Bioconjugate Chemistry

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Glycoform Remodeling Generates a Synthetic T Cell Phenotype

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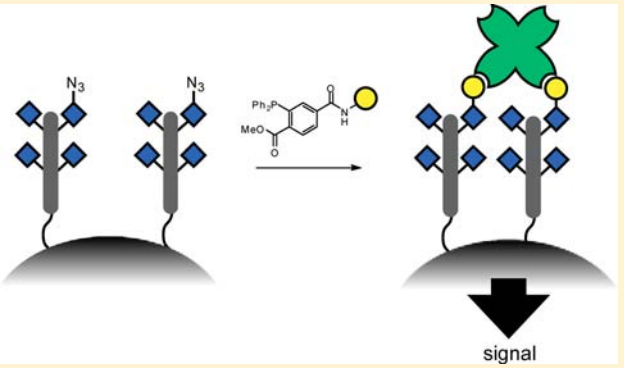
Supporting Information

ABSTRACT: The glycan of specific proteins can dictate the response of cells to stimuli, and thus their phenotype. We describe a chemical strategy to modify the cellular glycoform of T cells, which resulted in a modified cellular response. Our data indicate that chemical modification of the phosphatase CD45 is responsible for the observed differences in response to receptor cross-linking. By increasing the content of galactose epitopes in the glycocalyx of a lymphoma cell line, we were able to increase the response of the cell to lectin stimulation through the glycoprotein receptor, CD45. The method described here exploits metabolic labeling of a cell to reprogram the cellular response to external stimuli though changes in the number of lectin binding sites on the cell surface.



INTRODUCTION

Glycosylation of cellular receptors can act as a critical determinant of cell fate and response to stimuli. However, the inherent complexity of mammalian glycosylation presents a major barrier to understanding the role of specific glycan epitopes. In lymphocytes, glycosylation plays a major role in cell maturation and signaling.[1] The leukocyte common antigen, CD45, is a critical glycoprotein receptor on the T cell surface.[2,3] CD45 has a large, glycosylated, ectodomain and an intracellular domain with protein tyrosine phosphatase (PTPase) activity. The enzymatic activity of CD45 plays a central role in T cell activation, and is the major phosphatase activity found in human lymphocytes.[4,5] Glycosylation of CD45 is extensive, constituting approximately 25% of the glycoprotein mass.[2] The CD45 glycan is microheterogeneous,[6] and differences in the glycan structure are known to be critical for cellular response to physiological stimuli.[7] The enzyme activity of the receptor-like PTPases, including CD45, are sensitive to oligomerization state,[8,9] and lectins which induce clustering of the receptor can directly regulate PTPase activity.[10] Baum and co-workers have shown that differential glycosylation of CD45 among T cell populations was responsible for the phenotypic response of effector cells to endogenous lectins, known as galectins.[11] These findings established that the glycosylation state of the receptor was essential to cellular responses. Due to its central role in lymphocyte regulation and the importance of its glycosylation state, we chose to study lectin-mediated CD45 phosphatase activity as a model of glycosylation dependent receptor-mediated signaling in T cells.

The cellular glycosylation machinery modulates the number and location of lectin binding sites, and the sensitivity of the CD45 response. For example, increased activity of the ST6GalI sialyltransferase reduces the response of the PTPase to the presence of galectin by masking its primary binding epitope, thus altering the cellular phenotype through a specific change in the glycan.[10] This process is most clearly demonstrated by the work of Toscano et al., who found that the CD45 glycoform was a major determinant of T cell effector function.[11] The balance of T cell effectors, for example, between T_H1 and T_H2 cells, has been proposed to play a role in autoimmune diseases.[12,13] As a result, methods which alter the balance of effector T cells would be of interest for immunotherapy.[14−17] We propose here that the glycosylation state of CD45 presents a specific biochemical target that can be used to alter the effector phenotype of cells through their response to stimuli. However, methods for controlling receptor glycosylation have not previously been applied to this problem.

There are a wide variety of methods which have been used to alter the glycosylation state of cell surface glycoproteins. Examples can be classified into genetic methods, such as modification of the expression level of transferase proteins,[18,19] or chemical methods,[20,21] such as transferase inhibitors.[22,23] Both of these strategies result in broad alteration of the glycosylation state of proteins in the cell. Additionally, these methods are restricted to the manipulation of native glycan epitopes. Several strategies for the chemoselective modification of cell surface glycans, often referred to as glycoform remodeling, have been reported.[24] Perhaps the earliest example was described by Tolvanen and Gahmberg, who generated neoglycoconjugates on the cell surface through chemical oxidation and conjugation of hydrazine derivatives.[25] The efficiency of this method has recently been improved upon through the use of aniline catalysis at low pH.[21,26] Exogenous glycosyltransferases tolerant of modified substrates have been

Received: November 9, 2012
Revised: May 10, 2013
Published: June 6, 2013

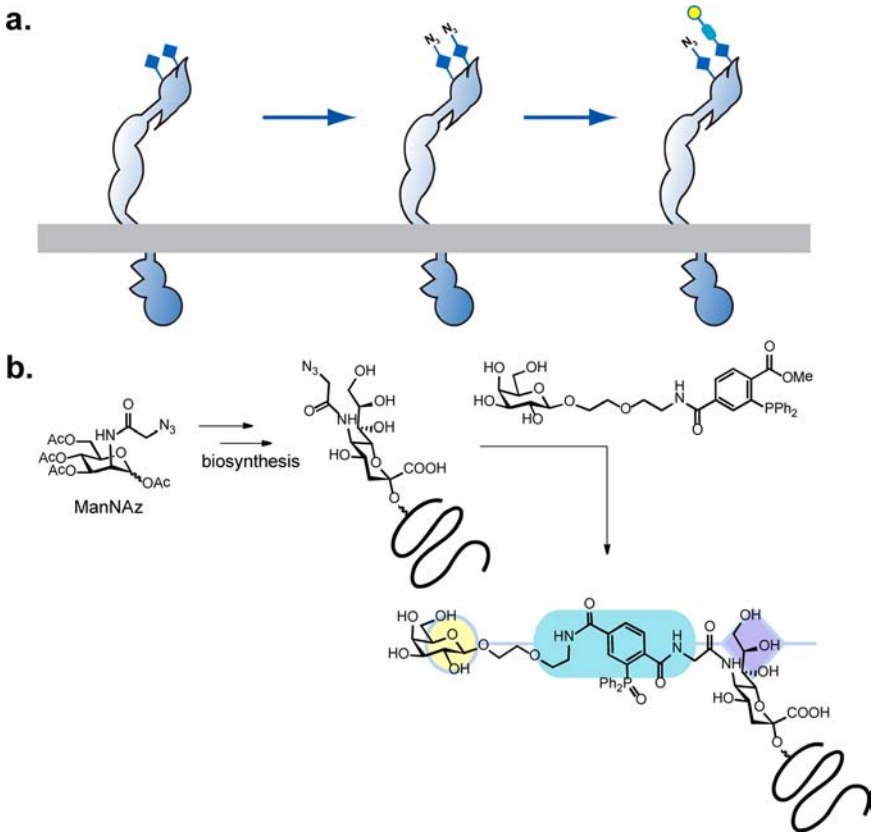


Figure 1. Synthetic glycoform remodeling. Glycoform remodeling of CD45 using the Staudinger ligation is shown (a) schematically and (b) with partial chemical detail. Cells were cultured in media containing the metabolic label, ManNAz, resulting in azide incorporation into sialic acid residues (filled diamonds) found at the termini of cellular glycans. Azide sites (N_3) at the cell surface were then modified by treatment with a Staudinger reagent containing a synthetic glycan epitope (open circle).

used to introduce synthetic glycan structures on intact cells.[27] Reutter and co-workers introduced the concept of metabolic incorporation of a chemically modified sialic acid into cellular glycans.[28] Metabolically labeled sialic acids have since been used to alter the number of viral receptor sites on cells,[29] as well as to probe the role of sialic acid in adhesion.[30] Bertozzi and co-workers expanded this strategy by introducing chemically reactive tags into metabolic precursors.[31,32] This technology has been used to introduce a range of groups including affinity tags,[31] carbohydrates,[33] thiols,[34] and synthetic antigens.[35] To date, biological applications of this technology have focused on the addition of binding epitopes, which are typically non-physiological, onto the cell surface to increase staining,[33] altering recognition by antibodies,[35,36] assembly of cellular clusters,[37] alteration of cell adhesion[38] and neurite growth.[39] However, there remains a lack of examples where glycoform remodeling has been used to probe the function of native glycan epitopes within a specific signaling pathway or to manipulate cellular response to external stimuli.

Glycoform remodeling strategies have the potential to reveal the role of specific glycan epitopes in complex signaling processes. However, to accomplish this task an experiment must be designed which can overcome the inherent nonspecific incorporation of metabolic labels into off-target glycoproteins and glycolipids.[38,40] We considered that CD45 is an ideal target for such an experiment based on the following reasons: (1) CD45 is the dominant source of PTPase activity within lymphocytes; (2) the dimerization, and subsequent suppression, of CD45 PTPase activity is easily assayed; (3) regulation of CD45 glycan microheterogeneity is physiologically relevant, and may enable the development of immunotherapies. Our group has previously employed the Staudinger ligation for chemoselective modification of surfaces and proteins *in vitro* with synthetic glycan epitopes.[41,42] We considered that the readily available lectin, jacalin, has been previously shown to target CD45 as its exclusive receptor on lymphocytes.[43,44] Furthermore, jacalin has been shown to alter cytokine production only in lymphocytes which express CD45. Taken together, we hypothesized that lectin stimulation of a lymphocyte cell could be manipulated through glycoform remodeling with a synthetic epitope recognized by jacalin (Figure 1). Using a metabolic precursor of sialic acid containing an azide group, we were able to introduce the desired synthetic epitopes onto Jurkat T cells. We show here that cells labeled with epitopes recognized by jacalin are sensitized to lectin stimulation as detected by IL-2 secretion. We also show that CD45 PTPase activity is altered under these manipulations, confirming that glycoform remodeling alters the phenotypic response of cells to external stimuli.

MATERIALS AND METHODS

Cell Culture and Reagents. Jurkat cells (clone E6.1 and J45.01) were obtained from ATCC (Mannassas, VA) and cultured in RPMI 1640 media supplemented with fetal bovine serum (FBS, 10% v/v), penicillin (10 units mL^{-1}), and streptomycin (10 mg mL^{-1}) in a humidified incubator (5% CO_2) at 37 °C. Clone E6.1 was used for most experiments unless otherwise noted. Staudinger reagents (**1**, **2**, and **3**; Figure

dx.doi.org/10.1021/bc300599w | *Bioconjugate Chem.* 2013, 24, 907−914

2) were prepared as reported.[42] Preparation of the azide labeling reagent, Ac_4-ManNAz (ManNAz), is described in Supporting Information.

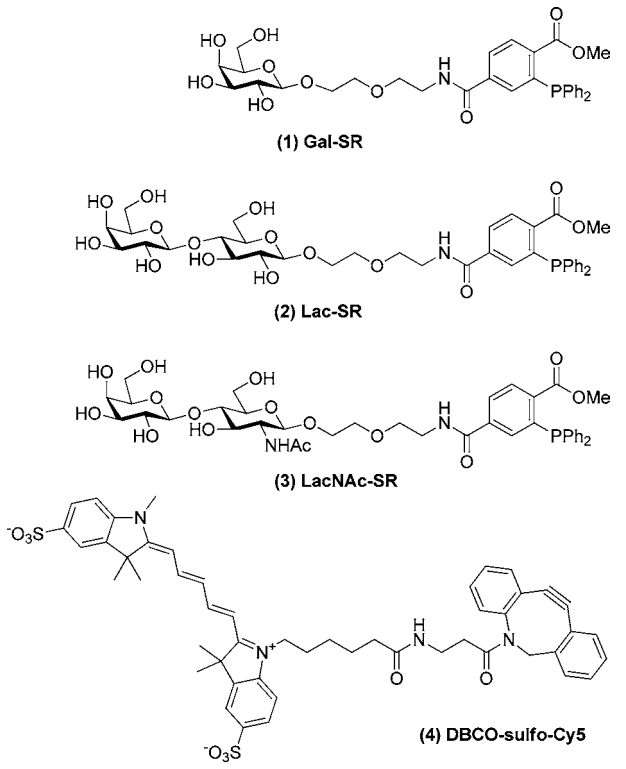


Figure 2. Compounds used in this study. Synthetic glycan epitopes were generated as conjugates containing a phosphane moiety for Staudinger ligation. Staudinger reagent (SR) compounds were synthesized with galactose (**1**), lactose (**2**), and (**3**) LacNAc epitopes.[42] A fluorophore attached to a dibenzylcyclooctyne (DBCO) moiety (**4**) was used for visualization of azide-incorporated glycoproteins.

Azide Labeling of Cells. Jurkat cells (1×10^6 cells mL^{-1}) were incubated at 37 °C with ManNAz (140 mM final concentration) in RPMI 1640 complete media for 24 h and washed with fresh media. Glycan-labeled cells were generated by subsequent treatment with the indicated Staudinger reagent (70 μM, 3 h) or vehicle control (DI water containing 1% DMSO). The cells were harvested, washed with PBS, and diluted to 1×10^7 cells mL^{-1} in PBS or PBS containing jacalin, and incubated for 30 min at 37 °C. At the end of the incubation, cells were cooled on ice, washed with cold PBS (4 °C), and then pelleted by centrifugation at 300*g* for 2 min. Cells were then either resuspended in PBS for further experiments, or lysed.

Flow Cytometry. Azide-labeled cells were generated as described above. Cells were then treated with the indicated Staudinger reagent (70 μM, 3 h) or vehicle control (DMSO). Cells were then harvested, washed with PBS, and diluted to 1×10^7 cells mL^{-1} in PBS containing FITC-labeled jacalin (50 ng mL^{-1}, 0.5 h). At the end of the lectin incubation, cells were cooled on ice, and washed with cold PBS (4 °C). Cell fluorescence was detected using an Accuri C6 Flow Cytometer (λ_{ex} 488 nm; λ_{em} 530 nm).

IL-2 Assays. Azide-labeled Jurkat cells were prepared as described above. Cells were then treated with the indicated Staudinger reagent (70 μM, 3 h) or vehicle control (DMSO) in PBS for 3 h. Cells were washed again in PBS and treated with jacalin (10, 20, or 50 μg mL^{-1}) for 18 h. Cells were spun down by centrifugation, and the supernatant was tested for IL-2 using an ELISA according to the manufacturers protocol (BD Biosciences, Mississauga ON). Briefly, an anti-IL-2 capture antibody was immobilized onto a polystyrene microwell plate. Microwells were washed and blocked, and samples were added and incubated for 2 h. Wells were washed again, and a biotinylated anti-IL-2 antibody was added to each well. After washing, the biotinylated antibody was detected using a streptavidin-HRP conjugate. Raw data were collected as pg mL^{-1} of IL-2, and normalized based on the number of cells in the sample, for each condition. The data shown were normalized to cells treated with the respective SR in the absence of ManNAz.

Fluorescence Imaging and Western Blot of Glycoproteins. Jurkat E6.1 cells were cultured on RPMI 1640 complete media, 2×10^7 cells were seeded into a 75 cm^2 T-flask. ManNAz (140 μM final concentration, from a 1.4 mM stock solution in DMSO) or vehicle control (DMSO) was added after dilution in 20 mL of fresh medium. The cells were incubated at 37 °C in a humidified incubator (5% CO_2) overnight. The medium was removed, and the cells were harvested by centrifugation and washed with cold PBS (3×). The cell pellet was flash frozen, resuspended in 400 μL lysis buffer (25 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, and 1% v/v Triton X100, protease inhibitor cocktail tablet), and incubated on ice for 15 min before centrifugation at 16 000 relative centrifugal force (rcf) and 4 °C. The supernatant was collected, and the protein concentration was determined using a Bradford assay. The lysate (100 μL) was diluted to 3 mg mL^{-1} in lysis buffer and treated with DBCO-sulfo-Cy5, **4** (20 μM dissolved in DMSO). The sample was incubated for 1 h at 37 °C. The reaction was stopped by chloroform/methanol precipitation. The pellet was redissolved in 20 μL of 2× Laemli's sample buffer. The proteins were resolved on a 7% SDS-PAGE gel. The glycoproteins were then transferred to polyvinylidene difluoride (PVDF) membranes for blotting. The membrane was blocked with blocking buffer (Pierce, Rockford, IL) for 1 h at 4 °C, incubated with anti-CD45 antibody (clone HI30; Biolegend, San Diego, CA) in blocking buffer for 2 h. The membrane was then washed with PBST (PBS, Tween 20 0.05% v/v) three times, then incubated with the secondary antibody (goat antimouse; FITC conjugate; BD Pharmingen) 1 h in blocking buffer, washed, and visualized on a Typhoon FLA9500 (GE Healthcare) at λ_{ex} 649 nm, λ_{em} 670 nm (DBCO-sulfo-Cy5) and λ_{ex} 495 nm, λ_{em} 528 nm (fluorescein-labeled secondary antibody).

Synthesis of DBCO-sulfo-Cy5. DBCO-Amine was purchased from Click Chemistry Tools (Scotsdale, AZ). Sulfo-Cy5 NHS ester was purchased from Lumiprobe (Hallandale Beach, FL). A solution of the sulfo-Cy5-NHS ester (5 mg, 0.006 mmol), DBCO-Amine (2.2 mg, 0.008 mmol), and DIPEA (1.7 μL, 0.01 mmol) in DMF (1 mL) was stirred overnight. The solvent was removed under reduced pressure, and the residue purified by silica gel flash chromatography (ethyl acetate:-MeOH; 1:1) to provide 4.2 mg (0.0045 mmol, 69%) of DBCO-SulfoCy5 as a purple oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (t, J = 13.2 Hz, 2H), 7.89−7.88 (m, 4H), 7.63 (d, J = 7.8 Hz, 1H), 7.47−7.41 (m, 4H), 7.34−7.27 (m, 5H), 6.64 (t, J = 12.6 Hz, 1H), 6.30 (dd, J = 13.8 Hz, 7.8 Hz, 2H), 5.12 (d, J = 14.4 Hz, 1H), 4.08 (t, J = 7.2 Hz, 2H), 3.67−3.58 (m, 4H), 3.24−3.23 (m, 1H), 3.12−3.10 (m, 1H), 2.45−2.43 (m, 1H), 2.02−

dx.doi.org/10.1021/bc300599w | *Bioconjugate Chem.* 2013, 24, 907−914

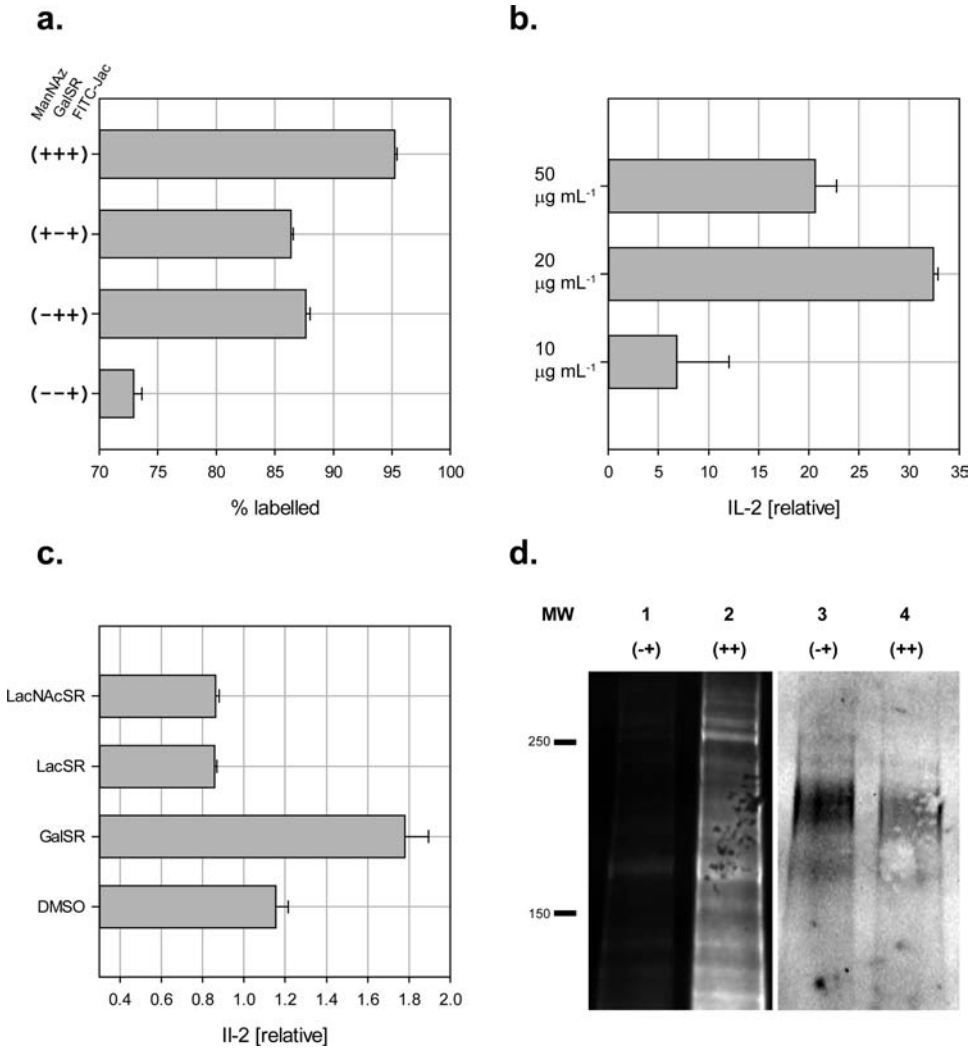


Figure 3. IL-2 response and labeling of Jurkat. (a) Jurkat cells treated with ManNAz and Gal-SR (**1**) showed significantly higher jacalin staining by flow cytometry than cells treated with ManNAz or Gal-SR alone. (b) We confirmed that Jurkat cells increased their secretion of IL-2 in response to jacalin treatment in the range of 10−50 μg mL^{-1}.[43] (c) The IL-2 response of Jurkat cells treated with jacalin (50 μg mL^{-1}) after treatment with ManNAz, followed by DMSO or the indicated SR. (d) Jurkat cell lysate was resolved by SDS-PAGE, and transferred to a PVDF membrane. Fluorescent imaging of Cy5-labeled conjugates is shown in lanes 1−2. Samples were treated with compound **4** alone (-+) or ManNAz with compound **4** (++). The membrane was also probed by an antibody for CD45 (HI30), which was visualized by fluorescence imaging after treatment with a fluorescein-conjugated secondary antibody (intensity inverted; lanes 3−4).

1.78 (m, 4H), 1.74−1.56 (m, 14H), 1.56−1.51 (m, 4H), 1.39−1.31 (m, 3H). HRMS (ESI) Calculated for $C_{50}H_{51}N_4O_8S_2$ [M-H]$^+$, 899.3154, found 899.3160.

Detection of Cellular CD45 Phosphatase Activity. Azide-labeled Jurkat cells were prepared as described above. Cells were then treated with the indicated Staudinger reagent (70 μM, 3 h) or vehicle control (DMSO) in PBS. Cells were washed again in PBS and treated with jacalin at the indicated concentration for 18 h. Cells were then centrifuged, the supernatant was removed by aspiration, and 200 μL of lysis buffer was added (100 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 5 mM DTT, 1% (v/v) Triton X100). The sample was then incubated for 15 min at room temperature with $MgCl_2$ (1 M, 2 μL), and RNase-free DNase I (10 units, 1 μL) added, with subsequent incubation for 15 min at room temperature. The sample was centrifuged at 9000*g* for 20 min at 4 °C. The supernatant was collected and could be stored at −80 °C or used immediately. Lysate samples (2 μL per well) were transferred to the wells of a 96-well black plate containing reaction buffer (50 μL, 50 mM HEPES, pH 7.2, 1 mM DTT, 0.1% (m/v) BSA). The phosphatase reaction was initiated by the addition of 6,8-difluoro-4-methyl-umbelliferyl phosphate (DiFMUP, 50 μL per well, 100 μM final concentration). Phosphatase activity was detected using a fluorescence plate reader (Molecular Devices, Sunnyvale, CA; λ_{ex} 358 nm, λ_{em} 450 nm). Approximately 90% of the phosphatase activity found in Jurkat cells was attributed to CD45, and a negative control containing a PTPase inhibitor ((1,10-phenanthraline)oxo-vanadate, bpV(phen); EMD Millipore Chemicals, Billerica, MA) was used to determine background phosphatase activity (final concentration 50 μM).

RESULTS

Labeling of Jurkat by Staudinger Ligation Alters Lectin Binding. We first set out to confirm the ability of the Staudinger reagent (SR) compounds to label Jurkat cells *in vitro*. Using flow cytometry, we observed the number of cells labeled in the presence of fluorescein isothiocyanate-conjugated

dx.doi.org/10.1021/bc300599w | *Bioconjugate Chem.* 2013, 24, 907−914

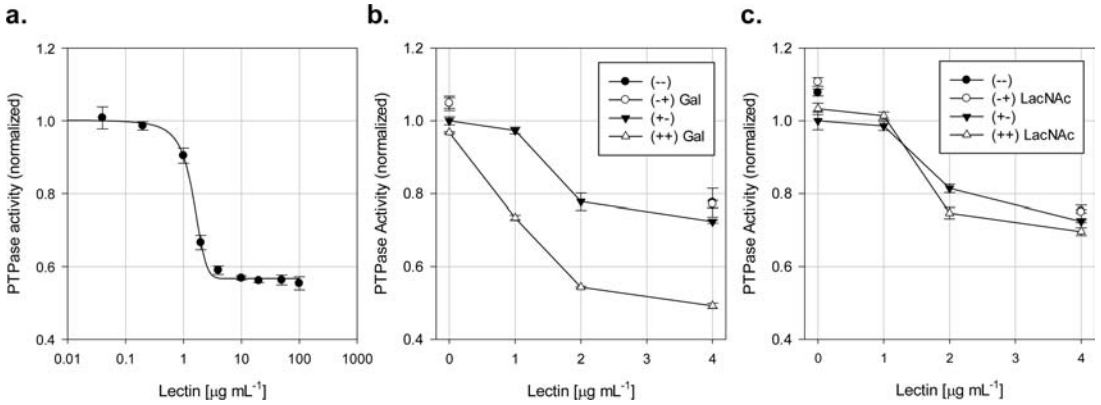


Figure 4. Synthetic manipulation of the PTPase response. CD45 PTPase activity was determined using the assay described by Amano et al.[10] (a) A titration of jacalin shows a dose-dependent inhibition of phosphatase activity, with an EC_{50} of 4 ± 1 μg mL^{-1}. PTPase activity was normalized to control cells treated with buffer and DMSO. (b) PTPase activity within the range of 1−4 μg mL^{-1} jacalin was measured for cells labeled with ManNAz. Cells were pretreated with DMSO alone (●, --) or Gal-SR, **1** (○, -+), at the highest lectin concentration to be used. To test the effect of glycoform remodeling on PTPase activity, cells were treated with ManNAz alone (▼, +-) or ManNAz and Gal-SR (Δ, ++). (c) Cells were treated with the same conditions as in (b), with the substitution of LacNAc-SR, **3**, for Gal-SR. PTPase activity shown in panels b and c were normalized to cells treated with ManNAz alone in the absence of jacalin. Error bars represent the standard deviation of four replicates for each point.

jacalin (FITC-Jac). Cells were first cultured with buffer, or buffer containing ManNAz. Cells were then treated with the Gal-SR (**1**) for 3 h, washed, and analyzed by cytometry (Figure 3a). As expected, cells treated with FITC-Jac alone (--+) showed significant background due to native jacalin receptors. Treatment of the cells with **1** (-++) or with ManNAz (+-+) alone caused only a partial increase in the binding of FITC-Jac. Near complete cell labeling was achieved with the combination of ManNAz and Gal-SR treatment (+++). These data confirmed that Gal-SR, in combination with ManNAz cell labeling, resulted in an increase in jacalin binding sites at the cell surface.

Glycoform Remodeling of Jurkat Alters Cellular Response to IL-2. Having confirmed that the labeling strategy could be used to alter the number of jacalin binding sites, we next examined the influence of specific glycoform epitopes on cellular response. Jurkat cells are known to constitutively secrete IL-2,[45] and the level of IL-2 production has been linked to CD45 signaling.[43] We confirmed that Jurkat cells stimulated with jacalin (10−50 μg mL^{-1}) secreted increased levels of IL-2, as detected by ELISA (Figure 3b). For this analysis we normalized secreted IL-2 to Jurkat cells treated with each SR alone, in the absence of ManNAz. The IL-2 response of Jurkat to jacalin, a multivalent lectin, was expected to be the result of CD45 cross-linking due to the presence of specific glycan epitopes on the receptor.[43] We confirmed that CD45 was required for IL-2 response to jacalin using a cell line lacking CD45 expression, J45.01, which gave <5% of the IL-2 response of E6.1 cells in the presence of 50 μg mL^{-1} jacalin and showed no dose-dependent response. We then sought to test if synthetic glycan epitopes could alter the IL-2 response of the cells by increasing CD45 cross-linking in the presence of the lectin. ManNAz-labeled Jurkat were treated with one of the three SR (Gal-SR, Lac-SR, or LacNAc-SR) or a DMSO control, followed by exposure to jacalin and detection of IL-2 levels (Figure 3c). We observed that DMSO treatment resulted in a minor increase in IL-2 secretion. Interestingly, the introduction of synthetic glycan epitopes had different effects depending on the identity of the glycan. In previous work we have demonstrated that jacalin binds specifically to the Gal-SR reagent, while both the Lac-SR and LacNAc-SR do not interact with the lectin.[41] Our IL-2 experiment revealed that treatment of the cell with Gal-SR resulted in an almost 2-fold increase in IL-2 secretion, while both Lac-SR and LacNAc-SR caused a measurable reduction in IL-2 levels. These results may indicate that noninteracting jacalin epitopes masked authentic epitopes and prevented receptor cross-linking. On the other hand, introduction of a synthetic epitope which acts as a jacalin receptor (Gal) significantly increased the response of the cells, effectively altering the cellular phenotype. This response was most likely due to increased interaction of jacalin with CD45, a known receptor on Jurkat.[43]

CD45 Incorporates the Azide Label. To confirm that CD45 is labeled in the ManNAz-SR treatment protocol, we used fluorescent imaging and Western blotting of the cell lysate. We first conjugated a dibenzylcyclooctyne (DBCO) group to a Cy5 dye (**4**), to be used for visualization of azide incorporation.[46] Cells were then treated with ManNAz and **4**, or compound **4** alone, followed by lysis and resolution by SDS-PAGE. After transfer to a membrane, and probing with an anti-CD45 antibody (HI30), fluorescent imaging allowed visualization of azide-incorporated glycoproteins and CD45 in two separate channels (Figure 3d). Imaging confirmed the ManNAz-dependent, broad incorporation of azide groups into a number of glycoproteins and the specificity of the DBCO-sulfo-Cy5 conjugate (lanes 1−2, Figure 3d). Blotting revealed that cells treated with DBCO-sulfo-Cy5 alone (-+) or ManNAz in combination with compound **4** (++) show a broad reactive band for CD45 at approximately 200 kDa (lanes 3−4); consistent with previous reports for CD45.[43] Analysis of the fluorescence images confirms that glycoproteins in the region of 200 kDa were labeled by ManNAz (see Figure SI1); thus, we concluded that CD45 is among the proteins which incorporate azide after ManNAz treatment in Jurkat. These data also suggest that the CD45 epitope recognized by the HI30 antibody includes sialic acid, which may be partly disrupted by the conjugation of the DBCO-sulfo-Cy5 conjugate or ManNAz treatment.

PTPase Activity Is Sensitive to Glycoform Remodeling. Although IL-2 secretion in Jurkat has been previously shown to be downstream of CD45 activation, we wanted to directly interrogate the PTPase activity of CD45. Increased

CD45 PTPase activity is known to negatively regulate cytokine signaling, thus attenuation of CD45 signaling should result in increased levels of IL-2 secretion.[47] To detect changes in the phosphatase activity of Jurkat cells, we employed an assay based on the work of Amano et al.[10] This strategy takes advantage of the fact that CD45 constitutes >90% of PTPase activity in lymphocytes[4,5] and Ser/Thr phosphatase activity is minimal.[10] Control experiments in Jurkat with a PTPase-selective inhibitor (bpV(phen)), confirmed that the major phosphatase activity in these cells was due to PTPase activity (data not shown). Furthermore, we were able to measure the dose-dependent suppression of PTPase activity in Jurkat in the presence of jacalin. We determined the EC_{50} of jacalin in these cells to be 4 ± 1 μg mL^{-1} (Figure 4a). To determine the effects of altering the cellular glycoform, we measured PTPase activity after jacalin treatment in cells that were treated with ManNAz alone (+-), SR alone (-+), or with both ManNAz and SR (++). In experiments with both the Gal-SR (Figure 4b) and LacNAc-SR (Figure 4c), we observed that the SR alone (-+) was identical to controls (--), even at high lectin concentration (4 μg mL^{-1}). Therefore, the compounds alone did not interfere with jacalin suppression of CD45. ManNAz treatment of the cells (+-) resulted in a minor, but consistent, drop in PTPase activity. Similar to our findings with IL-2, this result is consistent with partial masking of the jacalin receptor by azide-incorporation. Cells treated with SR after ManNAz treatment showed distinct effects due to the identity of the synthetic glycan epitope: cells treated with Gal-SR were sensitized to jacalin treatment (**4b**, ++), while cells treated with LacNAc-SR (**4c**, ++) were indistinguishable from control (**4c**, +-). As with the IL-2 results, we concluded that the change in PTPase activity was due to the specific recognition of the Gal-SR epitope by jacalin on the CD45 glycan. Thus, synthetic modification of the CD45 glycan attenuated the response of cells to an external stimulus. In this case, glycoform remodeling altered the number of receptor sites on CD45 for a multivalent lectin, thus altering CD45 oligomerization state and signaling.

DISCUSSION

In this study we examined the use of a chemoselective labeling strategy for manipulation of a cellular phenotype. We have shown that the response of a T cell line to stimulation by jacalin can be significantly attenuated by the selective introduction of a synthetic epitope (Gal-SR, **1**). We have provided evidence that CD45 is one of the glycoproteins modified through chemical ligation on these cells. While this labeling is not specific to CD45, measurement of IL-2 secretion confirms that the identity of the synthetic epitope is the determining factor in the modulation of cellular response to jacalin. Furthermore, our determination of CD45 PTPase activity confirms that the same treatment which results in IL-2 secretion alters CD45 enzyme activity. Therefore, we concluded that changes in jacalin-stimulated IL-2 secretion were modulated through changes in CD45 signaling, consistent with other studies.[43] Thus, due to the dominance of CD45 PTPase activity in T cells, we were able to discern the effects of a nonspecific glycan labeling strategy on a single biochemical pathway. These experiments confirm that chemoselective ligation can be used to manipulate a single cell surface receptor or signaling pathway.

Although previous work has used chemoselective labeling strategies to introduce binding epitopes into the cellular glycan, we believe this to be the first example where a specific signaling pathway has been manipulated with this method. In general, previous applications of chemoselective labeling in whole cells or in animals (also referred to as bioorthogonal labeling)[48] have focused on the introduction of nonphysiological tags or labels. Numerous examples of metabolic labeling strategies have demonstrated the introduction of unnatural chemical, glycan, fluorophore, or affinity tags.[21,49−54] In the few examples where native glycans have been introduced, these epitopes were used as lectin or immunoaffinity labels, rather than as a strategy to manipulate cellular response.[25,27,33] This is most likely due to the fact that applications of metabolic incorporation must struggle to generate a specific response from the nonspecific incorporation of the tag into proteins and other biomolecules. While strategies using genetic encoding of the label could present an alternative solution to this problem, herein we were able to identify a pathway which could be specifically manipulated by metabolic labeling through its dominant role in cell signaling. In other words, the judicious choice of a signaling target and assay system was able to overcome the inherent nonspecificity of the metabolic labeling.

The introduction of biological epitopes into cells can be used to interrogate their functional roles *in vivo*. While non-physiological epitopes on probe molecules can be used as tags to probe the role and location of biomolecules, nonspecific metabolic labeling strategies have limited utility for this type of application. Based on our findings, we propose that metabolic labeling is uniquely suited to investigate the role of native epitopes within the cellular environment. In the specific case of glycosylation, the most common tools employed are chemical inhibitors or genetic manipulation of transferase enzymes. Each of these strategies suffers from issues of specificity—whereby multiple unintended targets will be modified. Although metabolic labeling lacks specificity in targeting specific glycoproteins, it does not disrupt existing glycan structures. This is in contrast to genetic or chemical disruption of transferase enzymes. Additionally, through the use of a synthetic label, elaborate glycans can be introduced which may not be achievable through modification of a single transferase or pathway. Finally, it is conceivable that metabolic labeling could be used *ex vivo* to manipulate cells. Although we chose to use relatively simple epitopes of known function in this system, the strategy could be easily extended to examine the role of more complex epitopes whose function in CD45 regulation are not as clear. One could imagine the introduction of specific N-link, O-link, or glycosaminoglycan oligosaccharides as a way to probe the role of these complex structures *in vivo*.

The synthetic manipulation of a cellular phenotype could be a potent strategy for medical applications. In the work presented here, we have altered the phenotype of a cell line (cytokine production) through introduction of a synthetic epitope (Gal). T cell cytokine production is intricately linked to the functional role of lymphocytes. For example, the balance of $T_H1/T_H2/T_H17$ cells has received a great deal of attention in relation to autoimmune diseases.[55−57] Several groups have pursued *ex vivo* strategies to alter the functional composition of lymphocytes.[58−60] Results from Baum and co-workers have clearly demonstrated that CD45 glycosylation is the key determinant of $T_H1/T_H2/T_H17$ effector cells through interactions with galectins.[11] Combined with our findings, these results suggest that *ex vivo* metabolic labeling of T cells could allow manipulation of effector cell populations with an appropriate synthetic glycan. It is important to note that this type of manipulation would not alter the signaling machinery of

the cell but, instead, would alter its response to a native stimulus.

■ ASSOCIATED CONTENT

Supporting Information

Analysis of the fluorescence imaging in Figure 3d and additional PTPase replicates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge Prof. Linda G. Baum (UCLA) for helpful discussions. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and the Alberta Glycomics Centre. Infrastructure support was provided by the Canadian Foundation for Innovation and the Alberta Glycomics Centre.

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