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CARBOHYDRATES AND OTHER CELL ADHESION MOLECULES. CLINICAL AND DIAGNOSTIC IMPORTANCE

Magne BRYNE (*), Jahn M. NESLAND (*)

SUMMARY : **AND FUTURE DİRECTİONS.** Cell adhesion is of fundamental significance for fetal development, tissue organization, wound healing, inflammation and most pathological conditions. Increased understanding of cell adhesion at molecular level is necessary for development of a new potent treatments of various diseases. Alterations of expression of adhesion molecules will soon be of high diagnostic value for various diseases, including cancer.

Adhesion of cells to each other or to extracellular matrix is very important for various processes like embryonal development, maintenance of tissue architecture, angiogenesis, inflammation and cancer progression. Our knowledge about of cell adhesion has been very sparse, but the recent characterization of some families of cell adhesion molecules has significantly increased our understanding of these processes. Some of these families of adhesion molecules are cadherins, integrins, immunoglobulin superfamily, selectins and a few oligosaccharides. Three possibilities of cell adhesion are reported: 1) adhesion between similar molecules (homophilic binding), adhesion between different types of molecules (heterophilic binding) and cell-cell adhesion by means of a secreted linker molecule (1,2).

Expression of various cell adhesion molecules has proven to be of high diagnostic value for various diseases, including cancer. Promising animal studies have shown that blocking of cell adhesion can be a useful treatment of various inflammatory diseases and cancer.

In our department we work with carbohydrates as adhesion molecules and diagnostic markers and in the present review we will therefore mainly focus on the role of cell surface carbohydrates in biology, but also briefly describe other adhesion families.

CADHERİNS

Cadherins are proteins expressed on the surface most human cells and most of the cadherins are responsible for Ca2+ dependent homophilic adhesion. They play crucial roles during embryonal development and hold neighbouring cells together in epithelial and nervous tissues. Significant alterations in cadherin synthesis are observed during progression of carcinomas and these findings have proven to be of prognostic value for various carcinomas (1,3,4)

SELECTİNS

Selectins (E-, P-, and L-selectin) are lectinlike carbohydrate binding proteins present on endothelial cells and some leucocytes (1,5). Endothelial selectins are activated by some cytokines and bind to carbohydrate containing counter-receptors on leucocytes. A small oligosaccharide structure, sialyl-Lewis X, is an important part of the counter-receptors for all selectins. Siallyl-Lewis X is chemically closely related to the ABH blood group antigens. The selectin-carbohydrate bindings occur in the initial phase ("rolling phase") of the trapping of leucocytes and possibly cancer cells into tissues. Several in-vivo studies show that blocking of selectin binding can inhibit inflammation to a certain extent (6-9).

IMMUNOGLOBULIN SUPERFAMILY

This group of glycoproteins have some biochemical similarities with antibodies and are involved in both homophilic and hèterophilic cell-cell adhesions. One example is NCAM which bind nerve cells together in homophilic manner. Other molecules like ICAM, VCAM and MAdCAM are expressed on endothelial cells and are involved in homing of leucocytes in inflammation. They are counter-receptors for integrins (see below) on leucocytes and are important for successful adhesion and transmigration of leucocyte across the endothelial wall (1,5).

INTEGRINS

This is a group of transmembrane proteins which are found on many cell types like leucocytes and epithelia. They have various adhesive functions. In inflammation, they bind to adhesion molecules of the immunoglobulin family on endothelial cells during the adhesion cascade necessary for recruitment of leucocytes into the tissues. Integrins have also important functions in the binding of cells to extracellular matrix proteins. Alterations in integrin expression are frequently observed in cancer and inflammatory disorders (2,5).

THERAPEUTIC POTENTIAL OF BLOCKING ADHESIVE PROCESSES INVOLVİNG CAHBOHYDRATES

Adhesion of leucocytes or blood platelets

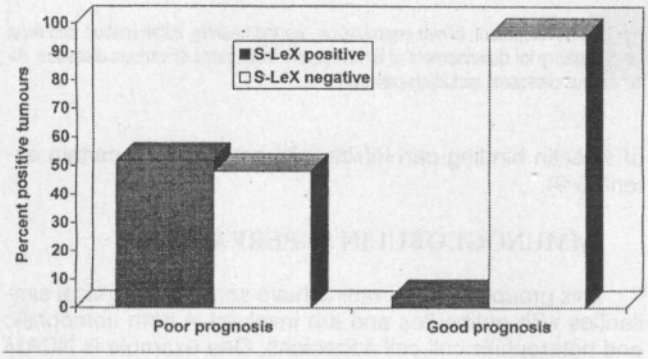
Leucocytes and blood platelets are necessary for various biological processes. However, they can also cause significant pathological damage. We will give some examples of studies showing that blocking of carbohydrate based adhesion can be clinically useful.

a) Reperfusion injury.

Reperfusion following ischemia often causes significant tissue injury and is a serious complication after myocardial infarction, organ transplantations, thermal skin injury and organ hypoperfusion (7). Reperfusion injury is associated with increased adhesion of neutrophilic granulocytes and activation of inflammatory cascades. It is mainly tissue destructive molecules from neutrophils that are responsible for the tissue injury. Several excellent animal studies have now shown that this injury can be significantly reduced by

* *Dept. of Pathol., The Norwegian Radiumhospital, Oslo, Norway*

FİG 1 : EXPRESSİON OF THE ADHESİON RELATED OLİGOSACCHARİDE SİALYL-LEWİS X İS STRONGLY ASSOCİATED WİTH POOR PROGNOSİS OF PATİENTS HAVİNG ADVANCED PROSTATE CANCER. NEARLY ALL PATİENTS WİTH SİALYL-LEWİS X EPRESSİON İN THE PRİMARY TUMOUR SHOWED A VERY POOR PROGNOSİS.



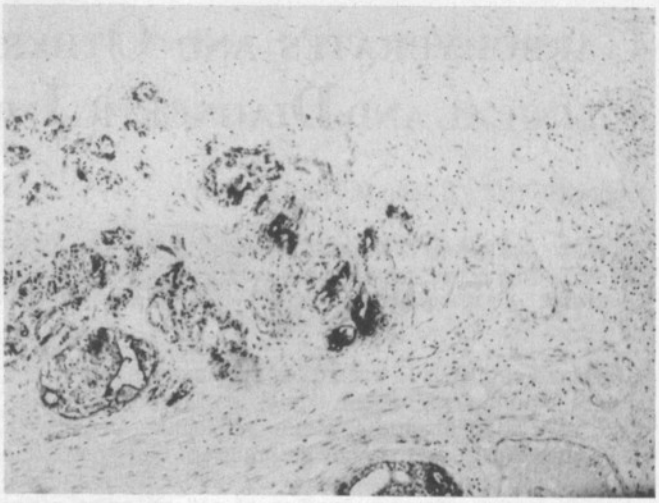


Fig 2 : Heterogenous expression of sialyl-Lewis X (indicating poor prognosis) in the primary tumour of a patient having advanced prostatic carcinoma. Benign epithelium did not express this structure.

blocking the adhesion of neutrophils to the endothelium by means of inhibiting carbohydrate-selectin interactions (7-10). For example, Buerke et al. (9) recently reported a 80% reduction of the myocardial necrosis with injection of the oligosaccharide sialyl-Lewis X after experimental ischemia compared with control animals. Thus, blocking of adhesion molecules may improve the prognosis for heart attack patients.

b) Transplantations.

Upregulation of adhesion molecules probably plays an importance during rejection reactions of transplanted organs (11,12). E-selectin is upregulated in various transplants, but no clinical studies of blocking of carbohydrate interactions during transplantation have so far been published. However, promising in-vivo results have been obtained by blocking other adhesion interactions (11). A major problem today is the lack of suitable organs and one possible strategy for successful xenotransplantation (transplantation across species) will be blocking of adhesive interactions (11).

c) Sepsis and septic shock.

Vascular injury and following hyperpermeability is one major cause to the serious complications from sepsis and septic shock (13). This endothelial injury is partly caused by adhesion of neutrophils and their local production of tissue destructive molecules. Activation of selectins and thus adhesion of neutrophils through their carbohydrate counterrepectors is thought to be important for this damage. Blocking of adhesion molecules is one potential newtreatment of sepsis (14). Interestingly. it has recently been shown that E-selectin probably is of significant importance for the development of ARDS (acute respiratory distress syndrome). This is a very serious complication of sepsis and some other conditions (15).

d) Thrombosis and atherosclerosis.

Various adhesion molecules on platelets, leucocytes and endothelial cells are most likely crucial for the development of thrombi and atherosclerotic plaques (6,16-18). Platelets synthesize P-selectin, which bind to the oligosaccharide sialyl-Lewis X on leucocytes (16). In addition, E-selectin is reportedly upregulated on endothelial cell in relation to atheromatous plaques (19). These and many other findings suggest a potential role for carbohydrate dependant cell adhesion in these important diseases. The hope for therapy based on modification of cell adhesion in thrombosis and atherosclerosis is considerable, but further clinical research is needed before anti-adhesion therapy can improve the prognosis for these patients (18).

e) Rheumatoid arthritis and multiple sclerosis.

Rheumatoid arthritis and multiple sclerosis are both chronic diseases of unknown etiology. Recent research suggests that adhesion molecules are important for the homing of leucocytes to the tissue and for progression of the disease (20-22). Endothelial selectins are also upregulated in these chronic diseases. No clinical study using anti-carbohydrate therapy has been published yet. However, Ochi et al. (23) have recently demonstrated a 63% reduction of clinical symptoms in patients with rheumatoid arthritis by injecting an oligosaccharide (dimeric Lewis X) intradermally as a vaccine. This carbohydrate epitope is found on a rare myeloid cell which is upregulated in patients with rheumatoid arthritis.

INFECTIONS

For many years it has been known that several microorganisms use carbohydrates for adhesion to tissues. Helicobacter Pylori, Mycoplasma pneumonia, HIV, Influensaevirus and E-coli well documented are examples (24-28). A few in-vivo studies show that infection (24) for example, urinary E-coli infection and diarrhoea caused by Clostridium difficile, can be inhibited by addition of various oligosaccharides (24,29). It is considered likely that anti adhesion therapy of some infections will be possible soon (24).

CANCER

Probably all human cancers show significant alterations of glycoconjugates on the cell surface compared to their normal counterparts (30). The biological functions for sug-

ars on the cell surface of cancer cells are mainly unknown, but some are probably involved in adhesive interactions important for tumour progression (30-33). These carbohydrate changes can be used as diagnostic markers for various carcinomas (34). Upregulation or downregulation of some carbohydrate structures have prognostic value for example colo-rectal carcinomas (32), head and neck carcinomas (35) and prostate carcinomas (36) (Fig 1 and 2).

Carbohydrate based adhesion is most likely important during all steps in tumour progression (32,33). Many in-vitro studies have demonstrated that blocking of oligosaccharides can inhibit cancer cell adhesion to endothelium (5,31-33). Furthermore, some studies have shown that injection of carbohydrate blocking antibodies can inhibit tumour progression in mice (24,37). However, a lot of basic and clinical research is still needed before the dream of an anti-cancer "sugar-pill" is a reality

In addition, recent in vitro studies suggest that carbohydrates have an important role during angiogenesis as adhesion molecules between endothelial cells (38). Thus, it is speculated that a novel anti-angiogenetic therapy based on blocking of carbohydrate interactions will be clinically useful as a cancer treatment.

DEVELOPMENT OF CARBOHYDRATE BLOCKING PHARMACEUTİCALS

The potential high clinical importance of carbohydrate adhesion has stimulated many pharmaceutical companies to work hard for creating clinically useful therapeutics (33,39,40). Carbohydrate based adhesion to selectins on endothelium may in theory be blocked by a) antibodies, b) free oligosaccharides, c) free selectinlike peptides, d) "glycomimetics" (molecules mimicking oligosaccharide), and e) blocking biosynthesis of adhesion molecules or cytokines important for activation of adhesion molecules. Several problems are still to be solved; free oligosaccharides are expensive and have a short half life, the revelant monoclonal antibodies are rarely humen and have thus a short half life and can only be administered intravenously, and several side effects are to be expected because some of the relevant molecules are present on some normal cells. In addition, blocking of biosynthesis of carbohydrates by means of glycosylation inhibitors or gene-therapy is possible, but will also most likely have side effects depending partly on how selective the blocking can be done. Some promising results from the pharmaceutical companies have, however, been recently published (39,40). For acuted and life threatening conditions like severe infections. septic chock, ARDS and reperfusion injury, the potential side effects and cost of the treatment are not necessarily important. For more chronic conditions, like rheumatoid arthritis and cancer, the side effects will probably be a greater problem.

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