Recombinant Glycoproteins That Inhibit Complement Activation and Also Bind the Selectin Adhesion Molecules*

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Soluble human complement receptor type 1 (sCR1, TP10) has been expressed in Chinese hamster ovary (CHO) DUKX-B11 cells and shown to inhibit the classical and alternative complement pathways in vitro and in vivo. A truncated version of sCR1 lacking the long homologous repeat-A domain (LHR-A) containing the C4b binding site has similarly been expressed and designated sCR1(desLHR-A). sCR1(desLHR-A) was shown to be a selective inhibitor of the alternative complement pathway in vitro and to function in vivo. In this study, sCR1 and sCR1(desLHR-A) were expressed in CHO LEC11 cells with an active α(1,3)-fucosyltransferase, which makes possible the biosynthesis of the sialyl-Lewisα (sLeα) tetrasaccharide (NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc) during post-translational glycosylation. The resulting glycoproteins, designated sCR1sLeα and sCR1(desLHR-A)sLeα, respectively, retained the complement regulatory activities of their DUKX B11 counterparts, which lack α(1→3)-fucose. Carbohydrate analysis of purified sCR1sLeα and sCR1(desLHR-A)sLeα indicated an average incorporation of 10 and 8 mol of sLeα/mol of glycoprotein, respectively. sLeα is a carbohydrate ligand for the selectin adhesion molecules. sCR1sLeα was shown to specifically bind CHO cells expressing cell surface E-selectin. sCR1(desLHR-A)sLeα inhibited the binding of the monocytic cell line U937 to human aortic endothelial cells, which had been activated with tumor necrosis factor-α to up-regulate the expression of E-selectin. sCR1sLeα inhibited the binding of U937 cells to surface-adsorbed P-selectin-IgG. sCR1sLeα and sCR1(desLHR-A)sLeα have thus demonstrated both complement regulatory activity and the capacity to bind selectin and to inhibit selectin-mediated cell adhesion in vitro.

Soluble human complement receptor type 1 (sCR1,1 TP10)

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1 The abbreviations used are: sCR1, soluble complement receptor type 1; BSA, bovine serum albumin; CHO, Chinese hamster ovary; CHO/E-selectin, CHO cells expressing recombinant cell surface E-selectin; ES-MS, electrospray ionization mass spectrometry; FACSE, fluoroembossed-assisted carbohydrate electrophoresis; FITC, fluorescein isothiocyanate; LHR, long homologous repeat; mAb, monoclonal antibody; PBS, Dulbecco's phosphate-buffered saline without Ca2+ and Mg2+; sCR1(desLHR-A), sCR1 deletion mutant lacking LHR-A; sCR1(desLHR-A)sLeα, sCR1(desLHR-A) with sLeα has been shown to inhibit the effects of complement activation in a variety of disease models, including ischemia-reperfusion injury (1–3), burns and acute lung injury (4), hyperacute rejection of transplant organs (5), autoimmune diseases (6, 7), and many others. sCR1(desLHR-A), a truncated version of sCR1 lacking the C4b binding site, was shown to be a selective inhibitor of the alternative complement pathway (8) and was protective in several models of myocardial injury (9–11).

The selective adhesion molecules, in particular P- and E-selectin, have been shown to mediate cellular interactions in the required rolling phase, which precedes the adherence and extravasation of leukocytes from the vasculature at sites of inflammation. The selectins, and in particular P-selectin, also mediate the adherence of platelets to lymphocytes (12) as well as the adherence of platelets to neutrophils.

The significance of selectin-mediated processes to various immune and inflammatory responses in vivo has been demonstrated using selectin-deficient animals, anti-selectin antibodies, soluble bivalent selectin-IgG chimeric proteins, and the selectin ligand sLeα tetrasaccharide as well as its analogs. Many of the same animal disease models, which have been shown to be complement-dependent using sCR1 as described above, have also been shown to be selectin-dependent using the sLeα tetrasaccharide. For example, myocardial ischemia-reperfusion injury (13), cobra venom factor-induced rat lung injury (14), and IgG immune complex-induced rat lung injury (15) have all been ameliorated by the use of the sLeα tetrasaccharide.

Sialated, fucosylated oligosaccharides, including the sLeα tetrasaccharide, are carbohydrate ligands for the P- and E-selectin adhesion molecules (16) and as such represent a potential means to combine anti-selectin activity with the anti-complement activity of a glycoprotein such as sCR1. Furthermore, sCR1 and sCR1(desLHR-A) expressing the sLeα tetrasaccharide should have the potential not only to inhibit complement activation and selectin-mediated cellular interactions, but also to localize these activities at sites of inflammation where activated endothelium has up-regulated the expression of P- and E-selectin.

EXPERIMENTAL PROCEDURES

Complement Proteins, Antibodies, and Cell Lines—Purified sCR1 and sCR1(desLHR-A) were prepared as described previously (Refs. 2 and 8, respectively). Concentrations of sCR1, sCR1sLeα, sCR1(desLHR-A), and sCR1(desLHR-A)sLeα were determined by immunoassay in a glycosylation; sCR1sLeα, sCR1 with sLeα glycosylation; sLeα, sialyl Lewis x; TP10, Avant designation for sCR1 used in clinical studies; HPLC, high performance liquid chromatography.
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microtiter plate format using two mouse monoclonal antibodies (mAb) specific for CR1. Microtiter plates were coated with antibody (mAb 6B1.H12 (17)), and detection employed a horseradish peroxidase-conjugated antibody (mAb 4D6.1 (17)). Alternatively, concentrations of the purified proteins were determined by absorbance at 280 nm using a molar extinction coefficient of 2.2 \times 10^4 liter mol^-1 cm^-1 for sCR1, sCR1-sLe\textalpha, and of 2.0 \times 10^4 liter mol^-1 cm^-1 for sCR1[desLHR-A]-sLe\textalpha, obtained from quantitative amino acid analysis (Cornell Biotechnology Program, Ithaca, NY). The human histiocyte lymphoma cell line U937 (18) was obtained from ATCC. The cloned cell line PRO LEC11.E7, referred throughout this paper simply as the LEC11 cells, was obtained from Dr. Pamela Stanley of the Albert Einstein College of Medicine.

CHO Cell Lines Expressing Soluble P-selectin-IgG Chimera and Cell Surface E-selectin—The plasmid CDMT B (20), which codes for a chimeric protein of amino acids 1–292 of human P-selectin fused with the H-CH2-CH3 heavy chain region of human IgG1, was a gift from Dr. Brian Seed of the Massachusetts General Hospital. The chimeric gene was cloned into the eukaryotic expression vector pTCsLneo, which is cotransfected with pTCSLdhfr* and the plasmid coding for sCR1. Microtiter plates were coated with antibody (mAb 4D6.1 (17)). Alternatively, concentrations of the released glycans from a C18 Sep-Pak. The Sep-Pak cartridge ( Waters number 20515) was attached to a 5-ml syringe (Hamilton number 1005) and rinsed with 7–8 ml of acetonitrile followed by 7–8 ml of 90% H\textsubscript{2}O, 10% AcCN by use of gentle air pressure. The digest was loaded onto the cartridge, and the glycans were eluted with 4 ml of 90% H\textsubscript{2}O, 10% AcCN. The volume was reduced on a Speed Vac Concentrator (Savant), and the sample transferred to a 1.5-ml glass Reacti-Vial, dried, and vacuum-desiccated prior to permethylation.

For permethylation, desiccated samples were dissolved in 100 \mu l of NaOH/Me\textsubscript{2}SO suspension, prepared by vortexing Me\textsubscript{2}SO and powdered sodium hydroxide. After 1 h at room temperature, 30 \mu l of methyl iodide was added, and the solution was held for 1 h at room temperature with constant vortexing. The permethylated glycans were partitioned by addition of 100 \mu l of chloroform, and the suspension was back-extracted six times with approximately 0.5 ml of H\textsubscript{2}O and the chloroform layer taken to dryness. Permethylation was repeated. All samples were stored at \(-20^\circ\text{C}.

Periodate oxidation was performed using a 90 ml solution of NaIO\textsubscript{4} buffered with 0.1 m sodium acetate at pH 5.3 in a dark cold room (4 °C) for 3 days. The reaction was quenched with 5 \mu l of ethylene glycol and allowed to stand overnight under the same conditions. The sample was neutralized with 0.1 m NaOH and reduced by the direct addition of 5 mg of solid NaB\textsubscript{1H4} or NaB\textsubscript{2H4} (which remained at room temperature for an additional 16 h). Excess reducing agent was destroyed by the addition of 5 \mu l of acetic acid and the solution dried in a vacuum centrifuge. Borate was removed as its ester by repeated addition and drying with methanol. The sample was vacuum-desiccated overnight prior to methylation.

Electrospray ionization mass spectrometry (ES-MS) was performed on a Finnigan-MAT TSQ-700 (Finnigan-MAT Corp., San Jose, CA) instrument equipped with an electrospray ion source. Methylated glycans were dissolved in water/methanol solutions (40:60 v/v) containing 0.25 m Na\textsubscript{2}O\textsubscript{2} and analyzed by syringe pump flow injected at a rate of 0.5 \mu l/min into the electrospray ionization interface. The sheath gas was nitrogen. The capillary voltage was 4000 \textsuperscript{v}, the capillary temperature 250 °C, and the source temperature 200 °C.

Unlike fast atom bombardment mass spectroscopy, which has difficulty in obtaining spectra of high molecular weight glycans, multiple charg-
FIG. 1. Western blots of sCR1 (lanes 1, 5, 9), sCR1sLeX* (lanes 2, 6, 10), sCR1[desLHR-A] (lanes 3, 7, 11), and sCR1[desLHR-A]sLeX* (lanes 4, 8, 12) with anti-sLeX mAb KM93 or 2F3 and anti-sCR1 mAb 3C6.11 as indicated. To the left of the blot are the apparent molecular masses from markers run and blotted in the left-most unlabeled lanes of each blot.

Results

Western Blot Analysis—The presence of sLeX on the purified glycoproteins derived from LEC11 cell expression is clearly evident by staining with two different anti-sLeX mAbs (Fig. 1, lanes 2, 4, 10, 12). The glycoproteins derived from both the LEC11 and DUKX B11 cells show comparable reactivity using anti-sCR1 mAbs (lanes 5–8).

Monosaccharide Analysis—Table I summarizes the monosaccharide content for typical lots of each glycoprotein. As might be expected, the most striking difference between the LEC11- and DUKX B11-derived proteins was found in the fucose content, which was more than doubled for the LEC11-derived proteins, most likely due to α(1,3)-fucosyltransferase activity, which is a primary distinction between these cell lines. In general, the LEC11 versions of the proteins also had higher sialic acid content. This higher sialic acid content may reflect the fact that, in addition to sCR1 expression, the LEC11 clones have a greater number of complex oligosaccharide chains on the larger protein.

Oligosaccharide Analysis by Mass Spectrometry—Table II summarizes the N-linked oligosaccharide content of typical lots of each glycoprotein. Only complex type N-linked oligosaccharides were observed.
NA1, NA2, NA3, and NA4 refer to no, a single, two, three, and four biantennary, triantennary, and tetraantennary, respectively. NA0, indicated in the abbreviation. BiNA0-(Gal) and BiNA1-(Gal) refer to rides from sCR1[desLHR-A]sLex were treated with a fucosidase that removes α(1–3)-fucose only from branches that lack terminal sialic acid. ES-MS analysis of the biantennary glycans with a single sialic acid (BiNA3, BiNA4, and BiNA5) after such fucosidase treatment yielded quantities for each form that were within 10% of the expected values, based on the quantities prior to treatment and an assumption of random distribution (data not shown). Using the assumption of random sialic acid and α(1–3)-fucose distribution for the sample lots reported here, both sCR1sLex and sCR1[desLHR-A]sLex yielded an sLeα mole percent of 60% (Table II).

Carbohydrate Analysis by Fluorescence-assisted Carbohydrate Electrophoresis—Typical profiles of the intact oligosaccharide chains for sCR1[desLHR-A] (Fig. 2, lane 1) and sCR1 (lane 3) were dominated by three major bands corresponding to biantennary structures, which have zero (BiNA0), one (BiNA1), or two (BiNA2) terminal sialic acid moieties. This was consistent with ES-MS results described above in which biantennary structures were the predominant species (Table II). The sCR1[desLHR-A]sLex (Fig. 2, lane 2) and sCR1sLex (lane 4) FACE® profiles included these same bands at lower relative intensities, but had additional bands from various fucosylated forms of these structures. The lower intensities of the nonfucosylated bands for the LEC11 glycans occur because the predominant biantennary species have been apportioned among various fucosylated versions having zero, one, or two branched α(1–3)-fucoses. Thus the FACE® oligosaccharide profiles clearly demonstrated the striking differences in oligosaccharide structures obtained from glycoproteins expressed by DUXK-B11 cells (lanes 1 and 3) as compared with those derived from LEC11 cells (lanes 2 and 4). These differences can be attributed to α(1,3)-fucosyltransferase activity.

In addition, the individual bands from the oligosaccharide profile were cut out and subjected to further digestion and separation in order to sequence and quantitate the various species. By such methods, the mole percent of each oligosaccharide species, as well as the mole percent of sLeα per oligosaccharide chain, were determined and shown to be in good agreement with data obtained by ES-MS for these glycoproteins (31).

The sialic acid linkage was determined by digestion with the neuraminidases NANase I (α2–3-specific) and NANase III (α2–3,6,8-specific). The banding patterns of both digestions were identical (31), and the changes in mobility were consistent with the loss of NeuNaC, indicating that the NeuNaC linkage is α2–3.

Fucose linkage was also determined using a combination of fucosidase and galactosidase digestions. After neuraminidase treatment, fucosase was removed using FUCase III, indicating the linkage to be either α1–3 or α1–4 to GlcNAc. After removing Fuc, galactosidases were used to determine the position of the remaining Gal and, by inference, the linkage of Fuc (31). Digestion with GALase III (Galβ1–4-specific) changed the band migration pattern, indicating that Gal is linked β1–4, consistent with the presence of Fuco1–3. Digestion with αGLase (Galα1–3-specific) did not change the banding pattern, indicating that Gal is not α1–3-linked, also consistent with the presence of Fuco1–3 (31).

Inhibition of Complement Activation in Vitro—Having established the presence of the sLeα tetrasaccharide on the LEC11 glycoproteins, it was important to examine the effects of such glycosylation on complement inhibitory function. In general, the concentrations required to inhibit human complement-mediated lysis of erythrocytes were similar for glycoproteins expressed by LEC11 cells (sLeα versions, open squares and circles, Fig. 3) compared with those expressed by DUXK-B11 cells (non-sLeα version, closed squares and circles). A representative
titration of each of the four glycoproteins is depicted in Fig. 3 in both an assay of classical pathway mediated lysis (Fig. 3, A and C) as well as in an assay of alternative pathway mediated lysis (Fig. 3, B and D). As expected, both versions of sCR1 (squares) and sCR1[desLHR-A] (circles) were similar in their capacity to inhibit alternative pathway mediated lysis, but the sCR1 versions were much more effective than the sCR1[desLHR-A] versions in the inhibition of classical pathway lysis.

A single such titration can be characterized by the concentration of glycoprotein required for 50% inhibition of maximal lysis (IC\(_{50}\)) so that a lower IC\(_{50}\) value would be indicative of a more effective inhibitor in these assays. From an examination of the means and S.D. values of the IC\(_{50}\) values from numerous such titrations, the mean concentration of sCR1[desLHR-A] required to yield half-maximal lysis (IC\(_{50}\) = 0.27 ± 0.082 nM, n = 31) of sensitized sheep erythrocytes was somewhat higher than required for sCR1 (IC\(_{50}\) = 0.21 ± 0.060 nM, n = 65). The difference in mean IC\(_{50}\) values between sCR1[desLHR-A] and sCR1 is statistically significant (p = 10\(^{-5}\) by analysis of variance), indicating a possible effect of differing glycosylation in these assays. The actual difference, however, is smaller than the differences observed between lots of either glycoprotein or even between multiple assays of a single lot. The most important aspect of these results is that both sCR1[desLHR-A] and sCR1 are potent inhibitors of classical complement activation as indicated by the subnanomolar IC\(_{50}\) values in these in vitro assays.

**FIG. 2. Oligosaccharide profile using FACE®.** From the left, the four lanes profile the carbohydrate from sCR1[desLHR-A] in lane 1, sCR1[desLHR-A][desLe\(^a\)] in lane 2, sCR1 in lane 3, and sCR1[desLe\(^a\)] in lane 4. The predominant structures associated with each band in lanes 1 and 3 are indicated to the left of the image; those associated with bands in lanes 2 and 4 are to the right of the image. The structural abbreviations are defined in the footnote to Table II. Sequencing of the individual bands indicated that some bands include contributions from minor components. For example, the band indicated to be predominantly BiNA\(_{1}F_{2}\) includes the minor component TriNA\(_{2}F_{1}\). Similarly, the BiNA\(_{2}F_{2}\) band includes the minor component TriNA\(_{3}F_{1}\). The band labeled BiNA\(_{1}\), BiNA\(_{2}F_{2}\) includes contributions from roughly equivalent amounts of the components BiNA\(_{1}\) and BiNA\(_{2}F_{2}\).

**FIG. 3. Representative assays of complement regulatory activity.** Inhibition of cell lysis by the classical (A and C) or alternative (B and D) pathway as a function of the concentration of sCR1 (filled squares, A and B), and sCR1[desLe\(^a\)] (open squares, A and B) or of sCR1[desLHR-A] (filled circles, C and D) and sCR1[desLHR-A][desLe\(^a\)] (open circles, C and D).
Similarly, in the assay of alternative pathway lysis of guinea pig erythrocytes, sCR1sLe^x (IC_{50} = 140 ± 32 nM, n = 3) was somewhat less effective than sCR1 (IC_{50} = 19 ± 6.6 nM, n = 10), which again was statistically significant (p = 0.003) but, as expected, both were much less effective than either version of sCR1 which yielded subnanomolar IC_{50} values. In the alternative pathway assay, sCR1[desLHR-A]sLe^x (IC_{50} = 46 ± 9.1 nM, n = 4) was again somewhat less effective than sCR1[desLHR-A] (IC_{50} = 37 ± 6.2 nM, n = 4, p = 0.16) but comparable with either version of sCR1.

A general observation was that in all of these assays, the sLe^x versions were somewhat less effective inhibitors of complement mediated cell lysis than were the non-sLe^x versions, but that these differences in mean IC_{50} values were in all cases modest (less than 60%).

Radioligand binding of sCR1sLe^x to CHO cells expressing cell surface E-selectin—Having established the presence of sLe^x in the N-linked complex oligosaccharide of sCR1sLe^x, it was important to demonstrate its capacity to function as a selectin ligand. The binding of ^125^I-sCR1sLe^x and ^125^I-sCR1 to CHO/E-selectin cells as a function of glycoprotein concentration is shown in Fig. 4. Specific sCR1sLe^x binding was obtained by subtracting sCR1 binding, which was assumed to be nonspecific, from the observed sCR1sLe^x binding. A K_{d(app)} for the binding of sCR1sLe^x was estimated from the concentration yielding half-maximal specific binding and found to be 1.4 μM.

Binding of sCR1sLe^x to CHO Cells Expressing E-selectin by Flow Cytometry—Because the low affinities of sCR1sLe^x were near the limits of typical radioligand binding methods, a second method was used to confirm the binding of sCR1sLe^x to cell surface E-selectin on transfected CHO cells. Nearly identical staining by sCR1sLe^x-FITC and sCR1-FITC indicated no binding of either labeled protein to the control CHO cells (Fig. 5A). Similarly, anti-E-selectin mAb-FITC and an irrelevant control mAb-FITC showed no evidence of binding to the control CHO cells (not shown). As expected, the anti-E-selectin mAb-FITC stained the CHO/E-selectin cells compared with the control mAb-FITC (not shown), confirming the presence of cell surface E-selectin. Significantly greater staining of CHO/E-selectin cells was observed for sCR1sLe^x-FITC as compared with sCR1-FITC (Fig. 5B), demonstrating specific binding of sCR1sLe^x-FITC to cell surface E-selectin. The addition of 1.0 mM EDTA essentially eliminated the selective staining of CHO/E-selectin by sCR1sLe^x-FITC (data not shown), which is consistent with Ca^{2+}-dependent binding as expected for the selectins. In similar experiments, sCR1[desLHR-A]sLe^x-FITC, but not sCR1[desLHR-A]-FITC, was also shown to bind CHO/E-selectin.

In related flow cytometry experiments, the mean fluorescence from CHO/E-selectin cells incubated with 0.1 μM sCR1sLe^x-FITC was reduced by approximately 50% by competition with 0.5 μM sCR1[desLHR-A]sLe^x, with 1.5 μM BSAsLe^x, or with 0.7 μM anti-E-selectin mAb, but was unaffected by 0.4 μM sCR1 (not shown).
**Inhibition of U937 Cell Adhesion to Activated Endothelial Cells**—In addition to binding cell surface E-selectin, the blocking of E-selectin-mediated cell-cell interactions was examined in static cell binding assays. The capacity of sCR1(desLHR-A)sLe x to inhibit the binding of the monocyte-like human lymphoma line U937 to activated human aortic endothelial cells was examined in static binding assays. Activation of endothelial cells leads to the up-regulation of E-selectin after 4 h, which was confirmed by flow cytometry using anti-E-selectin mAb-FITC. As shown in Fig. 6, sCR1(desLHR-A)sLe x, but not sCR1(desLHR-A), inhibited the binding of U937 cells in a concentration-dependent manner (IC50 = 0.7 μM). 

**Inhibition of U937 Cell Adhesion to Immobilized P-selectin-IgG Chimeras**—The capacity of sCR1sLe x to inhibit P-selectin-mediated cell adhesion was examined in static binding assays. As shown in Fig. 7, the increased fluorescence obtained upon lysing the adherent U937 cells, which had been incubated with PBS buffer or with 12 μM sCR1, reflects binding to the immobilized P-selectin-IgG. As expected, this U937 binding was inhibited by removing Ca2+ using 20 mM EDTA or by blocking with the sLe x-specific mAb CSLEX1. Similar inhibition of U937 binding to immobilized P-selectin-IgG was obtained using 12 μM sCR1sLe x (Fig. 7). Inhibition of U937 binding was dependent on sCR1sLe x concentration with an IC50 = 3.1 μM (not shown).

**DISCUSSION**

We have expressed the recombinant human protein sCR1, and its deletion mutant sCR1(desLHR-A), in the LEC11 cell line in order to incorporate sLe x glycosylation in the resulting mature glycoproteins. Cotransfection with a plasmid coding for a mutant mouse dihydrofolate reductase enabled gene amplification to increase protein expression using methotrexate. This approach is a practical method enabling the large scale production of recombinant glycoproteins bearing a desired, specific carbohydrate moiety, namely, the sLe x tetrasaccharide. Alternative approaches, such as chemical (32) or enzymatic (33) glycosylation of purified proteins, may not be as suitable for the preparation of biological drugs. The general applicability of the approach has been exemplified using two different, although related, glycoproteins.

sCR1 appears especially well suited for the incorporation of multiple sLe x moieties because of its elongated, flexible structure bearing 25 potential N-linked glycosylation sites, which should facilitate multivalent binding to cell surface selectins. This elongated flexible structure is characteristic of proteins comprised of short consensus repeat domains such as CR1, CR2, membrane cofactor protein (MCP, CD46), decay accelerating factor (DAF, CD55), C4-binding protein, and even the selectins themselves.

Because only N-linked, complex-type oligosaccharides were observed in the mass spectroscopy analysis, an estimate of the number of oligosaccharide chains per glycoprotein can be made from the mannose content, assuming a trimannosyl core structure for each chain. Such a calculation yields 12.1 and 13.7 oligosaccharide chains on sCR1(desLHR-A) and sCR1(desLHR-A)sLe x, respectively, and 20.3 and 16.2 chains on sCR1 and sCR1sLe x. Combined with the mole percent sLe x (Table II), the average incorporation is 8 and 10 moles of sLe x per sCR1(desLHR-A)sLe x and sCR1sLe x, respectively.

sCR1(desLHR-A)sLe x inhibited the E-selectin-dependent binding of U937 cells to tumor necrosis factor-α-activated human aortic endothelial cells in a concentration-dependent manner with an IC50 of 1.3 μM. These results are similar to the IC50 values of 1 μM reported for the inhibition of HL60 cell binding to immobilized E-selectin by SLe x-BSA and by SLe x-BSA, bovine serum albumin conjugate with 16 and 11 mol of sLe x tetrasaccharide/mol of protein, respectively (32). It should be noted, however, that the sLe x on these serum albumin conjugates was not part of a natural oligosaccharide. The IC50 values obtained for blocking E-selectin mediated cell adhesion reflect dissociation constants for multivalent sLe x binding to E-selectin, which was directly confirmed by radioligand binding studies of sCR1sLe x (Kd(app) = 1.4 μM). sCR1sLe x also inhibited the binding of U937 cells to surface-adsorbed P-selectin-IgG chimeras in a concentration-dependent manner (IC50 = 3.1 μM).

In summary, sCR1sLe x and sCR1(desLHR-A)sLe x have been expressed in LEC11 cells and purified from culture supernatants. Carbohydrate analysis presented here and elsewhere (31) confirmed the presence of the desired sLe x tetrasaccharide. These sLe x-containing glycoproteins retain potent complement inhibitory activity that is comparable with that of the non-sLe x versions expressed by DUKK-B11 cells. The sLe x-containing glycoproteins functioned as selectin ligands in vitro by binding to cells expressing surface E-selectin and by blocking selectin-mediated cell adhesion in a static system. The in vivo targeting and anti-inflammatory activities of these glycoproteins are currently being examined in animal models of lung injury, stroke, and myocardial ischemia-reperfusion injury.

*Note Added in Proof*—A manuscript comparing the four glycoproteins described here in a rat lung injury model by Mulligan, M. S., Warner, R. L., Rittershaus, C. W., Thomas, L. J., Ryan, U. S., Foreman, K. E., Crouch, L. D., Till, G. O., and Ward, P. A. and entitled *Eendo-
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Thea Therapeutic Targeting and Enhanced Anti-Inflammatory Effects of Complement Inhibitors Possessing sLeα Moieties has been accepted for publication in The Journal of Immunology.

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