myeloma cell lines with a phenotype close to that of terminally differentiated plasma cells. It should be noted that LCL could be divided into 2 groups according to their sialyltransferase activity: 5/7 LCL (group 1) had a high activity whereas 2/7 had a lower activity (group 2). Gb3 synthetase activity was not detectable in pre-B cell lines nor in group 1 LCL, whereas group 2 LCL had a low but significant activity. Among myeloma cell lines, one had the highest Gb3 synthetase activity observed and the other one a moderate activity. Gb4 synthetase was active only in LCL with a higher specific activity in group 2 than in group 1 cell lines. Finally, GM2 synthetase activity was low or even undetectable in all cell lines except myelomas which presented a very high specific activity. These results allowed us to conclude that a coordinate regulation of key glycosyltransferases is responsible for glycolipid core structure assembly and terminal chain modifications observed during B cell differentiation.

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S1.27

Synthesis of Inhibitor for *N*-Acetylglucosaminyltransferase V

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N-Acetylglucosaminyltransferase V, whose increase in activity is concerned with the metastatic potential of tumor cells, is the most interesting among seven GnTases from a viewpoint of inhibitors, because the inhibitors of GnTase V could be used in cancer therapy.

Thus, we designed an inhibitor which contains both the donor (*N*-acetylglucosamine) and the acceptor molecule (mannose) involved in the enzymatic glycosylation process. In our opinion, the five-membered azasugar mimic of *N*-acetylglucosamine is the most suitable transition-state analog of the *N*-acetylglucosamine part.

The ozonolysed compound of 4-phenyl-2-azido-3-butenylacetoamide derived from cinnamic aldehyde in 5 steps (overall 80%) is condensed with dihydroxyacetone phosphate by using FDP-aldoalse (80%). The condensed product is dephosphorylated and hydrogenated to afford the five-membered azasugar of *N*-acetylglucosamine (85%).

Linking the acceptor and UDP analog to this azasugar to yield the proposed compound as selective transition-state analog inhibitor and evaluation of the inhibition are in progress.

S1.28

Release of Oligomannoside Type Glycans as a Marker of the Degradation of Newly Synthesized Glycoproteins

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The *N*-glycosylation of proteins is accompanied by the release of soluble oligosaccharide material. Besides oligosaccharide phosphates originating from the cleavage of the pyrophosphate bond of lipid intermediates, this material is predominantly formed of neutral oligosaccharides which are heterogenous depending on their reducing end with either one or two *N*-acetylglucosamine residues. The present study is focused on cellular aspects of the origin of neutral oligosaccharides in a CHO cell line. Kinetic and pulse chase experiments clearly indicate that oligosaccharides possessing a chitobiosyl unit derived from oligosaccharide-PP-Dol degradation whereas oligosaccharides possessing one single *N*-acetylglucosamine residue are related with neoglycoprotein degradation. This relationship is confirmed by comparing the glycosylation pattern of lipid donors and glycoproteins with those of neutral oligosaccharides in various incubation conditions (inhibition of protein synthesis, presence of processing inhibitors, presence or absence of glucose). Newly synthesized glycoprotein degradation and formation of neutral oligosaccharides with one GlcNAc residue are inhibited at 16°C but not affected by lysosomotropic agents as leupeptin or NH_4Cl. Moreover, newly synthesized glycoproteins and the subsequent release of the glycan are recovered with permeabilized cells suggesting that this phenomenom occurs in the rough endoplasmic reticulum or in a closely related compartment.

S1.29

Purification and Characterization of Bovine Lysosomal Alpha-Mannosidase

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Lysosomal alpha-mannosidase from bovine kidney was purified 50000 × to apparent homogeneity using ammonium-sulfate fractionation, heat-treatment, Concanavalin A, Hydroxylapatite, Mono Q anion exchange and Superose 6 gel filtration chromatographies. The native molecular weight was estimated to 220 kDa by gel filtration. In unreduced state alpha-mannosidase exhibited two bands on SDS/PAGE corresponding to Mr 40 and 60 kDa, whereas in reduced state the 60 kDa band was replaced by two faster migrating bands of Mr 20 and 40 kDa. Neither aminoterminal sequencing nor Western blot analysis indicated any homologies between these three peptides. Thus, bovine acid alpha-mannosidase seems to be a dimer of which each unit consists of three different peptides with one intermolecular disulfide bridge. The enzyme was stable up to 65°C at pH 7, pH optimum was 4.5 and the isoelectric point 5.8.

We are presently studying the clearance and uptake into various tissues after intravenous injection of alpha-mannosidase into rats.

S1.30

Analysis of Carbohydrate Moieties of Microsomal β-Glucuronidase from Rat by Lectin Affinity Chromatography

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Microsomal rat liver β-glucuronidase (β-D-glucuronide-