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High Mannose Type N-Linked Oligosaccharides on Endothelial Cells May Influence β2 Integrin Mediated Neutrophil Adherence In Vitro

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Abstract We report herein on the role of N-linked oligosaccharide processing of endothelial cell surface proteins on the adhesion of neutrophils. Monolayers of human umbilical vein endothelial cells were treated for 24 h with deoxymannojirimycin (DMJ), an inhibitor of golgi mannosidase I, which results in changes in glycoprotein processing, and then incubated with neutrophils to examine their ability to adhere to the treated endothelial cells. Treatment with DMJ, which leads to accumulation of high mannose type oligosaccharides, resulted in a twofold increase in adherence of phorbol ester (PMA) activated neutrophils compared to attachment to untreated endothelial cells. This adherence was likely mediated by the β2 integrin, Mac-1, and could be specifically inhibited with monoclonal antibodies to ICAM-1 and to the integrin β2 subunit. Similarly, IL-1 treatment resulted in a β2 integrin mediated increase in neutrophil adherence to the DMJ treated endothelial cells in a dose dependent manner. However, the IL-1 induced adherence was not significantly inhibited by the anti-ICAM-1 antibody, thus, suggesting the presence of other inducible components on the endothelial cell surface. Our results demonstrate that alterations in glycosylation of N-linked oligosaccharides, resulting in the synthesis of high mannose type sugars on molecules that may interact with the β2 integrins, leads to an increased adherence of PMA activated neutrophils to endothelial cells.

Key words: β2 integrins, carbohydrate processing, neutrophil attachment, endothelial cell ligands

Polymorphonuclear leukocytes must initially adhere to an endothelial cell surface before they can extravasate from the vasculature and reach sites of infection or inflammation. Vascular endothelial cells normally exhibit a low affinity for circulating leukocytes [Harlan et al., 1985]. However, the release of cytokines at sites of inflammation results in the increased expression of endothelial cell surface adhesion molecules [Bevilacqua et al., 1987]. Cell adhesion events seem to be mediated by receptors or molecules that may be induced, activated, or expressed constitutively on cells [Stoolman and Ebling, 1987; Osborn, 1990]. The leukocyte β_2 integrins which consist of the CD11/CD18 receptors, are high molecular weight glycoproteins that have broad distribution on leukocytes [Sanchez-Madrid et al., 1983] and are important for activation-induced cell adhesion during an immune response [Kishimoto et al., 1989]. Leukocyte integrins are composed of a common 95 kD, β-subunit (β_2, CD18) combined non-covalently with a specific α-subunit (αL; 180 kD, αM; 160 kD, and αX; 150 kD). Recent studies have shown that Mac-1 and LFA-1 are largely responsible for the adhesion of neutrophils to endothelial cell surfaces [Lo et al., 1989a,b; Diamond et al., 1990, 1991; Smith et al., 1988, 1989; Luscinskas et al., 1989].

Although much is known about the structure and synthesis of N-linked oligosaccharides, the importance of such structures in the biological functioning of various cell adhesion molecules is now being realized. Consequent to structural modifications involving cotranslational addition of core oligosaccharides to asparagine residues in the golgi complex, glucosyl residues are trimmed from $Glc_3Man_9GlcNAc_2$ structures by glucosidase I and II. The subsequent removal of

Abbreviations: DMJ: deoxymannojirimycin; DNJ: deoxynojirimycin; ICAM-1,2: intercellular cell adhesion molecule-1,2; LFA-1: lymphocyte function-associated antigen-1.

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mannose residues by mannosidase I and II enzymes is followed by addition of oligosaccharides such as sialic acid and GlcNAc which results in the formation of complex glycoproteins with high mannose, or a hybrid type terminal containing carbohydrate structures with similar core structures [Elbein, 1987; Kornfeld and Kornfeld, 1980]. Specific inhibitors that block the maturation of N-linked oligosaccharides are useful for understanding the effects of post-translational processing of carbohydrates in the golgi apparatus and endoplasmic reticulum. Treatment of glycoproteins with castonospermine and DNJ, inhibitors of glucosidase I and II, or DMJ and swainosinine, inhibitors of mannosidase I and II, results in the synthesis of blocked carbohydrates [Diamond et al., 1991; Bischoff et al., 1986; Fuhrmann et al., 1984; Peyrieras et al., 1983; Renkonen and Ustinov, 1991]. These altered glycoproteins can then be tested for their ability to affect certain properties of the treated cells.

The purpose of the present study was to elucidate the possible role of asparagine-linked oligosaccharides in the structure and function of human umbilical vein endothelial cell adhesion molecules that bind the β2 integrin class of neutrophil receptors. Endothelial cells were treated with PBS or DMJ for 24 h, and the adhesion of PMA activated neutrophils was measured. A significant increase in the binding of activated neutrophils to DMJ treated compared to control (PBS) treated endothelial cells was observed. This binding could be specifically inhibited with Mabs to the β2 subunit of Mac-1/LFA-1 (Mab 60.3 or IB4) and with Mab to ICAM-1, an endothelial cell adhesion molecule that is a counter receptor for Mac-1/LFA-1 [Diamond et al., 1990, 1991]. Our data demonstrates that intracellular trimming of high mannose oligosaccharides on endothelial cell surface proteins may play an important role in promoting or regulating leukocyte-endothelial cell interactions.

MATERIALS AND METHODS

Cell Culture

Neutrophils were purified from whole blood after dextran sedimentation followed by centrifugation on a Percoll gradient (Pharmacia, Uppsala, Sweden) [Hjorth et al., 1981]. Neutrophils were suspended to 5×10^6 cells/ml in M199 medium containing HBSS and 0.1% BSA. Human umbilical cord endothelial cells were isolated and propagated as described earlier [Jaffe et al., 1973] and maintained in Medium 199 with 10% fetal bovine serum, 10% fetal calf serum (both from Whittaker Bioproducts Inc., Walkersville, MD), 30 μg/ml endothelial cell growth supplement (Collaborative Research Inc., Bedford, MA), and 100 μg/ml bovine lung heparin (Sigma Chemical Co., St. Louis, MO). Cells were used between passages 2 and 3 and endothelial cell identity was established by the presence of Factor VIII and a typical cobblestone morphology at confluence. For the adhesion assays, endothelial cells were passaged by scraping gently and then cultured into gelatin coated 48 well plates (Costar). Cells were allowed to grow to semi-confluence prior to addition of inhibitors.

Monoclonal Antibodies

Purified Mab IB4 [Wright et al., 1983] and Mab 60.3 [Arfors et al., 1987] which are directed against the common β2 chain (CD 18) of LFA-1, Mac-1, and p150,95 were used. Mab to ICAM-1 was obtained from Dr. M. Bevilacqua (Brigham and Women's Hospital, Boston). Mab P4C10 directed against the integrin β1 subunit was a kind gift from Dr. Elizabeth Wayner. All antibodies were used as purified IgG. Mabs were added simultaneously with neutrophils on endothelial cell monolayers and were present during the assay.

N-Linked Oligosaccharide Processing

Inhibitors of golgi mannosidase I (DMJ) and N-linked glycosylation (tunicamycin) were used at concentrations determined elsewhere [Spiro et al., 1989; Chatterjee et al., 1990] and were all obtained from Sigma. Endothelial cells were incubated for 24 h with 2 mM of either DMJ or 50 ng/ml of tunicamycin. PBS and DNJ (inhibitor of golgi glucosidase I) were used as controls. Four hours before the end of the incubation, the cells were treated either with 100 U/ml of IL-1 (Genentech) or the equivivalent volume (25 μl) of PBS.

Flow Cytometric Analysis

Upregulation of the surface expression of Mac-1 on isolated neutrophils and whole blood after PMA activation was analyzed by flow cytometry as previously described [von Andrian et al., 1991]. Heparinized blood (1 ml) or isolated neutrophils were incubated with or without PMA (10^{-8} M) for 10 min at 37°C. Fifty microliter

aliquots of the unstimulated and PMA stimulated neutrophils were incubated with 50 μl of ice cold HEPES buffer containing 5 μg/ml of FITC-labeled Mab IB4. Further activation of the neutrophils was terminated by cooling the samples to 0°C. The cells were then incubated on ice for 40 min to permit interaction with the antibody. Fifty microliters of the samples was then mixed with equal volumes of saponin solution (10 μg/ml PBS) for 30 s on ice to lyse the erythrocytes present in the whole blood. The binding of FITC Mab to the neutrophils was analyzed on a FACScan flow cytometer (Becton Dickinson) after gating for neutrophils by characteristic forward and side light scatter. The number of antibody molecules bound per cell was calculated as previously described [Simon et al., 1991] after calibration of both flow cytometer and fluorescent antibody with standard microparticles (Flow Cytometry Standards Corp., Research Triangle Park, NC).

Cell Surface Labeling of Endothelial Cells

Endothelial cells from 3rd passage were treated with DMJ (2 mM) or PBS for 24 h. The cells were then harvested by washing with Dulbecco's PBS and incubated with a PBS based Enzyme Free Cell Dissociation Medium (Speciality Media, Inc., Lavallette, NJ) for 30 min by shaking in a 37°C incubator. The single cell suspension was resuspended in PBS, counted, and equal number of cells surface labeled with ^{125}I as previously described [Gehlsen et al., 1992]. Briefly, cells were labeled for 10 min on ice with 1 mCi/ml Na ^{125}I (New England Nuclear, Burbank, CA) in PBS containing 100 U/ml of lactoperoxidase (Sigma Chemical Co.) and 0.05% hydrogen peroxide. The cells were sequentially washed with PBS to remove the unbound ^{125}I and then extracted in TBS, pH 7.4, containing 50 mM octyl-β-D-glucopyranoside (OG), 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM PMSF for 30 min at 4°C. The cell lysates were then electrophoresed on a 7.5% SDS-PAGE under nonreducing conditions [Laemmli, 1970]. The gel was dried and exposed on Kodak X-OMAT film for 24 h.

Neutrophil Adherence Assay

Prior to the adherence assay [Ley et al., 1989], the cultured endothelial cells were washed two times with PBS and the culture media replaced with assay buffer (M199/HBSS/0.1% BSA). Neutrophils (5×10^5) were added to the wells and incubated at 37°C for 30 minutes in the presence or absence of PMA (1×10^{-8} M final). Nonadherent neutrophils were removed by gently washing the wells 2× with PBS and the bound cells were solubilized by the addition of hexadecyltrimethylammonium bromide (HTAB) detergent. Inhibition of neutrophil adherence was studied by incubating the neutrophil-endothelial mixture with increasing concentrations (0–5.0 μg/ml) of the Mabs 60.3, IB4, or anti-ICAM-1. Neutrophil adherence was quantitated as the mean myeloperoxidase extracted from triplicate samples of adherent cells in experiments repeated at least three times.

Myeloperoxidase Assay

Myeloperoxidase was determined as described earlier [Suzuki et al., 1983]. Briefly, neutrophils were solubilized by addition of 0.5 ml of HTAB in 50 mM potassium phosphate buffer (pH 6.0) to each well. Samples of the extracts were added to 96 well microtiter plates (Costar) containing 80 mM potassium phosphate buffer (pH 5.4), 0.5 mM hydrogen peroxide, 0.16 mM tetramethylbenzidine, and 8% N,N-dimethylforamide. The samples were incubated for 15–20 min at 37°C on an orbital shaker and the reaction was stopped by addition of 0.2 M sodium acetate (pH 3.0). The absorbance of the developed color was measured at 650 nm in a microtiter plate reader (Flow Laboratories, McLean, VA). A known number of neutrophils in suspension was serially diluted, extracted, and analyzed for myeloperoxidase content on the microtiter plates and used for calculation of a standard curve. Adherence is expressed as a percentage of the total number of cells present in the adherence assay.

RESULTS

The role of N-linked oligosaccharide trimming on the cell surface expression of altered glycoproteins involved in neutrophil-endothelial interactions was examined using a neutrophil adherence assay. Unactivated neutrophils were initially examined for their binding to unstimulated endothelial cells that were treated either with PBS (control) or DMJ, an inhibitor of golgi mannosidase I. No significant adherence of unactivated neutrophils to any of the treated or control endothelial cell surfaces was observed (Fig. 1a). However, incubation of unstimulated endothelial cells with neutrophils activated with

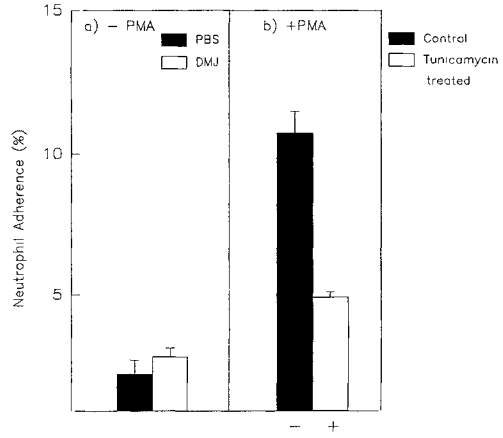

Fig. 1. Neutrophil adherence to unstimulated endothelial cells. **a**: Non-PMA activated neutrophils were incubated with unstimulated endothelial cell monolayers that were either treated with PBS or DMJ for 24 h. **b**: PMA activated neutrophils were incubated with either PBS (–) or tunicamycin (+) treated endothelial cell monolayers. Unbound cells were removed and the adherent cells quantitated using the myeloperoxidase assay.

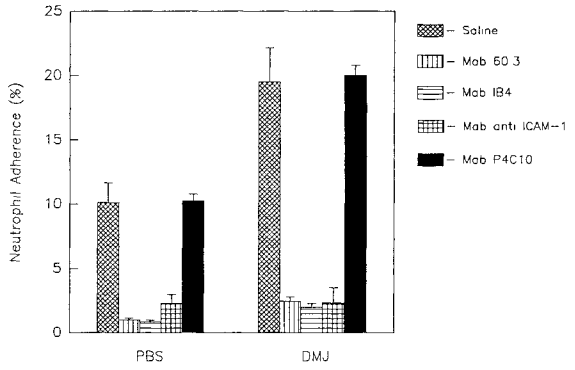

Fig. 2. Effect of inhibitors of oligosaccharide processing on neutrophil adherence to unstimulated endothelial cells. Monolayers of endothelial cells were treated with PBS (control) or DMJ for 24 h. The cells were then washed and PMA activated neutrophils were added to the cultures. The adherent cells were quantitated as described earlier. DMJ treatment of endothelial cells increased neutrophil adherence by 1.9-fold. Incubation of neutrophil-endothelial cells with Mab 60.3 or IB4 at a concentration of 5 μg/ml resulted in a complete inhibition of neutrophil adhesion. Profiles from a single representative experiment out of five are presented. The error bars indicate standard deviation of the mean values.

PMA resulted in significant binding. Furthermore, treatment of endothelial cells with tunicamycin for 24 h resulted in a 60% decrease in the adherence of PMA activated neutrophils to unstimulated endothelial cell monolayers (Fig. 1b).

To characterize a more definitive function of N-linked glycosylation in cell-cell adhesion, endothelial cells were incubated with PBS or DMJ for 24 h and tested for their ability to promote adhesion to activated neutrophils. These experiments resulted in a twofold increase in adherence of activated neutrophils to endothelial cells following DMJ treatment, compared to neutrophil adherence to untreated (control) endothelial cells (Fig. 2). This increase in adherence of PMA activated neutrophils to endothelial monolayers could be completely blocked when cells were incubated with Mab 60.3 or IB4 (anti-β_2 chain of Mac-1 and LFA-1) at a concentration of 5 μg/ml, while a control antibody (Mab P4C10 anti-β_1 integrin) at the same concentration failed to inhibit this binding (Fig. 2). The anti-ICAM-1 Mab was able to effectively inhibit the β2 integrin mediated binding of activated neutrophils in both cases thus supporting a possible role for ICAM-1 in these interactions.

The surface expression of β2 integrins on isolated neutrophils as well as whole blood and its activation by PMA was further confirmed by a flow cytometric analysis (Fig. 3). Stimulation of neutrophils with PMA for 10 min resulted in significant upregulation of surface bound β2 integrins on isolated neutrophils as well as on cells from whole blood (Fig. 3). The binding of activated neutrophils to treated as well as untreated endothelial cells could be inhibited with Mab 60.3 in a concentration dependent manner (0–5.0 μg/ml) (Fig. 4). When the cells were incubated with Mab 60.3 at a concentration of 0.5 μg/ml there was a twofold difference in neutrophil adherence between DMJ and PBS treated endothelial cells (Fig. 4). At this concentration only 6% of the neutrophils were found to adhere to control endothelial cells, while 13% of the neutrophils were adherent to the DMJ treated cells. When incubated with Mab 60.3 at a concentration of 1 μg/ml, there was 55 and 70% inhibition in neutrophil adherence to DMJ and PBS treated cells, respectively.

The effects of these treatments on the cell surface expression of glycosylation modified proteins were next examined using detergent extracts of ^{125}I-surface labeled endothelial cells treated with PBS or DMJ, and subjected to electrophoresis on 7.5% SDS gel under non reducing conditions (Fig. 5). As compared to PBS, DMJ treatment resulted in expression of proteins with increased electrophoretic mobilities in the molecular weight range of 90–160 kd, thus demonstrating the presence of proteins with altered molecular masses as a probable consequence of high mannose N-linked sugars

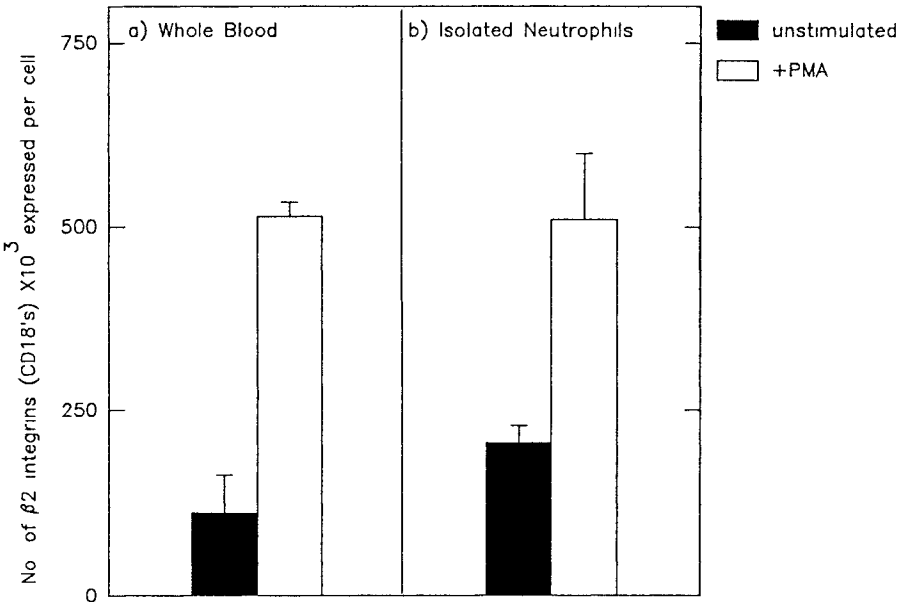

Fig. 3. Expression of β2 integrins (CD18) on the cell surface by flow cytometry FITC Mab-IB4 binding to (a) erythrocyte lysed whole blood and (b) isolated neutrophils was determined on a FACScan flow cytometer (Becton Dickinson) Data represents the number of β2 integrins expressed per cell The error bars indicate the standard deviation of the mean values of six replicate determinations from two independent experiments

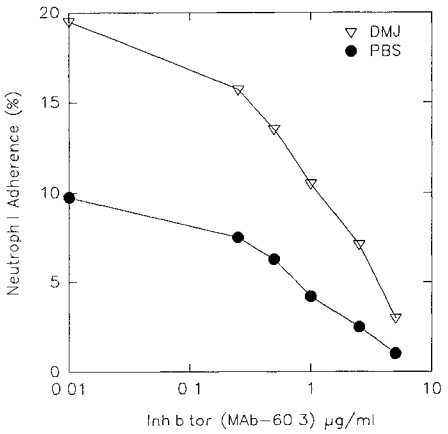

Fig. 4. Mab 60 3 inhibition studies of neutrophil adhesion to unstimulated endothelial cells Endothelial cells were pretreated with PBS (●) or DMJ (▽) for 24 h prior to the neutrophil adherence assay Incubation of neutrophil-endothelial cells with different concentrations of Mab 60 3 resulted in a dose dependent decrease in adhesion Data representative of a single experiment out of three is presented

synthesized as a result of the inhibition of golgi mannosidase I.

The binding of activated neutrophils to IL-1 stimulated endothelial cells treated with DMJ or PBS was then examined. Both control and treated endothelial cells were stimulated with IL-1 in a concentration dependent manner (0–100 U/ml) before performing the neutrophil adherence assay. There was a dose dependent increase in neutrophil adherence to IL-1 stimulated endothelial cells compared to unstimulated endothelial cells (Fig. 6). A significant increase in neutrophil adherence to DMJ treated cells compared to control endothelial cells was observed. This difference was highly significant at lower concentrations (5 U/ml) of the IL-1 stimulation. In contrast, stimulation of the DMJ treated cells with IL-1 at higher concentrations (>10 U/ml) resulted only in a marginal discernable increase in neutrophil adherence compared to PBS treated endothelial cells (Fig. 6) and DNJ treated cells (data not shown). More than 90% inhibition in the adherence of activated neutrophils to IL-1 stimulated endothelial cells was observed when cells were incubated with 5 μg/ml of Mab 60.3 or IB4 (Fig. 7). However, anti-ICAM-1 Mab at the same concentration did not significantly inhibit neutrophil adherence to the treated or control endothelial cells stimulated with IL-1.

Although, incubation of the activated neutrophils with different concentrations of Mab 60.3 led to a dose dependent inhibition in binding, there were significant differences in neutrophil adherence to DMJ or PBS treated endothelial cells that were stimulated with IL-1 (Fig. 8). At a concentration of 1 μg/ml of Mab 60.3, there was a 72% inhibition in neutrophil adherence to

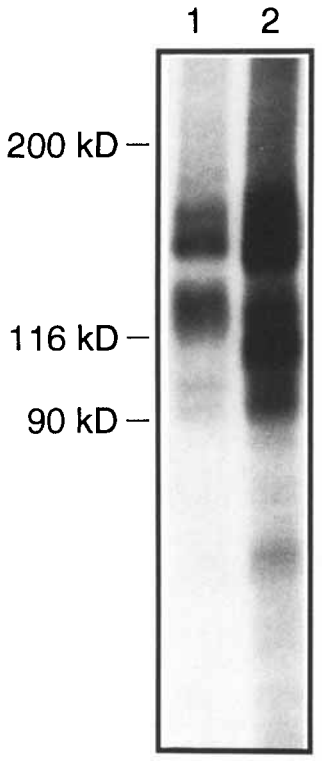

Fig. 5. ^{125}I-surface labeling of endothelial cells. Prior to the iodination endothelial cells were treated with (1) PBS or (2) DMJ for 24 h and harvested as described in Materials and Methods. Equal number of the single cell suspension was subsequently labeled with ^{125}I. The cells were then detergent extracted and aliquots containing equal counts (cpm) were subjected to electrophoreses on a 7.5% SDS-PAGE under non-reducing conditions.

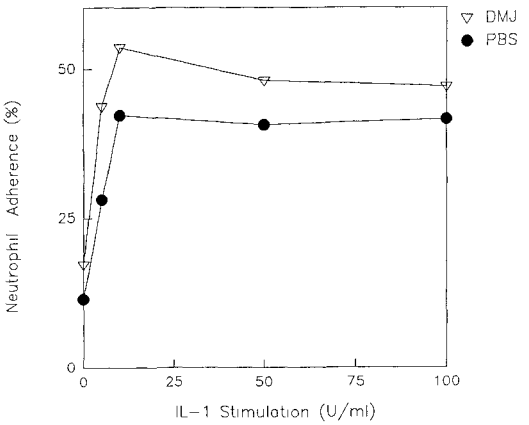

Fig. 6. Titration curves for IL-1 stimulation of endothelial cells. Monolayers of endothelial cells were treated with PBS or DMJ for 24 h prior to stimulation with different concentrations of IL-1 for 4 h. PMA activated neutrophils were then incubated with the endothelial cells and the adherence was quantitated as described earlier.

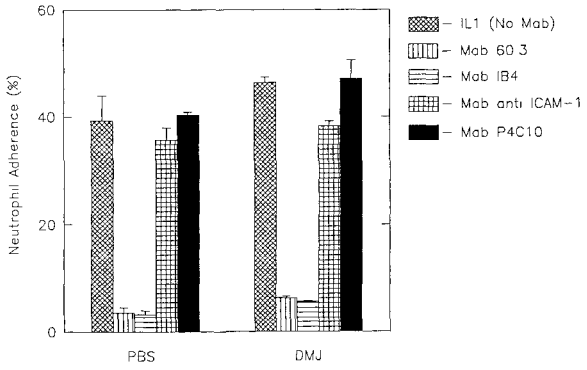

Fig. 7. Neutrophil adherence to endothelial cells stimulated with IL-1. Endothelial cells were treated with PBS or DMJ for 24 h and incubated with IL-1 for 4 h prior to the neutrophil adherence assay. For the inhibition experiments, PMA activated neutrophils and endothelial cells were incubated along with Mabs (5 μg/ml). The cells were then washed and the bound neutrophils were quantitated as described earlier. Data shown is representative of above experiment repeated 5 times. Error bars indicate the standard deviation of the mean.

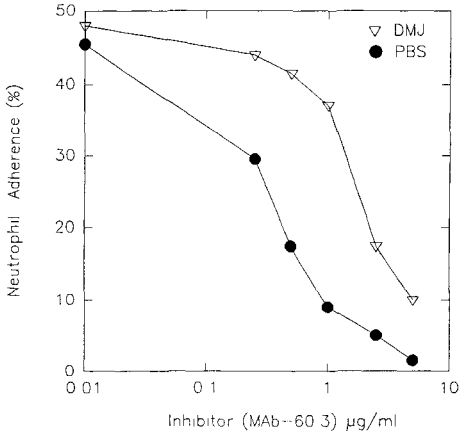

Fig. 8. Dose response curves of neutrophil adherence to treated endothelial cells stimulated with IL-1. Endothelial cells were treated with PBS (●) or DMJ (▽) for 24 h and stimulated for 4 h with 100 units/ml IL-1 prior to the neutrophil adherence assay. PMA activated neutrophils and different concentrations of Mab 60.3 were added to the endothelial cell monolayers. Neutrophil adherence was quantitated as described in Figure 1. The data presented here is a single representative out of five.

PBS treated endothelial cells stimulated with 100 U/ml of IL-1. In comparison, DMJ treatment resulted in only a 32% inhibition in neutrophil adherence, suggesting a significantly increased adherence to the DMJ treated endothelial cells. Furthermore, the binding of PMA activated neutrophils to IL-1 stimulated endothelial cells was not inhibited when the cells were incubated with a β1 integrin Mab (P4C10), thus confirming the specificity of these

interactions to the ligands on endothelial cell surfaces for the β2 integrins.

DISCUSSION

The expression of leukocyte adhesion molecules—GMP-140, ELAM-1, ICAM-1, ICAM-2, and VCAM-1—on endothelial cells appears to be important in regulating leukocyte traffic into inflammatory tissues and facilitating cell-cell interactions [Springer, 1990; Lo et al., 1991]. ICAM-1, a heavily glycosylated adhesion molecule [Tomassini et al., 1989] and ICAM-2 are constitutively expressed on endothelial cells [Springer, 1990], but only ICAM-1 is upregulated by IL-1, TNF, LPS, and less so by interferon gamma (IFNγ) [Dustin and Springer, 1988]. ICAM-1 and 2 have been reported to be counter receptors for LFA-1 [Rothlein et al., 1986; Marlin and Springer, 1987] while Mac-1 is known to bind only ICAM-1 [Diamond et al., 1990, 1991; Smith et al., 1988, 1989]. Preliminary studies have recently shown that the levels of carbohydrate on ICAM-1 transfected COS cells can modify its interaction with soluble Mac-1 [Diamond et al., 1991]. However, the overall effects of carbohydrate processing on the interaction of intact neutrophils and endothelial cells has not been investigated. Furthermore, the importance of N-linked oligosaccharides in endothelial-neutrophil interactions is not well understood. Herein, it is demonstrated that high mannose type N-linked oligosaccharides may promote neutrophil adherence to endothelial cells.

Activation of neutrophils with PMA resulted in their adherence to monolayers of endothelial cells that were treated either with PBS (control) or DMJ. This was associated to the upregulation of β2 integrins on the neutrophil cell surface as demonstrated by the binding of FITC-Mab IB4 using flow cytometry. The correlation in cell adherence associated with the upregulation of cell surface β2 integrins and a subsequent inhibition of this adherence with Mab IB4 or 60.3 is strongly suggestive of a role for the involvement of β2 integrins in endothelial cell-neutrophil interactions. Treatment of endothelial cells with tunicamycin resulted in a 60% decrease in the binding of PMA activated neutrophils to endothelial cells. The lack of complete inhibition in neutrophil adherence might be due to the inhibition in cell surface expression of some of the molecules mediating these interactions or due to the incomplete processing of N-linked oligosaccharides.

Incubation of endothelial cells with DMJ, which is expected to abolish the formation of lactosaminyl branches and leads to the accumulation of high mannose variants ($Man_{5-9}GlcNAc_2$) [Bischoff et al., 1986; Fuhrmann et al., 1984; Peyrieras et al., 1983], resulted in a twofold increase in the adherence of neutrophils as compared to control endothelial cells. Treatment of endothelial cells with DNJ, which prevents the removal of three glucose residues from $Glc_3Man_9GlcNAc_2$, resulted in only a marginal increase in neutrophil adherence as compared to control endothelial cells (data not shown). The presence of high mannose oligosaccharides on endothelial surface proteins was also demonstrated by ^{125}I-surface labeling of the treated endothelial cells and subjecting the detergent extracts to electrophoresis on SDS-PAGE under nonreducing conditions. DMJ treatment resulted in the synthesis of a number of proteins in the molecular weight range of 90–160 kd with faster electrophoretic mobilities. Although not conclusive, it is suggestive of the presence of high mannose $Man_{5-9}GlcNAc_2$ N-linked sugars on the surface of some proteins [Elbein, 1987; Spiro et al., 1989]. Similarly, DMJ treatment resulted in expression of the vitronectin receptor (αVβ3) with increased surface high mannose sugars which was demonstrated by an increase in electrophoretic mobility [Spiro et al., 1989].

Stimulation of DMJ or PBS treated endothelial cells with IL-1 resulted in an dose dependent increase in neutrophil adherence. The adherence of PMA activated neutrophil to DMJ treated endothelial cells was significantly higher compared to PBS treated cells when stimulated with IL-1 at concentrations of 5 U/ml and lower. However, at higher concentrations of IL-1, only a marginal increase in binding of the activated neutrophils to DMJ treated cells compared to PBS treated endothelial cells was observed. Differences in results between the unstimulated and stimulated endothelial cells might be due to the induction of not only ICAM-1 but also ELAM-1, VCAM-1, GMP-140, and/or other unknown ligands which contribute to neutrophil binding to endothelial cells [Bevilacqua et al., 1987; Lo et al., 1991]. This is particularly evident as the anti-ICAM-1 Mab inhibited neutrophil adherence to unstimulated endothelial cells but not significantly to IL-1 stimulated endothelial cells. However, when the cells were incu-

bated with Mab 60.3 at a concentration of 1 μg/ml only 28% of the neutrophils were adherent to the PBS treated endothelial cells. In contrast, nearly 70% of the neutrophils adhered to DMJ treated endothelial cells thus indicating a significantly increased adherence of the activated neutrophils to high mannose type oligosaccharides. Incubation of these cells with Mab 60.3 or IB4 at a concentration of 5 μg/ml resulted in a > 90% inhibition in neutrophil adherence in all three treatments.

In view of the significant inhibition of neutrophil adhesion by Mab 60.3 and IB4, it is clear that Mac-1 and/or LFA-1 are the principle integrin receptors involved in neutrophil-endothelial cell binding (Figs. 2, 7). The two fold difference in neutrophil adherence to DMJ treated endothelial cells in comparison to control cells when inhibited in a concentration dependent manner with Mab 60.3 suggests that the alterations in N-linked glycosylation could be specific for either ICAM-1 and possibly ICAM-2. Recent studies have, however, shown that LFA-1 but not Mac-1 is the receptor for ICAM-2 [Diamond et al., 1990]. Since Mab 60.3 and IB4 were able to inhibit the binding of activated neutrophils to IL-1 stimulated endothelial cells, these results implicate ICAM-1 as a possible ligand for β2 integrins in these interactions. Inhibition of neutrophil adherence to unstimulated endothelial cells by Mab to ICAM-1 is also supportive of this argument. However, our results showing a lack of significant inhibition of activated neutrophils to IL-1 stimulated endothelial cells by Mab to ICAM-1 are also suggestive of the existence of an additional Mac-1/LFA-1 dependent ICAM-1-independent ligand(s) for neutrophil interactions with endothelial cells.

The overall increase in neutrophil adherence observed in the present investigation is consistent with previously published studies [Yagel et al., 1990; Muchmore et al., 1990]. Pretreatment of first trimester trophoblast cells with swainosinine, an inhibitor of alpha mannosidase, resulted in these cells attaching more avidly to amnion basement membrane and to an extracellular matrix preparation when compared to control trophoblasts [Yagel et al., 1990]. An additional study on the effects of inhibitors of N-linked oligosaccharide processing revealed that DMJ and swainosinine could not only raise intracellular levels of $Man_{5-9}GlcNAc_2$ but also enhance the in vitro biological activity of IL-1 and rTNF [Muchmore et al., 1990]. This is consistent with the increased neutrophil adherence to DMJ treated endothelial cells, an effect more clearly discernable when stimulated with lower concentrations of IL-1. This stimulation conceivably augments the effects of DMJ induced IL-1 stimulation as observed by an increased adherence of the non-IL-1 stimulated endothelial cells by DMJ. It is therefore envisioned that the increased neutrophil adherence to DMJ treated endothelial cells after stimulation with IL-1 might be due to the cumulative effects of not only the synthesis of high mannose type structures of ICAM-1 or other ligands, but also to a substantially increased expression of the ligands for β2 integrins caused by enhanced biological activity of IL-1. However, treatment of endothelial cells with DMJ did not result in attachment of non-PMA stimulated neutrophils suggesting that the surface expression of activated β2 integrins is important in binding to even treated endothelial cells. Taken together our results demonstrate a possible role for N-linked glycosylation and high mannose type oligosaccharides in neutrophil-endothelial interactions. Efforts are currently underway to identify and characterize these high mannose type surface ligands for the β2 integrins involved in endothelial cell interactions to activated neutrophils.

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