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MODULATION OF TWO DISTINCT GALACTOSYLTRANSFERASE ACTIVITIES IN POPULATIONS OF MOUSE PERITONEAL MACROPHAGES[1]

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We have examined two galactosyltransferase activities in membrane preparations obtained from resident macrophages, from resident macrophages maintained in culture for 24 hr, and from thioglycollate (TG)-elicited macrophages. Transfer of galactose from uridine diphosphate (UDP)-galactose to *N*-acetylglucosamine is 2.6 times higher in membranes prepared from TG macrophages (107 ± 5.5 nmol/hr/mg) than in membranes prepared from resident macrophages (41 ± 2.0 nmol/hr/mg). Membranes obtained from resident macrophages cultured for 24 hr exhibit a 2.5 times higher activity (102 ± 4.4 nmol/hr/mg) than membranes from resident cells plated for 4 hr. Transferase activity in membranes derived from TG macrophages is not significantly affected by overnight culture. The transferase reaction product, isolated on Bio-Gel P-4 and analyzed by galactosidase treatments, was identified as galactosyl-β1,4-*N*-acetylglucosamine. The enzyme, therefore, is UDP-galactose:2-acetamido-2-deoxy-D-glucose 4β-galactosyltransferase. This is supported by the fact that this galactosyltransferase activity is specifically inhibited by high concentrations of *N*-acetylglucosamine (200 mM). We have also examined the transfer of galactose to *N*-acetyllactosamine. Membranes from TG-elicited macrophages contain a UDP-galactose:galactosyl-β1,4-*N*-acetylglucosamine 3α-galactosyltransferase which synthesizes the trisaccharide, galactosyl-α1,3-galactosyl-β1,4-*N*-acetylglucosamine. This product was identified by gel filtration chromatography, high performance liquid chromatography, and galactosidase digestions. This α-galactosyltransferase activity was not detected in membranes prepared from resident macrophages. These results indicate that glycosyltransferase activities are modulated in populations of mouse macrophages, and that these changes correlate with changes in cell surface lactosaminoglycans reported previously.

The diverse functional capabilities associated with populations of elicited and activated macrophages are mediated, in large part, by their cell surfaces (1–4). This observation is congruent with many reports indicating that constituents of the macrophage plasma membrane undergo numerous modifications in response to inflammation and as a consequence of tumoricidal activation (1–4). Most likely, these changes are initiated by the interaction of external stimuli with the macrophage surface, and represent the culmination of a series of biochemical events that occur intracellularly. Such events must be elucidated in order to understand fully the complex process of macrophage activation and its functional ramifications.

In recent work, we compared protein-bound carbohydrates on the surfaces of resident, elicited, and activated mouse peritoneal macrophages (5). The results obtained indicated that elicited and activated macrophages exhibit an enrichment in surface expression of a specific class of carbohydrates, lactosaminoglycans, compared with resident macrophages. Lactosaminoglycans are oligosaccharide chains that contain the repeating lactosamine dissacharide, Gal-β1,4-GlcNAc.[3] In addition to increased surface expression, structural differences were observed in lactosaminoglycans obtained from elicited and activated macrophages compared with resident macrophages. Specifically, GlcNAc-β1,6-Gal branching and the addition of terminal α-linked galactose residues were observed in the elicited and activated populations but not in the resident population. One postulate that can be derived from these observations is that the activities of distinct glycosyltransferases, which are involved in the biosynthesis of specific oligosaccharide sequences, differ among macrophage populations. As an initial test of this hypothesis, we examined the activities of two distinct galactosyltransferases in populations of resident and elicited macrophages. The results obtained indicate the activity of a uridine diphosphate (UDP)-Gal:GlcNAc 4β-galactosyltransferase is significantly increased in populations of elicited macrophages compared with resident macrophages. Also, populations of elicited macrophages contain a UDP-Gal:*N*-acetyllactosamine 3α-galactosyltransferase activity that is not detectable in resident cells. These observations support an enzymatic basis for the alterations in cell surface carbohydrates reported previously (5).

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[3] Abbreviations: used in this paper: GlcNAc, *N*-acetylglucosamine; Gal, galactose; *N*-acetyllactosamine, galactosyl-β1,4-*N*-acetylglucosamine; HVPE, high voltage paper electrophoresis; UDP-Gal:GlcNAc 4β-galactosyltransferase, UDP-galactose: 2-acetamido-2-deoxy-D-glucose 4β-galactosyltransferase (EC 2.4.1.22; 2.4.1.38); UDP-Gal:*N*-acetyllactosamine 3α-galactosyltransferase, UDP-galactose:galactosyl-β1,4-2-acetamido-2deoxy-D-glucose 3α-galactosyltransferase (EC not designated); TG, thioglycollate broth.

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MATERIALS AND METHODS

Materials. Unless otherwise indicated, reagents were obtained from commercial sources at the highest purity available. [^{3}H]-Gal-α1,3-Gal-β1,4-GlcNAc was kindly supplied by Dr. D. Van den Eijnden, Vrije Universiteit; *Diplococcus pneumoniae* β-galactosidase was a generous gift of Dr. R. L. Hill, Duke University Medical Center Durham, NC; *Escherichia coli* α-galactosidase was from Boehringer Mannheim; and UDP-[^{14}C]galactose (340 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, IL.

Mice. Female C57/BL6/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 7 wk of age.

Macrophages. Peritoneal cells were harvested by lavage of the peritoneal cavity as described previously (5). Resident macrophages were obtained from untreated mice, and thioglycollate (TG)-elicited macrophages were obtained from mice that had been injected i.p. 4 days previously with 1.5 ml of TG broth. The peritoneal exudates were maintained in minimal essential medium containing 10% heat-inactivated fetal bovine serum for 4 hr at 37°C. The adherent monolayers were then washed vigorously with warm phosphate-buffered saline (10 mM phosphate (pH 7.2) and 150 mM sodium chloride) and they were used for the membrane preparations described below. In some experiments, the washed monolayers were maintained in culture in minimal essential medium containing 10% fetal bovine serum for an additional 20 hr.

Membrane preparation. Total macrophage membranes were prepared as follows. Monolayers of adherent macrophages that had been washed with phosphate-buffered saline were removed from the culture dishes by mechanical scraping in ice-cold 10 mM Tris-HCl (pH 8.0) containing 2 mM phenylmethylsulfonyl fluoride. The macrophage suspension was sonicated for 30 sec at 4°C with a Branson probe sonicator (model 185; Branson Sonic Power Co., Danbury, CT) set at the lowest setting. The suspension was then centrifuged at 100,000 × G in an Angle 40 rotor (Beckmann Instruments) for 60 min at 4°C. The resultant pellet was resuspended in the Tris-buffer and an aliquot of this suspension was removed for protein determination by the method of Lowry et al. (6). The remaining suspension was stored at −20°C until it was used in the transferase assays described below.

Galactosyltransferase assay. Galactosyltransferase assays were performed under conditions where product formation was linear with time and protein concentration. Membrane protein (10 to 25 μg) was incubated at 37°C in a total volume of 50 μl containing 0.1 M 2-(*N*-morpholino)ethane sulfonic acid (pH 6.3), 0.5% Triton X-100, 25 mM $MnCl_2$, 1 mM UDP-galactose (1×10^3 cpm/nmol) and 10 mM acceptor (unless otherwise stated). The acceptor for the UDP-Gal:GlcNAc 4β-galactosyltransferase assays was *N*-acetylglucosamine, and for the UDP-Gal:*N*-acetyllactosamine 3α-galactosyltransferase assay it was *N*-acetyllactosamine (Gal-β1,4-GlcNAc). Reactions were terminated, generally after 3 hr by the addition of 10 μl of 7.5% sodium tetraborate/0.2 M EDTA. Samples were subjected to high voltage paper electrophoresis (HVPE) for 1 hr at 1700 V in 1 M sodium tetraborate, pH 9.0, as described previously (7). Quantitation of ^{14}C-labeled products was determined by liquid scintillation counting. In this electrophoresis system, di- and trisaccharide products remain near the origin whereas galactose and UDP-galactose migrate approximately 7 and 11 cm, respectively. Galactosyltransferase activities are expressed as nanomoles of galactose incorporated/per hour per milligram of protein. All data reported are mean values (± SD)of at least three separate determinations.

Isolation of galactosyltransferase reaction products. Galactosyltransferase reactions were carried out as described above, except the volume and components of each tube were increased two- to fourfold. The amount of membrane protein added to each tube was 25 to 100 μg and the reactions were allowed to proceed for 4 to 8 hr. The reaction mixtures were then diluted with 500 μl of distilled water and applied to a 1-ml column of AG1X8 (chloride form) to remove reacted UDP-galactose. The column was eluted with water until 5 ml of eluant had been collected. Each sample was freeze-dried and resuspended in 200 μl of 100 mM pyridinium acetate (pH 6.0) and applied to a Bio-Gel P-4 column (1 × 100 cm, −400 mesh). The column was eluted with the pyridinium acetate buffer and 0.5 ml fractions were collected. Bovine serum albumin and [^{3}H]galactose were used to determine the void (V_0) and inclusion (V_i) volumes of the column, respectively.

The relative elution coefficient (K_d) was determined from the elution volume (V_e) as shown.

$$K_d = \frac{V_e - V_0}{V_0 - V_i}$$

Substances containing [^{14}C]galactose were identified by liquid scintillation counting and they were pooled for further analyses.

Glycosidase digestions. Fractions pooled from Bio-Gel columns were lyophilized and resuspended in 50 μl of 100 mM citrate, pH 6.0. Samples were digested for 4 hr at 37°C with the appropriate glycosidase. After digestion, 10 μl of borate-EDTA was added to each tube and the digestion products were resolved by HVPE.

RESULTS

Transfer of galactose to N-acetylglucosamine. Total membranes obtained from resident and TG macrophages were assayed for their ability to transfer Gal from UDP-Gal to GlcNAc. Membranes derived from TG macrophages incorporated 2.6 times more [^{14}C]galactose into product than an equivalent amount of membrane protein obtained from freshly explanted (4 hr) resident cells (Fig. 1). Similar results were obtained when the assay was performed using membranes obtained from equivalent numbers of cells. This indicates that the observed differences in specific activity cannot be attributed to differences in cell number between the two populations. The ability of membranes obtained from freshly isolated resident macrophages and from resident macrophages maintained in culture for 24 hr to transfer Gal to GlcNAc was also compared. As shown in Figure 1, macrophages cultured for 24 hr exhibited a 2.5-fold increase in this transfer relative to freshly isolated macrophages. The activity observed in cultured resident macrophages is comparable to that observed in TG macrophages. However, 24-hr culture of TG macrophages did not significantly affect galactosyltransferase activity toward GlcNAc (Fig. 1).

Previously, we provided evidence for the existence of three distinct UDP-Gal:GlcNAc galactosyltransferases (8, 9), i.e., enzymes that transfer Gal to GlcNAc. The activity of one of these enzymes, component A of the lactose synthetase complex, is specifically inhibited by GlcNAc at high concentrations (8). However, the other two enzymes are not affected by increased concentrations of

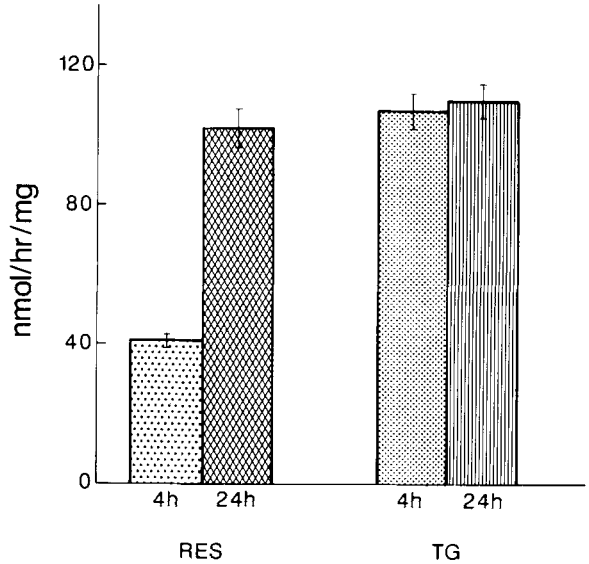

Figure 1. Transfer of galactose to *N*-acetylglucosamine by membranes obtained from resident and TG-elicited macrophages. Resident macrophages (RES) and macrophages elicited in response to TG were plated for 4 hr or 24 hr before preparation of a total membrane fraction as described in *Materials and Methods*. In each case, galactosyltransferase assays were performed with 10 μg of membrane protein and 10 mM *N*-acetylglucosamine for 3 hr at 37°C prior to HVPE. Galactosyltransferase activities were calculated from the amount of radioactivity remaining near the origin after electrophoresis as described in *Materials and Methods*.

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GlcNAc. To determine whether the increased galactosyltransferase activity associated with elicited macrophages was the result of an enzyme activity other than the lactose synthetase type enzyme, membranes derived from TG macrophages were assayed for transferase activity toward GlcNAc at concentrations of either 10 or 200 mM. The transfer of Gal to GlcNAc by TG membranes is 3.6-fold higher at 10 mM GlcNAc than at 200 mM GlcNAc (110 ± 5.1 and 30 ± 3.2 nmol/hr/mg, respectively). This substrate inhibition suggests, therefore, that the transfer to GlcNAc in elicited macrophages is accomplished exclusively by the lactose synthetase type enzyme.

Isolation and characterization of the UDP-Gal:GlcNAc galactosyltransferase reaction product synthesized by TG membranes. In order to isolate and identify the radiolabeled reaction product, membranes derived from TG macrophages were used in large scale transferase incubations. After removal of unreacted UDP-Gal by ion-exchange chromatography, the reaction mixture was resolved by gel filtration chromatography on Bio-Gel P-4. The major radiolabeled species in this mixture eluted from the column with a relative elution coefficient of 0.82 (Fig. 2), and it co-migrated with [^{14}C]-Gal-β1,4-GlcNAc synthesized by rat liver membranes. These data indicate that the reaction product is the disaccharide *N*-acetyllactosamine. The structure of this product was confirmed by glycosidase digestions of the purified disaccharide to determine the nature of the linkage between the constituent saccharides. The inset of Figure 2 shows the amount of radioactive product remaining near the origin after the glycosidase treatments and HVPE. The disaccharide product is not susceptible to hydrolysis by an *E. coli* α-galactosidase which is specific for α-linked galactose residues (10). A similar result was obtained after treatment with green coffee bean α-galactosidase (data not shown). The disaccharide product, however, was totally digested by *D. pneumoniae* β-galactosidase. This glycosidase will digest Gal-β1,4-GlcNAc, but not the 1,3- or 1,6-linked isomers (11). Therefore, the disaccharide product synthesized by membranes derived from TG macrophages is *N*-acetyllactosamine, Gal-β1,4-GlcNAc.

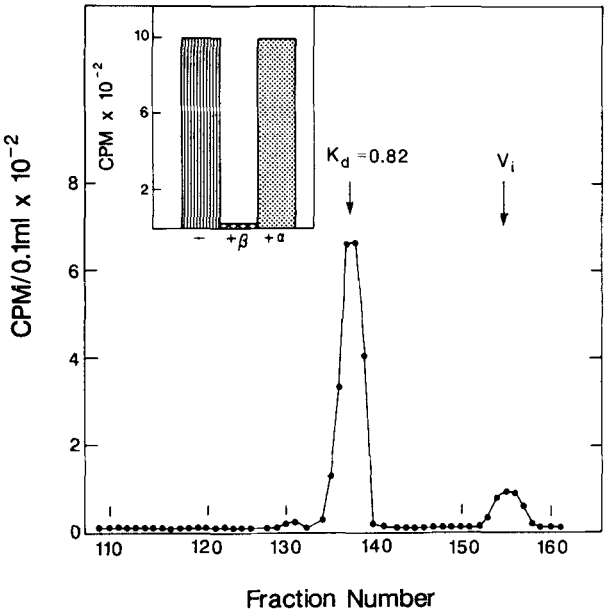

Figure 2. Isolation of disaccharide. Membranes (50 μg) prepared from TG macrophages were incubated for 4 hr at 37°C in a total volume of 100 μl containing 10 mM *N*-acetylglucosamine for synthesis of radiolabeled products as described under *Materials and Methods*. UDP-galactose was removed from reaction mixtures by chromatography on AG1X-8 prior to gel filtration chromatography on Bio-Gel P-4. Authentic [^{14}C]Gal-β1,4-GlcNAc synthesized by rat liver membranes eluted with a K_d of 0.82. Fractions 133–139 were pooled as purified dissacharide. The *inset* shows the effects of α- and β-galactosidase digestions on this purified product. The disaccharide was incubated in buffer alone (−) or in the buffer containing either *D. pneumoniae* β-galactosidase (β) or *E. coli* α-galactosidase(α). The galactosidase digestions were resolved by HVPE and radioactivity remaining near the origin was quantitated by liquid scintillation counting. Shown in the figure is the amount of radioactivity remaining at the origin after electrophoresis (intact disaccharide).

Transfer of galactose to N-acetyllactosamine. Elicited and activated macrophages contain terminal α-galactose residues on cell surface lactosaminoglycans (5). Such residues are not evident, or are markedly reduced, on the surfaces of resident macrophages. To determine whether the appearance of this sugar moiety could be correlated with the activity of an α-galactosyltransferase, membranes from resident and TG-elicited macrophages were prepared and assayed for their ability to transfer galactose to *N*-acetyllactosamine. Membranes prepared from resident macrophages incorporated very little [^{14}C]galactose into product remaining near the origin after HVPE (1.6 ± 0.7 nmol/hr/mg). In contrast, membranes derived from TG macrophages displayed a significant galactosyltransferase activity toward *N*-acetyllactosamine (7.3 ± 1.2 nmol/hr/mg).

Isolation and characterization of the UDP-Gal:N-acetyllactosamine 3α-galactosyltransferase reaction product. Membranes obtained from resident and TG macrophages were used for large scale synthesis of radiolabeled products using *N*-acetyllactosamine as the acceptor. After removal of UDP-galactose, the reaction products were resolved on Bio-Gel P-4 columns (Figs. 3 and 4). In addition to free [^{14}C]galactose eluting at the inclusion volume of the columns, both preparations synthesized a product that eluted with a relative elution coefficient of 0.82. This product is Gal-β1,4-GlcNAc as determined by its K_d and by its complete hydrolysis by *D. pneumoniae* β-galactosidase (data not shown). This di-

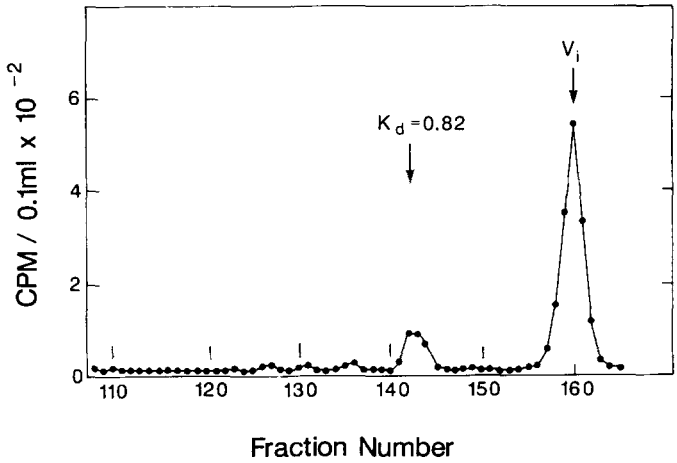

Figure 3. Gel filtration analysis of products synthesized by resident macrophages using *N*-acetyllactosamine as an acceptor. To obtain preparative amounts of radiolabeled products, membranes obtained from resident macrophages were incubated for 8 hr at 37°C in the presence of 10 mM *N*-acetyllactosamine. Incubations (80 μg) were carried out in a total volume of 200 μl. Chromatography on AGX-8 was performed before gel filtration chromatography on Bio-Gel P-4 as described in the legend of Figure 2. The standard saccharide, [^{14}C]Gal-β1,4-GlcNAc, elutes with a K_d of 0.82.

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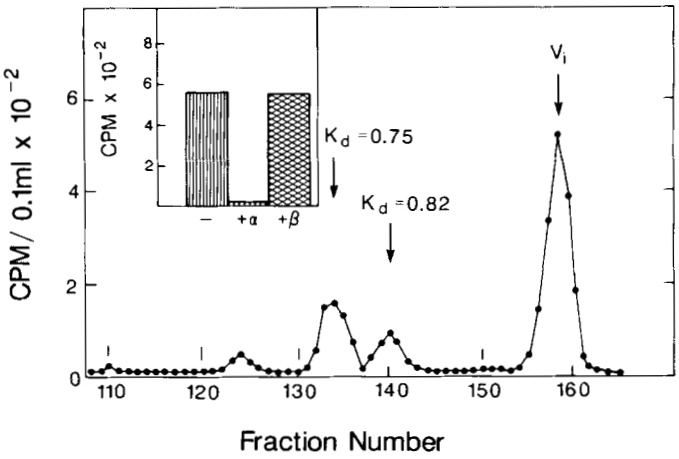

Figure 4. Gel filtration analysis of products synthesized by TG macrophages using *N*-acetyllactosamine as an acceptor. Preparative amounts of radiolabeled products were synthesized using membranes derived from TG macrophages and chromatagraphed on Bio-Gel P-4 as described in the legend of Figure 3. The standard saccharides, [^{14}C]Gal-β1,4-GlcNAc and [^{3}H]Galα1,3-Galβ1,4-GlcNAc eluted with K_d values of 0.82 and 0.75, respectively. Fractions 131–136 were pooled as purified trisaccharide. The inset shows the effects of α- and β-galactosidase digestions on the purified products. The trisaccharide was incubated in buffer alone (–), or in buffer containing either *E. coli* α-galactosidase (α) or *D. pneumonia* β-galactosidase (β). The digestion products were resolved by HVPE and the amount of radioactivity remaining near the origin was determined by liquid scintillation counting (intact trisaccharide).

saccharide product is likely synthesized by transfer of radiolabeled galactose by the 4β-galactosyltransferase to free GlcNAc generated by an endogenous β-galactosidase present in the incubation mixtures.

A larger radiolabeled product was synthesized by membranes from TG macrophages but not resident macrophages. This product eluted from the column with a K_d of 0.75 (Fig. 4). This material co-migrates with [^{3}H]-Gal-α1,3-Gal-β1,4-GlcNAc on Bio-Gel P-4 columns (Fig. 4), as well as on Licrosorb-NH_2 high performance liquid chromatography columns (data not shown). Thus, we conclude that TG membranes, but not resident membranes, synthesize the trisaccharide Gal-α1,3-Gal-β1,4GlcNAc. This trisaccharide was completely digested by cloned *E. coli* α-galactosidase, but it was totally resistant to digestion by β-galactosidase from *D. pneumoniae* (Fig. 4 *inset*). These data support our conclusions that elicited macrophages contain a UDP-Gal:*N*-acetyllactosamine 3α-galactosyltransferase that synthesizes Gal-α1,3-Gal-β1,4-GlcNAc, and that this activity is not present in resident macrophages.

DISCUSSION

Although modifications of cell surface carbohydrates are a salient feature of many biologic processes including differentiation (e.g., Kannagi et al. (12)), development (e.g., Lennarz (13)), and immune cell activation (e.g., Morrison et al. (14)), biochemical data to support an enzymatic basis for such alterations are scarce. Within this context, we have focused our own work on cell surface modifications in populations of resident, elicited, and activated mouse macrophages. In a previous study (5), we demonstrated that populations of TG-elicited and bacillus Calmette-Guérin-activated macrophages differ from resident macrophages in the structure and surface expression of a distinct class of protein-bound oligosaccharides, lactosaminoglycans. This observation, in conjunction with our other studies on macrophage glycolipids (15), has led us to propose that modulations in the structure and surface expression of specific glycoconjugates are an integral component of macrophage activation. In the present study, we provide evidence that the activities of distinct glycosyltransferases, which are involved in the biosynthesis of specific carbohydrate sequences, are modulated in populations of elicited and resident macrophages. Specifically, the activity of UDP-Gal:GlcNAc 4β-galactosyltransferase, which synthesizes Gal-β1,4-GlcNAc, is markedly increased in populations of elicited macrophages compared to resident macrophages. The Gal-β1,4-GlcNAc structure is the repeating disaccharide present in mouse macrophage lactosaminoglycans (5), as well as in other types of protein-bound carbohydrates (16). In addition, a UDP-Gal:N-acetyllactosamine 3α-galactosyltransferase activity, which synthesizes Gal-α1,3-Gal linkages, is detectable in membranes obtained from TG-elicited macrophages but it is not detectable in membranes obtained from resident macrophages. This latter finding correlates with our previous observation that α-linked galactose residues are present on the nonreducing termini of lactosaminoglycan chains on the surfaces of elicited and activated macrophages, but that they are not present on the surfaces of resident macrophages (5). It also supports the lectin-binding data of Maddox et al. (17) which indicated that α-linked galactose residues are present on the surfaces of TG-elicited mouse macrophages but not on resident macrophages.

In this study, we compared the galactosyltransferase activities in membranes obtained from TG-elicited macrophages and resident macrophages maintained in culture for 24 hr to freshly explanted resident macrophages. Both TG-elicited macrophages and resident macrophages maintained in culture for 24 hr differ from unstimulated resident macrophages in a number of biochemical properties (1–4, 18), including alterations in cell surface lactosaminoglycans (5). The data presented in this paper provide additional evidence that these macrophage populations exhibit many similarities and do indeed differ from unstimulated resident macrophages. However, significant differences in the activities of these two galactosyltransferases were not noted between TG-elicited macrophages and TG macrophages activated for tumoricidal activity by recombinant interferon-γ and lipopolysaccharide (data not shown). This observation agrees with our previous structural studies which indicated that TG-elicited and bacillus Calmette-Guérin-activated macrophages exhibit similar surface-labeling profiles of protein-bound carbohydrates (5). In addition, recent studies on macrophage gangliosides in our laboratory suggest that differences between TG-elicited and interferon-γ-activated macrophages reside not in ganglioside composition, but rather they reside in ganglioside surface expression (A. M. Mercurio and G. A. Schwarting, unpublished results). According to current models of macrophage activation (19), inflammatory or responsive macrophages, but not resident macrophages, respond to lymphokines such as interferon-γ. Such "primed" macrophages respond to a second signal such as endotoxin to become tumoricidally activated. The development of responsive macrophages to the activated state is relatively rapid (4 to 6 hr). Thus, it is not surprising that changes in glycoconjugate biosynthesis are evident betwen resi-

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dent and inflammatory macrophages, and are less evident between inflammatory and activated cells. The transition from responsive to activated macrophages may involve modulations in the surface expression of conformation of specific glycoproteins and oligosaccharide sequences, a possibility in agreement with our ganglioside results discussed above.

The most extensively studied glycosyltransferase to date is the A component of the lactose synthetase complex (20). This enzyme which, synthesizes Gal-β1,4-GlcNAc, is found in soluble form in milk, colostrum, and serum and it is membrane-bound in most tissues. In addition to this enzyme, we have previously identified two other enzymes that transfer Gal to GlcNAc (8, 9). These three distinct enzymes are readily distinguished by specific enzymatic properties, as well as by the products they synthesize. The approximate 2.6-fold increase in the incorporation of Gal into GlcNAc by membranes from TG-macrophages and from resident macrophages maintained in culture for 24 hr compared with membranes from freshly explanted resident cells is due to the activity of the lactose synthetase A component activity in the cells and not to the presence of another UDP-Gal:GlcNAc 3β- or 4β-galactosyltransferase. Moreover, the increase in the amount of [^{14}C]galactose incorporated cannot be accounted for by a higher β-galactosidase activity in freshly explanted resident macrophage membranes. In reaction mixtures used to determine the specific activity of the UDP-Gal:GlcNAc 4β-galactosyltransferase in the two membrane preparations (Fig. 1), the amount of free galactose that can be attributed to galactosidase activity is not significant. The activity that is present is lower in resident than in TG-membranes and represents less than 2% of the radioactivity transferred to GlcNAc in both cases (data not shown).

A second type of galactosyltransferase is the enzyme that transfers Gal in α-linkage to *N*-acetyllactosamine. This enzyme has been purified from two sources (21, 22), and appears to be present in many tissues. In long term (8 hr) incubations with *N*-acetyllactosamine, membranes derived from both macrophage populations synthesize [^{14}C]-Gal-β1,4-GlcNAc (Figs. 3 and 4). This suggests that a β-galactosidase present in both membrane preparations converts some of the disaccharide acceptor to free monosaccharides during long incubation periods. The free GlcNAc produced by this galactosidase can be used as a substrate by the 4β-galactosyltransferase present in both membrane preparations thereby producing labeled *N*-acetyllactosamine. It is also important to note that the α-galactosyltransferase activity in TG-membranes is substantially lower (approximately 15-fold) than the activity of the 4β-galactosyltransferase in the same membranes. This might be explained by the fact that α-galactosyl residues only occur as terminal nonreducing sugars, whereas the β-linked galactose moieties are added to several branches in a repeating fashion. At present, the mechanism by which these two galactosyltransferase activities are modulated is not known. The 4β-galactosyltransferase has recently been cloned from three sources (23–25). Such cDNA clones could be employed in studies on the transcription, translation, and stability of the mRNA encoding this transferase in populations of resident, elicited, and activated macrophages. Studies of this sort could serve as a prototype for analyzing alterations in cell surface glycosylation at the molecular level.

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REFERENCES

1. **Cohn, Z. A.** 1980. The activation of mononuclear phagocytes. Fact, fancy and future. *J. Immunol. 121:813.*
2. **Karnovsky, M. L., and J. K. Lazdins.** 1978. Biochemical criteria for activated macrophages. *J. Immunol. 121:809.*
3. **North, R. J.** 1978. The concept of the activated macrophage. *J. Immunol. 121:806.*
4. **Cohn, Z. A.** 1983. The macrophage—versatile element of inflammation. *Harvey Lect. 77:63.*
5. **Mercurio, A. M., and P. W. Robbins.** 1985. Activation of mouse peritoneal macrophages alters the structure and surface expression of protein-bound lactosaminoglycans. *J. Immunol. 135:1305.*
6. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193:265.*
7. **Sheares, B. T., J. T. Y. Lau, and D. M. Carlson.** 1982. Biosynthesis of Galactosyl-β-1,3-*N*-acetylglucosamine. *J. Biol. Chem. 257:599.*
8. **Sheares, B. T., and D. M. Carlson.** 1983. Characterization of UDP-galactose:2-acetamido-2-deoxy-D-glucose 3β-galactosyltransferase from pig trachea. *J. Biol. Chem. 253:9893.*
9. **Sheares, B. T., and D. M. Carlson.** 1984. Two distinct UDP-galactose: 2-acetamido-2-deoxy-D-glucose 4β-galactosyltransferases in porcine trachea. *J. Biol. Chem. 259:8045.*
10. **Schmid, K., and R. Schmitt.** 1976. Raffinose metabolism in *E. coli* K 12. Purification and properties of a new α-galactosidase specified by a transmissible plasmid. *Eur. J. Biochem. 67:95.*
11. **Paulson, J. C., J. P. Prieels, L. R. Glasgow, and R. L. Hill.** 1978. Sialyl and fucosyltransferases in the biosynthesis of asparaginyl-linked oligosaccharides in glycoproteins. *J. Biol. Chem. 253:5617.*
12. **Kannagi, R., S. B. Levery, and S. Hakomori.** 1983. Sequential change of carbohydrate antigens associated with differentiation of murine leukemic cells: i-I antigenic conversion and shifting of glycolipid synthesis. *Proc. Natl. Acad. Sci. USA 80:2844.*
13. **Lennarz, W. J.** 1985. Regulation of glycoprotein synthesis in the developing sea-urchin embryo. *Trends Biochem. Sci. 10:248.*
14. **Morrison, M. H., R. A. Lynch, and W. J. Esselman.** 1986. Poly-*N*-acetyllactosamine alterations of Thy-1 glycoprotein in lymphocyte differentiation. *Mol. Immunol. 23:63.*
15. **Mercurio, A. M., G. A. Schwarting, and P. W. Robbins.** 1984. Glycolipids of the mouse peritoneal macrophage. Alterations in amount and surface exposure of specific glycolipid species occur in response to inflammation and tumoriadal activation. *J. Exp. Med. 160:1114.*
16. **Cummings, R. D., and S. Kornfeld.** 1984. The distribution of repeating [Galβ1-4 GlcNAcβ1-3] sequences in asparagine-linked oligosaccharides of the mouse lymphoma cell lines BW5147 and PHA^{R}2.1. *J. Biol. Chem. 259:6253.*
17. **Maddox, D. E., S. Shibata, and I. J. Goldstein.** 1982. Stimulated macrophages express a new glycoprotein receptor reactive with *Griffonia simplicifolia* I-B$_4$ isolectin. *Proc. Natl. Acad. Sci. USA 79:166.*
18. **Bianco, C., F. M. Griffin, and S. C. Silverstein.** 1975. Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation. *J. Exp. Med. 141:1278.*
19. **Adams, D. O., and T. A. Hamilton.** 1984. The cell biology of macrophage activation. *Annu. Rev. Immunol. 2:283.*
20. **Schachter, H., and S. Roseman.** 1980. *The Biochemistry of Glycoproteins and Proteoglycons.* W. J. Lennarz ed. Plenum Press, New York, pp. 85–160.
21. **Blanken, W. M., and D. H. Van den Eijnden.** 1985. Biosynthesis of terminal Gal-α1,3-Gal-β1,4-GlcNAc-R oligosaccharide sequences on glycoconjugates. *J. Biol. Chem. 260:12927.*
22. **Elices, M. J., D. A. Blake, and I. J. Goldstein.** 1986. Purification and characterization of a UDP-Gal:β-D-Gal(1,4)-D-GlcNAc α(1,3)-galactosyltransferase from Ehrlich ascites tumor cells. *J. Biol. Chem. 261:6064.*
23. **Shaper, N. L., J. H. Shaper, J. L. Meuth, J. L. Fox, H. Chang, I. R. Kirsh, and G. F. Hollis.** 1986. Bovine galactosyltransferase: identification of a clone by direct immunological screening of a cDNA expression library. *Proc. Natl. Acad. Sci. USA 83:1573.*
24. **Narimatsu, H., S. Sinha, K. Brew, H. Okayoma, and P. K. Qasba.** 1986. Cloning and sequencing of a cNDA of bovine *N*-acetylglucosamine (β1,4)galactosyltransferase. *Proc. Natl. Acad. Sci. USA 83:4720.*
25. **Humphreys-Beher, M. G., B. Bunnell, P. van Tuinen, D. H. Ledbetter, and V. J. Kidd.** 1986. Molecular cloning and chromosomal localization of human 4β-Galactosyltransferase. *Proc. Natl. Acad. Sci. USA 83:8918.*

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