-null and the CMAH-null/*St6gal1*-null recipients receiving wild-type platelet transfusions were also similar on the neutrophils in peripheral blood (supplemental Fig. 2). To further confirm that platelet-mediated extrinsic sialylation can occur even in the complete absence of functional ST6Gal-1, platelets from mice that lack α2,6-sialylation are still able to donate Neu5Gc, as demonstrated in transfusion of *St6gal1*-null but $Neu5Gc^+$ platelets into CMAH-null/*St6gal1*-null recipients (Fig. 2*D3*). To further demonstrate the mechanism of *de novo* synthesis of sialyl-glycans rather than redistribution of platelet sialyl-glycans, WT platelets that are both SNA^+ and $NeuGc^+$ were transfused into CMAH-null/*St6gal1*-null recipients. Thus, if there were a simple redistribution of platelet sialyl-glycans, we would observe acquisition of SNA reactivity in the absence of functional ST6Gal-1. Instead, we observed the complete absence of SNA signal upon airway inflammation (Fig. 2*D4*), strongly indicating that passive redistribution of platelet sialyl-glycans is not a significant event. In summary, these observations show that not only are platelets an important *in vivo* source of sialic acids for extrinsic sialylation, but importantly, extrinsic sialylation is also mediated by other sialyltransferases in addition to ST6Gal-1.

Discussion

In 1970, Dr. Roseman postulated the existence of extracellular and cell-surface glycosyltransferases, or ecto-glycosyltransferases (18), and he further speculated a role of these externally localized enzymes in intercellular interactions by the formation of transferase–acceptor binding pairs that could be dissolved by supplying the sugar donor substrate to drive the glycosyltransferase catalysis to completion (19). Since then, there have been sporadic reports of ecto-glycosyltransferases on the surfaces of a diverse number of cells including fibroblasts (20, 21), neuronal cells (22), lymphocytes (23), as well as hematopoietic progenitors (24). However, only a cell-surface β1,4-galactosyltransferase participating in mammalian fertilization and in neuronal development became well-established (25–27). The cell-surface residence of other glycosyltransferases, including ST6Gal-1, remained controversial, due mainly to inherent difficulties in distinguishing those enzymes residing on the surfaces of intact cells from the contributions of contaminating membrane debris. Regardless of whether or not extracellular glycosyltransferases actually reside on cell surfaces, it is well-established that enzymatically active ST6Gal-1 and other glycosyltransferases are present in the extracellular spaces and that they circulate freely in the blood (1, 28). Moreover, hematopoietic progenitor cells are extensively, if not exclusively, modified by the systemic ST6Gal-1 (12), and result in attenuated granulopoiesis by blunting the ability of the common myeloid progenitor to respond to G-CSF signaling (3).

The observations presented here confirm that, despite the availability of extracellular ST6Gal-1 and other sialyltransferases, extrinsic sialylation does not appear to be a constitutive process. Our observations show that stressful insults, such as irradiation or acute peripheral inflammation, are necessary to trigger extrinsic sialylation, at least in the periphery. Platelet activation appears to be a minimally sufficient condition for extrinsic sialylation, as demonstrated by the $FeCl_3$ model, which can induce artery thrombosis without inflammation.

The observations described here confirm the role of platelets as an *in vivo* source of the donor substrate, made available upon platelet activation (9), and suggest that access to the sialic acid donor substrate is the limiting factor for extrinsic sialylation *in vivo*. Although there is ample evidence for extrinsic sialylation also occurring within the bone marrow hematopoietic microenvironment (3, 12), the present approach of tracking sialic acids from transfused platelets is uninformative as to how extrinsic sialylation in the bone marrow might be regulated. The marrow is the site of endogenous thrombopoiesis; any transfused platelets that manage to enter the marrow are likely diluting into a vast excess of newly synthesized platelets and platelet progenitors.

There has been much focus on extrinsic sialylation mediated by blood-borne ST6Gal-1, but other sialyltransferases, most notably the α2,3-sialyltransferases, are also abundantly present in systemic circulation (9). The transfer of platelet Neu5Gc onto host cells natively lacking Neu5Gc was followed to visualize overall extrinsic sialylation regardless of specific sialyltransferases. A punctate Neu5Gc signal was observed on the surface of targeted cells, predominantly infiltrating granulocytes, within 3 h of an inflammatory challenge (Fig. 2*D1*). A similar pattern of Neu5Gc staining was observed in ST6Gal-1–deficient animals, identifying other extracellular sialyltransferases in addition to ST6Gal-1 as participants in extrinsic sialylation *in vivo* (Fig. 2, *D2* and *D3*). Furthermore, the intensity of the observed platelet–Neu5Gc transfer in the complete absence of functional ST6Gal-1 infers strongly that ST6Gal-1 may not even be the dominant enzyme mediating extrinsic sialylation.

The caveat that platelet sialyl-glycans are passively absorbed onto recipient cell surfaces can be discounted based on the following observations. First, the short time course of 3 h after inflammatory stimulus to the airway or within 15 min of $FeCl_3$-induced mesenteric thrombosis minimized the extent of phagocytosis or pinocytosis of platelet-derived sialyl-glycans and subsequently the cell-autonomous recycling of platelet sialic acids onto host cell surfaces. Moreover, sialylation of recipient cells was observable within 15 min of $FeCl_3$-induced mesenteric thrombosis (Fig. 2*C3*). Second, although wild-type platelets (*e.g.* platelets containing α2,6-sialyl-glycans) were transfused into an ST6Gal-1–null host, SNA signal was not observed upon induction of inflammation, demonstrating an absolute requirement for functional ST6Gal-1 (Fig. 2*D4*). Moreover, even wild-type platelets are not abundantly endowed with SNA-reactive sialyl-glycans, which is quite evident in Fig. 2*D4*, and passive transfer of these glycans onto target cells is unlikely to account for the intensity of the observed SNA signals. Finally, third, we had previously observed that *St6gal1*-null platelets, which do not have α2,6-sialyl-glycans, are able to transfer sialic acids to target cells in *ex vivo* assays (9).

In addition to sialyltransferases, members of the galactosyl and fucosyl families of glycosyltransferases are also present in systemic circulation (1). Although it remains to be demonstrated directly that extrinsic galactosylation and fucosylation occur *in vivo*, platelets also contain releasable caches of other sugar precursor substrates such as UDP-GalNAc and UDP-Gal (8). The emerging picture is that platelets may serve as crucial regulators in the extrinsic remodeling of cell-surface glycans by extracellular glycosyltransferases, with the potential to impact a wide variety of pathologic processes. The propensity of platelets to adhere to granulocytes and other leukocytes, to the vascular endothelium, and to cancer cells may additionally facilitate extrinsic glycan remodeling by bringing the source for sugar donor substrates into the closest proximity of the targeted cell surfaces (29, 30).

Cancer is often associated with changes in platelet count. Thrombocytosis occurs in 10–50% of patients with solid malignancies and is associated with shortened survival times (31, 32). Alternatively, platelet insufficiency is a common side effect of cancer treatment, and extreme thrombocytopenia is often the reason for premature cessation from the full course of treatment. Although platelets are typically known to be critical for blood clotting, the current observations highlight a distinct possibility that changes in platelet homeostasis and activation can alter the overall tumor microenvironment by extrinsic glycan remodeling. Removal of sialic acid from immune cell surfaces such as dendritic cells enhances their anti-tumor activity (33). By inference, platelet-mediated extrinsic sialylation may dampen immune cell function, leading to poor clinical outcomes. Extrinsic remodeling of the tumor cell-surface glycans may also contribute to altered metastatic cell behaviors such as invasion, enhanced cell survival, and evasion from immune surveillance (34–37).

Experimental procedures

Animals

The Institute Animal Care and Use Committee of Roswell Park Cancer Institute approved all animal studies. The *St6gal1*-null mouse with globally inactivated ST6Gal-1 gene, unable to make α2,6-linked sialic acid, was described previously (5). The CMAH-null mouse, with a defect in the cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase rendering it unable to express Neu5Gc, was obtained from The Jackson Laboratory. Adoptive bone marrow transfers were performed as described previously (12). To assess transfer of platelet-derived Neu5Gc, 1×10^9 C57/BL6 platelets were transfused into each CMAH-null mouse. Platelets were isolated as described previously (9). Platelets were transfused by intravenous injection into the tail vein. Pre-depletion of recipient platelets was not performed to avoid inducing an unintended inflammatory or stress response in the animals. Transfused platelets remained in circulation for at least 2 days, declining to 70% of the starting level after 2 days without inflammation. Animals were used immediately after transfusion. To induce acute airway inflammation, mice were anesthetized with isoflurane, and 100 ng of LPS (Sigma) in 25 μl of saline was delivered by intratracheal instillation. Lung and blood were collected 3 h after LPS instillation, unless stated otherwise. To induce platelet activation without inflammation, mice were anesthetized with isoflurane, the intestines were exposed, and 6% $FeCl_3$ was applied to the mesentery artery via filter paper (30). Mice between ages of 50 and 70 days were used; differences between sexes were not noted.

Analytical procedures

All antibodies were purchased from BioLegend (San Diego, CA). Direct FITC-conjugated lectins (SNA, 8 μg/ml, Vector Laboratories) were also used. Bone marrow cells were collected from femurs of mice, resuspended in RBC lysis buffer (0.8% NH_4Cl, 0.1 mM EDTA buffered with $KHCO_3$ to pH 7.4), washed, and resuspended in PBS with 0.5% BSA or fetal bovine serum and 2 mM EDTA, and then passed through a 100-μm cell strainer (BD Biosciences). Samples were analyzed on a Fortessa B flow cytometer and processed using FlowJo software. For immunocytochemistry, nuclei were routinely labeled using DAPI (BioLegend 0.5 μg/ml), and platelets were routinely labeled using CD41 (BioLegend). Lung and artery tissue were collected from the different models, and frozen sections were prepared and probed for anti-Neu5Gc (clone Poly21469, BioLegend). Slides were mounted using FluorSave Reagent (Calbiochem), and then imaged on a Nikon Eclipse TE-2000E fluorescent microscope with a 60× objective and a CoolSNAP HQ camera (Photometrics, Tucson, AZ). Image acquisition was controlled by MetaMorph software. Statistical evaluations for differences between mean values were determined by Student's *t* test with post-test comparisons on GraphPad Prism 6 software (La Jolla, CA). A $p < 0.05$ was considered significant.

Author contributions—C. T. M. conceived, designed, and performed experiments and wrote the manuscript. C. T. M., P. R. P., and C. W. L. D. contributed to design and execution of experiments. J. T. Y. L. provided critical oversight, conceived and designed research, and wrote the manuscript.

Acknowledgments—The core facilities of Roswell Park Cancer Institute used in this work were supported in part by National Institutes of Health NCI Cancer Center Support Grant CA076056. The expert technical assistance of Valerie Andersen and Joseph Hassett is gratefully acknowledged. We also thank Dr. Himangi Marathe for help in flow cytometry.

References

1. Lee-Sundlov, M. M., Ashline, D. J., Hanneman, A. J., Grozovsky, R., Reinhold, V. N., Hoffmeister, K. M., and Lau, J. T. (2017) Circulating blood and platelets supply glycosyltransferases that enable extrinsic extracellular glycosylation. *Glycobiology* **27,** 188–198
2. Jamieson, J. C., McCaffrey, G., and Harder, P. G. (1993) Sialyltransferase: a novel acute-phase reactant. *Comp. Biochem. Physiol. B* **105,** 29–33
3. Dougher, C. W. L., Buffone, A., Jr, Nemeth, M. J., Nasirikenari, M., Irons, E. E., Bogner, P. N., and Lau, J. T. Y. (2017) The blood-borne sialyltransferase ST6Gal-1 is a negative systemic regulator of granulopoiesis. *J. Leukoc. Biol.* 10.1189/jlb.3A1216–538RR
4. Jones, M. B., Nasirikenari, M., Feng, L., Migliore, M. T., Choi, K. S., Kazim, L., and Lau, J. T. (2010) Role for hepatic and circulatory ST6Gal-1 sialyltransferase in regulating myelopoiesis. *J. Biol. Chem.* **285,** 25009–25017
5. Nasirikenari, M., Segal, B. H., Ostberg, J. R., Urbasic, A., and Lau, J. T. (2006) Altered granulopoietic profile and exaggerated acute neutrophilic inflammation in mice with targeted deficiency in the sialyltransferase ST6Gal I. *Blood* **108,** 3397–3405
6. Jones, M. B., Nasirikenari, M., Lugade, A. A., Thanavala, Y., and Lau, J. T. (2012) Anti-inflammatory IgG production requires functional P1 promoter in β-galactoside α2,6-sialyltransferase 1 (ST6Gal-1) gene. *J. Biol. Chem.* **287,** 15365–15370
7. Jones, M. B., Oswald, D. M., Joshi, S., Whiteheart, S. W., Orlando, R., and Cobb, B. A. (2016) B-cell-independent sialylation of IgG. *Proc. Natl. Acad. Sci. U.S.A.* **113,** 7207–7212
8. Wandall, H. H., Rumjantseva, V., Sørensen, A. L., Patel-Hett, S., Josefsson, E. C., Bennett, E. P., Italiano, J. E., Jr, Clausen, H., Hartwig, J. H., and Hoffmeister, K. M. (2012) The origin and function of platelet glycosyltransferases. *Blood* **120,** 626–635
9. Lee, M. M., Nasirikenari, M., Manhardt, C. T., Ashline, D. J., Hanneman, A. J., Reinhold, V. N., and Lau, J. T. (2014) Platelets support extracellular sialylation by supplying the sugar donor substrate. *J. Biol. Chem.* **289,** 8742–8748
10. Esmon, C. T. (2005) The interactions between inflammation and coagulation. *Br. J. Haematol.* **131,** 417–430
11. Gros, A., Ollivier, V., and Ho-Tin-Noé, B. (2014) Platelets in inflammation: regulation of leukocyte activities and vascular repair. *Front. Immunol.* **5,** 678
12. Nasirikenari, M., Veillon, L., Collins, C. C., Azadi, P., and Lau, J. T. (2014) Remodeling of marrow hematopoietic stem and progenitor cells by non-self ST6Gal-1 sialyltransferase. *J. Biol. Chem.* **289,** 7178–7189
13. Zhong, W., Cui, Y., Yu, Q., Xie, X., Liu, Y., Wei, M., Ci, X., and Peng, L. (2014) Modulation of LPS-stimulated pulmonary inflammation by Borneol in murine acute lung injury model. *Inflammation* **37,** 1148–1157
14. Chou, H. H., Takematsu, H., Diaz, S., Iber, J., Nickerson, E., Wright, K. L., Muchmore, E. A., Nelson, D. L., Warren, S. T., and Varki, A. (1998) A mutation in human CMP-sialic acid hydroxylase occurred after the *Homo-Pan* divergence. *Proc. Natl. Acad. Sci. U.S.A.* **95,** 11751–11756
15. Hedlund, M., Tangvoranuntakul, P., Takematsu, H., Long, J. M., Housley, G. D., Kozutsumi, Y., Suzuki, A., Wynshaw-Boris, A., Ryan, A. F., Gallo, R. L., Varki, N., and Varki, A. (2007) *N*-Glycolylneuraminic acid deficiency in mice: implications for human biology and evolution. *Mol. Cell. Biol.* **27,** 4340–4346
16. Li, W., McIntyre, T. M., and Silverstein, R. L. (2013) Ferric chloride-induced murine carotid arterial injury: a model of redox pathology. *Redox Biol.* **1,** 50–55
17. Neeves, K. B. (2015) Physiochemical artifacts in FeCl3 thrombosis models. *Blood* **126,** 700–701
18. Roseman, S. (1970) The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids* **5,** 270–297
19. Roth, S., McGuire, E. J., and Roseman, S. (1971) Evidence for cell-surface glycosyltransferases: their potential role in cellular recognition. *J. Cell Biol.* **51,** 536–547
20. Patt, L. M., and Grimes, W. J. (1975) Ectoglycosyltransferase activity in suspensions and monolayers of cultured fibroblasts. *Biochem. Biophys. Res. Commun.* **67,** 483–490
21. Porter, C. W., and Bernacki, R. J. (1975) Ultrastructural evidence for ectoglycosyltransferase systems. *Nature* **256,** 648–650
22. Matsui, Y., Lombard, D., Hoflack, B., Harth, S., Massarelli, R., Mandel, P., and Dreyfus, H. (1983) Ectoglycosyltransferase activities at the surface of cultured neurons. *Biochem. Biophys. Res. Commun.* **113,** 446–453
23. Gross, H. J., Merling, A., Moldenhauer, G., and Schwartz-Albiez, R. (1996) Ecto-sialyltransferase of human B lymphocytes reconstitutes differentiation markers in the presence of exogenous CMP-*N*-acetyl-neuraminic acid. *Blood* **87,** 5113–5126
24. Schwartz-Albiez, R., Merling, A., Martin, S., Haas, R., and Gross, H. J. (2004) Cell surface sialylation and ecto-sialyltransferase activity of human CD34 progenitors from peripheral blood and bone marrow. *Glycoconj. J.* **21,** 451–459
25. Shur, B. D. (1991) Cell surface β1,4 galactosyltransferase: twenty years later. *Glycobiology* **1,** 563–575
26. Hathaway, H. J., and Shur, B. D. (1992) Cell surface ·1,4-galactosyltransferase functions during neural crest cell migration and neurulation *in vivo*. *J. Cell Biol.* **117,** 369–382
27. Miller, D. J., Macek, M. B., and Shur, B. D. (1992) Complementarity between sperm surface β-1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. *Nature* **357,** 589–593

28. Yasukawa, Z., Sato, C., and Kitajima, K. (2005) Inflammation-dependent changes in α2,3-, α2,6-, and α2,8-sialic acid glycotopes on serum glycoproteins in mice. *Glycobiology* **15,** 827–837
29. Massberg, S., Enders, G., Leiderer, R., Eisenmenger, S., Vestweber, D., Krombach, F., and Messmer, K. (1998) Platelet-endothelial cell interactions during ischemia/reperfusion: the role of P-selectin. *Blood* **92,** 507–515
30. Gawaz, M., Langer, H., and May, A. E. (2005) Platelets in inflammation and atherogenesis. *J. Clin. Invest.* **115,** 3378–3384
31. Buergy, D., Wenz, F., Groden, C., and Brockmann, M. A. (2012) Tumor-platelet interaction in solid tumors. *Int. J. Cancer* **130,** 2747–2760
32. Yuan, L., and Liu, X. (2015) Platelets are associated with xenograft tumor growth and the clinical malignancy of ovarian cancer through an angiogenesis-dependent mechanism. *Mol. Med. Rep.* **11,** 2449–2458
33. Silva, M., Silva, Z., Marques, G., Ferro, T., Gonçalves, M., Monteiro, M., van Vliet, S. J., Mohr, E., Lino, A. C., Fernandes, A. R., Lima, F. A., van Kooyk, Y., Matos, T., Tadokoro, C. E., and Videira, P. A. (2016) Sialic acid removal from dendritic cells improves antigen cross-presentation and boosts anti-tumor immune responses. *Oncotarget* **7,** 41053–41066
34. Lu, J., and Gu, J. (2015) Significance of β-galactoside α2,6 sialyltranferase 1 in cancers. *Molecules* **20,** 7509–7527
35. Zhao, Y., Li, Y., Ma, H., Dong, W., Zhou, H., Song, X., Zhang, J., and Jia, L. (2014) Modification of sialylation mediates the invasive properties and chemosensitivity of human hepatocellular carcinoma. *Mol. Cell. Proteomics* **13,** 520–536
36. Korczak, B., and Dennis, J. W. (1993) Inhibition of *N*-linked oligosaccharide processing in tumor cells is associated with enhanced tissue inhibitor of metalloproteinases (TIMP) gene expression. *Int. J. Cancer* **53,** 634–639
37. Schultz, M. J., Swindall, A. F., and Bellis, S. L. (2012) Regulation of the metastatic cell phenotype by sialylated glycans. *Cancer Metastasis Rev.* **31,** 501–518