anti-DP monoclonal antibody (anti-Fy6) and an anti-glycophorin A monoclonal antibody. *N*-gly and Endo-F caused a shift in the molecular weight (m.w.) of DP from 35-43 to 32 kD. *O*-gly had no effect, although *O*-gly did cause a major shift downward in the m.w. of glycophorin A. DP was partially purified from SDS-solubilized membranes by Thiopropyl Sepharose 6B chromatography and preparative SDS-PAGE and then treated with trypsin (3). A 28 kD tryptic peptide of DP was formed which was purified by SDS-PAGE. *N*-gly and Endo-F treatment of the 28 kD peptide of DP caused a shift downward to 18 kD; *O*-gly had no effect. The 18 kD de-glycosylated peptide of DP appeared pure by gel staining. We conclude that DP is *N*-glycosylated; removal of *N*-linked sugars may aid in purification and sequencing.

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S12.13
Problem of Cross-Reactivity of Some Monoclonal Anti-M Antibodies with Nonglycosylated Variant M^g Antigen

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The *N*-terminal structures of blood group M, N, and rare variant M^g-type glycophorin A (GPA) differ in amino acid sequence as follows:

M — **Ser**-Ser*-Thr*-Thr*-**Gly**-Val-Ala-Met-His-
N — **Leu**-Ser*-Thr*-Thr*-**Glu**-Val-Ala-Met-His-
Mg — **Leu**-Ser-Thr-**Asn-Glu**-Val-Ala-Met-His-

The glycosylation of a.a. residues 2-4 in M and N antigens is indicated by asterisks. Replacement Thr4→Asn4 in M^g is connected with the defect in glycosylation of Ser2 and Thr3. It has been known that some anti-M MAbs crossreact with M^g antigen. To elucidate the character of the epitope recognized by these MAbs we used peptides synthesized on plastic pins which were tested in immobilized form for MAbs binding by ELISA (PEPSCAN method introduced by Geysen *et al.*). The initial screening of the antibodies was performed using 3 octapeptides corresponding to the *N*-terminal sequence of M, N and M^g antigens. Among several anti-M and anti-N MAbs tested, two anti-M MAbs only (425/2B and E6), which were known to agglutinate M^g RBC, showed binding to the 3 octapeptides tested. Testing of many other synthetic peptides ('window' and replacement analysis) showed that both M^g-reactive MAbs were specific for the peptidic epitope in which Met8 and Val6 were the most important residues. In nonglycosylated peptides replacements Gly5↔Glu5 or Thr4↔Asn4 did not change the MAbs binding. However, in reaction with fully glycosylated GPA these two MAbs show anti-M specificity dependent on Gly5 and on Gal-linked sialic acid residue(s) in the epitope. The anti-M/M^g MAbs are an interesting example of changing the specificity of antipeptide MAb by glycosylation of amino acid residues 'flanking' the most essential amino acid residues in the peptidic epitope.

S12.14
Differences in Significance of →3Gal-Linked and →6GalNAc-Linked Sialic Acid Residues in Blood Group M- and N-Related Epitopes Recognized by Various Monoclonal Antibodies

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The blood group M and N determinants of glycophorin A (GPA) are defined by amino acid residues at positions 1 and 5, and the a.a. residues 2-4 carry *O*-glycans with α2,3Gal-linked and α2,6GalNAc-linked sialic acid residues. Sialylation is required for activity of most epitopes recognized by various anti-M and anti-N antibodies. In order to check whether these two types of sialic acid residues differ in contribution to antigenic properties, the GPA-M and GPA-N preparations with monosialylated *O*-glycans were obtained and tested for binding of anti-M and anti-N MAbs specific for sialylated epitopes. The GPAs with sialic acid residues linked to Gal (GPA2,3) were obtained by selective resialylation of asialoGPAs with the use α2,3 sialyltransferase and their reactivity with the MAbs was tested by microtiter plate ELISA inhibition assay. The GPAs with sialic acid residues linked to GalNAc (GPA2,6) were obtained by treating GPAs adsorbed on ELISA plate with NDV isolate (expressing neuraminidase specific for α2,3Gal-linked sialic acid) and binding of the MAbs to untreated and NDV-treated antigens was compared. Among 12 MAbs tested (6 anti-M and 6 anti-N), 5 (2 anti-M and 3 anti-N) reacted strongly with GPA2,6 and showed undetectable or weak reaction with GPA2,3,4 MAbs (3 anti-M and 1 anti-N) showed opposite properties, and 3 MAbs reacted less strongly with both types of monosialylated GPA than with untreated antigen. In conclusion, the results obtained indicated that most MAbs recognizing sialic acid-dependent blood group M or N epitopes showed distinct requirement for either Gal-linked or GalNAc-linked sialic acid residues.

S12.15
Epitopic Structure of Tn Antigen

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A monoclonal antibody, designated MLS 128, which was raised against LS 180 cells, a human colorectal cancer cell line, was identified as an antibody directed to the Tn antigen. By using this antibody epitopic structure of Tn glycophorin A was investigated. Glycophorin A was digested with glycopeptidase (*Pasteurella haemolytica*) and the digest was fractionated by a combination of high pressure column chromatographies to produce the glycopeptides. The glycopeptides were treated with sialidase and β-galactosidase. The Tn antigenicity, as assayed by the binding to MLS 128, was found exclusively in the glycopeptides including three or four consecutive residues of GalNAc-Ser/Thr, whereas the glycopeptides containing two nonconsecutive GalNAc-Ser/Thr residues had practically