glycosylation sites per polypeptide, three in the receptor portion and one in the CH2 domain of IgG portion and is O-glycosylated in the hinge region. The proportion of O-glycosylated TNFR-IgG varies from batch to batch. Jacalin is a plant lectin which binds Tn antigen (Galb1,3GalNAc) even if the antigen is sialylated (at O-3 of the Gal residue and/or O-6 of the GalNAc residue). By jacalin-agarose affinity chromatography, TNFR-IgG was separated into column-bound and unbound fractions. Both fractions were separately digested with lysyl endopeptidase (Lys-C) and the digests were examined by reverse phase high-performance liquid chromatography (RP-HPLC) which showed that the O-glycosylated peptide was present almost exclusively in the bound fraction. The activities of the O-glycosylated and non-O-glycosylated forms were indistinguishable using a binding assay (ELIBA or enzyme linked immunobioassay) and a cell based TNF cell killing inhibition assay. The N-linked oligosaccharides from the bound and unbound fractions were, separately, released by peptide-N-glycosidase F (PNGase F). The released oligosaccharides were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for acidic and neutral oligosaccharides (Papac et al, Glycobiology, 8, 445-454, 1998). The mass spectra of the bound and unbound fractions were indistinguishable which confirms that jacalin-agarose does not recognize N-linked oligosaccharides. These results confirms that jacalin-agarose lectin affinity chromatography is a useful way to separate O-glycosylated molecules from non-O-glycosylated molecules and to study the biological significance of O-linked oligosaccharides.

(53) RANTES Suppresses HIV-1 Infection But Does Not Activate Cognate Receptors after Binding to Soluble Glycosaminoglycans

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Chemokines form a family of low molecular weight proteins that play a critical role in inflammatory immune processes. These proteins mediate a wide variety of effects, including intracellular calcium mobilization, chemotaxis and cell activation, as a result of interactions with 7 transmembrane spanning, G protein coupled receptors. A subset of chemokines, beginning with RANTES, MIP-1a, and MIP-1b, suppress infection of the human immunodeficiency virus type 1 (HIV-1) by binding receptors also required by HIV-1 for entry into susceptible host cells. It has become increasingly apparent that chemokine function is intimately related to interactions with glycosaminoglycans (GAG) and these interactions are also important for the antiviral activities of certain b chemokines. As part of efforts to characterize the dependence of various chemokine activities on GAG interactions, the ability of RANTES to stimulate intracellular Ca^{2+} mobilization in peripheral blood mononuclear cells (PBMC) treated with a glycanase cocktail was examined. When cell surface GAG on PBMC were removed RANTES was no longer able to signal. Additionally, incubating the chemokine with heparin in order to form a chemokine-GAG complex did not restore the ability of RANTES to stimulate Ca^{2+} mobilization on glycanase treated cells. The inability of RANTES-GAG complexes to activate cognate receptors prompted further investigation into the antiviral activity directed towards HIV-1 infection revealing enhanced antiviral activity by RANTES-GAG complexes. Despite the inability of RANTES-GAG to stimulate Ca^{2+} mobilization, along with the observation of enhanced antiviral activity, these complexes still bind to receptors as assessed by ^{125}I RANTES binding to glycanase treated PBMC eliminating a possible role of GAG itself in the absence of true receptor binding. Taken together, these results demonstrate that soluble complexes formed between b chemokines and GAG selectively retain antiviral activity yet lack the capacity to activate receptors. Given their novel properties, chemokine-GAG complexes appear to be especially promising candidates for strategies to treat HIV-1 infection.

(54) Mucin-biopolymer interactions for Mucoadhesion

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The interactions of mucin glycoproteins with cationic biopolymers underpin the phenomenon of "mucoadhesion" which is of considerable significance for the oral administration of drugs. The aim of mucoadhesion is to slow down the passage of drugs adminstered through the mouth as they pass through the gastrointestinal tract in order to enhance drug residence times at the sites of optimal absorption (1). Much of the pioneering work has been on mechanical or bulk measurements using for example the Tensiometer although little is known about the molecular nature of such adhesive phenomena (2). At Nottingham we have been applying a molecular hydrodynamics approach using primarily the analytical ultracentrifuge alongside molecular imaging techniques to probe the interactions between gastric mucins and two potentially powerful polysaccharide mucoadhesives: the polycationics DEAE-Dextran and Chitosan. Complexes are generally too large to be quantified by sedimentation equilibrium based methods and sedimentation velocity is shown to be the analytical ultracentrifuge method of choice. The superior behaviour of chitosans is demonstrated, and agrees with measurements from electron microscopy, scanning tunneling microscopy and atomic force microscopy. The conclusions are also consistent with the findings from the tensiometric mechanical studies. Attention is paid to the numbers of charged groups on the adherents and the affects of pH and bile salts on any potential mucoadhesive action, as well as the specific mucin involved. The potential of the highly basic mussel foot glue protein mefp-1 is also considered. (1) Fiebrig I, Davis SS, Harding SE (1995) Methods used to develop mucoadhesive drug delivery systems: Bioadhesion in the gastrointestinal tract. In (Harding SE, Hill SE, Mitchell JR eds) "Biopolymer Mixtures" Chap. 18, Nottingham University Press (2) Lehr CM, Bouwstra JA, Schacht EH, Junginger HE (1992) In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43-48

(55) Progenitor And Mature Oligodendroglial Cells Contain mRNA Transcripts Of ST8SIA II and IV.

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Polysialyltransferase activity is reduced by 50% when rat oligodendroglial cells develop into mature myelin producing cells (1). The activity of the enzyme in extracts of the progenitor oligodendrocytes was approximately 20 pmol/hr/mg protein using fetuin as substrate in the presence of CMP-[^{14}C]NeuAc. Polysialic acid was detected by an immunoaffinity column and Endo N, allowing the distinction of polymers DP-10 or larger and oligomers DP 5-9. A difference in polymer size of the synthesized ^{14}C-polysialic acid was noted between two clonal cell lines of progenitor oligodendroglial cells upon maturation. The fact that the cells were cloned led us to suggest that more than one polysialyltransferase was present in the oligodendrocytes (1). Recently it has been shown (2) that the cDNA from ST8Sia II (STX) and ST8Sia IV (PST-1) can direct the synthesis of polysialic acid containing a different number of sialyl residues when transfected into Neuro2a cells; ST8Sia II synthesized shorter polymers and ST8Sia IV synthesized longer polymers. The total RNA was extracted from the progenitor and mature oligodendrocytes and examined by northern blot analysis with probes for ST8Sia II and IV (2,3). Quantitation of the RNA was by Phosphor Imaging, normalizing to a G3PDH cDNA probe. One transcript, 5.2 kb, comprised 45-65% of the RNA detected but no consistent trends were noted between progenitor and mature oligodendrocytes using either probe. In contrast, after maturation of the oligodendroglial cells, the expression of a second transcript, 3.1 kb, was increased as detected by the ST8Sia IV probe. It is possible that the 3.1 kb transcript detected by the human (3) or mouse (2) ST8Sia IV probes represents a third polysialyltransferase for the biosynthesis of polysialic acid in mature oligodendrocytes.

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(56) In Vivo Autopolysialylation and Localization of the Polysialyltransferases PST and STX

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A select group of mammalian proteins have been shown to possess alpha 2, 8-polysialylated oligosaccharide chains. The best studied of these proteins is the neural cell adhesion molecule (NCAM). The Asn-linked oligosaccharides of NCAM are polysialylated during development of the