CD15: A STICKY SUGAR?

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SUMMARY

- 3-Fucosyl-N-acetyl-lactosamine is trisaccharide designated as CD15. Its most salient property is self recognition, which is seldom found in nature. Based on its transient feature in brain tissue and its differential expression during aging several functions have been proposed for this sugar. The most acceptable is still regulation of adhesion during development and aging. Sugar expression evolves from the production of the enzymes involved in the synthesis of the sugar. Therefore research has been directed fawards the CD 15 constituting enzymes alpha 3-fucosyltransferases and their chromosomal localization. Since CD 15 can be used in typing tumors, it's use in pathological research has increased in recent years.

INTRODUCTION

- CD15 (Fig. 1) is known by a series of trivial names: lacto-N-fucopentaose II (LFPII), X, Lewis x (Le$^a$), fucose-N-acetyl-lactosamine (FAL) and as a component of Stage Specific Embryonic Antigen-1 (SSEA-1). The various names reflect the history of the discovery of CD 15. The milk oligosaccharides lacto-N-fucopentaose I and II were first isolated and characterized in the 1950's. These sugars were soon related to blood group: lacto-N-fucopentaose II as Le$^a$ using haemaglutination inhibition assays. Le$^a$ was initially termed X, but later on replaced by Le$^x$ (Lewis x) to overcome confusion with antigens related to the x chromosomes (1). The term Stage Specific Embryonic Antigen was introduced by the Feizi group (2) as a consequence of their study of antigens important during mouse embryogenesis. They detected that SSEA antigens contained alpha 1-3 fucosylated types (3). Once it was established that N-acetyl lactosamine was involved, the term FAL was introduced and later on CD15.

The study of CD 15 was intensified after the demonstration of this sugar as a myeloid associated antigen and its localization on various granulocyte-membrane glycoproteins involved in cancer and inflammation (4). In the 80's a series of antibodies were reported that detected 3 alpha-fucosyl-N-acetyl lactosamine. At the First International Workshop on Human Leucocyte Differentiation Antigens held in Paris in 1982, a number of these antibodies were
identical reaction pattern (e.g. B4.3, VIM D5, FMC10, FMC12 and FMC13) (5). The fifth workshop expended the amount of antibodies that detected CD 15 (see Table 1). The establishment of an unifying nomenclature in conjunction with the specification of the pertinent antibodies detecting CD 15 contributed to an increase in quality of the papers involved.

**BIOCHEMISTRY OF CD15**

- The immunodominant structure is a trisaccharide consisting of a N-acetyl-lactosamine (Gal beta4GlcNAc) unit with a fucose (Fuc) residue attached.

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<th>Workshop no.</th>
<th>mAb name</th>
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* Organs tested for CD15 positivity are gut, bladder, breast, lung parenchyma, lymph node, pancreas, ovary, peritoneum, mesenchymal soft tissues, adrenal cortex, adrenal medulla, and testes. The three tissues most strongly positive for CD15 are denoted in this table (28). *
4GlcNAc) unit with a fucose (Fuc) residue attached to it in the alpha 1-3-linkage (6). This sugar can be linked to O- and N-glycoproteins and to glycolipids. The sialylated form and the non-sialylated form is found throughout the human body and in other organisms. Cell surface expression of both CD 15 and sialyl-CD 15 is developmentally regulated and is tumor-associated. The synthesis starts with LAG (Fig. 2), which is the synthesis of repeats of GlcNAcbeta-1-3 and Galbeta-4. This substance can be either alphaS-sialylated producing 3'-sialyl-LAG or alpha3-fucosylated which may yield the CD15 structure. 3'-Sialyl-LAG can be alpha-3 fucosylated to sialyl CD 15 (7). In the biosynthesis of the alpha3-sialylated structures sialylation has to precede fucosylation, because none of the known sialyltransferases to date is capable of acting on fucosylated substances (8). The action of the alphaS-fucosyltransferases appears to be the critical step in the synthesis of these determinants and therefore the regulation of expression of the genes coding for these enzymes is expected to play an important role in the expression of malignant phenotypes and in the control of cell adhesion events. In general three

Figure 2. Metabolic pathways leading to CD 15 and sialyl CD15.
Abbreviations used are:
(X3-FT: (x3-fucosyltransferase
G: Galpl-^4
GN: GlcNAcpl-3
F: Fucal-3
LAG: repeats ofGalpl->4GlcNAcpl+
SA: NeuAca2-*3
O3-SAT: a-sialyltransferase

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different alphaS-fucosyl transferases can be distinguished by criteria such as pH optimum, $K_m$ values, cation requirements and sensitivity to inhibitors. The main difference is in their acceptor specificity (9). These enzymes all are capable of transferring fucose from the donor substrate GDP-fucose to a beta 4-galacto-sylated GLcNAc residue in the N-acetyl-lactosamine type acceptor structures. It is in the specificity for additional structural features of acceptor molecules that alphaS-fucosyltransferases differ. The plasma type alphaS-fucosyltransferase and the Lewis type alpha 3/4-fucosyltransferase are both capable of functioning using sialylated sugars. The myeloid-type alphaS-fucosyltransferase cannot and it is this type that is predominantly present in nervous tissue (7).

**CHROMOSOMAL LOCALIZATION OF ALPHAS-FUCOSYLTRANSFERASES**

- The gene coding for the plasma-type alphaS-fucosyltransferase is syntenic, homologous but non-allelic to the Lewis locus on human chromosome 19 (10). The Lewis-type alphaS-fucosyltransferase is a product of the human Lewis blood group locus, which is localized on chromosome 19 (11). Of all individuals in the European population less than 10% are Lewis (CD 15) negative, and these persons do not express the Lewis fucosyltransferase (9).

The myeloid-type alphaS-fucosyltransferase has been located on chromosome 11 by somatic hybridization (12). Despite the differential chromosome localization of the cDNA this enzyme shows a considerable similarity to that of the plasma type and the Lewis type enzymes. It has therefore been suggested that the genes of these enzymes all belong to an evolutionarily related family (10).

**CELL ADHESION PROPERTIES OF CD15**

- Several sugars only slightly deviating from the structure of CD 15 exist. Le$^\beta$ has been the new name of Le$^b$ and differs from CD 15 (Le$^x$) in that an extra fucosylation (Fuc-alpha 1-2) is present. The H sugar is the Le$^\beta$ without the alphal-3 fucolysation. The interaction between various fucosylated glycosphingolipids or proteins is very intriguing (Fig. 3). Certain sugars will recognize themselves and in this respect are seldom found in nature. Sugars that contain receptor and ligand properties in itself are CD 15 and H. Other sugars among them produce weak bonding (eg H-CD15). Sialylated CD 15 is recently identified as target molecule for E-selectin (ELAM-1), linking this sugar to selectin-dependent leucocyte processes and therefore to inflammation. CD 15 is expressed during platelet activation (13). CD 15 functions as an adhesion molecule capable of $Ca^{2+}$ mediated homotypic binding. Cells with high surface expression of CD 15 therefore exhibit strong self-aggregation (based on CD 15-CD15 interaction) in the presence of $Ca^{2+}$ (13).

**TUMOR-ASSOCIATION OF CD15**

- Glycolipids bearing the CD 15, dimeric CD 15 and even trimeric CD 15 determinants are tumor associated. All the CD 15 and CD 15 related substances were named tumor-associated carbohydrate antigen (TAGA) (14). This includes sialosyl-CD15. The human cancers involved include colonic, lung, bladder, neural and gastric carcinoma. Bladder cancer in humans expresses CD15-related carbohydrate epitopes. Sialyl-CD15 possibly interacts with

![Figure 3. Interactions of various CD15 related antigens: CD15-to-CD15 and H-to-H interaction is strong. All other bondings are weak, while Le$^\beta$-to-Le$^\beta$ bonding is repellent.](image-url)
selectin, while bound to a 60 kD glycoprotein (15). Expression of CD 15 in tumors of the nervous system occurs too (16). CD15 was seldom reported in low grade gliomas, but present in metastatic carcinomas, menin giomas, germinomas and malignant melanomas. The same study also used rat glioma cell lines. In these rat cell lines CD 15 expression is absent. Myeloid leukaemia cells express CD 15 in a heterogeneous manner.

**ANITIBODIESTOCD15**

H Several monoclonal antibodies exist, that are claimed to demonstrate the presence of CD 15. Complicating the study of CD 15 is the fact that CD 15 may be sialylated at the terminal position in which case only neuraminergic treatment will reveal the CD 15 residue. Mostmurine monoclonal antibodies to CD 15 are IgM and do not react with sialylated CD 15. In the workshop on CD 15 from the fifth international workshop on leucocyte antigens four out of twenty-five of the tested monoclonal antibodies positive for CD 15 were IgG(17) (Table 1).

**CD15 IN THE HERVOUS SYSTEM**

- CD 15 is a good determinant in the study of aging of the brain. Age related expression patterns are demonstrated for the human lateral geniculate nucleus (18), but also for the monkey lateral geniculate body over years (19). The increase in expression of the CD 15 epitope in the human nucleus basalis of Meynert could be related to a network density change of astrocytes in the lateral part of the same nucleus in cases of Huntington's disease. In organic brain diseases and other psychiatric disorders changes in CD 15 expression could not be correlated to the specific diseases (20).

CDISisasugar whose expression in central nervous structures varies during the course of development. In the developing rat spinal cord CD 15 is restricted to alar plate and the dorsal root entrance zone. A transitional stage expression of CD 15 in this case is present, ending up with a mature expression in lamina II of Rexed. Although the dorsal root entrance zone is positive for CD 15, no CD 15 positivity could be found in the dorsal root ganglion cells (21), indicating a heterogenous expression of CD 15 over subunits of neural structure.

Differential expression of CD 15 was also studied in the vertebrate cerebellar cortex. CD 15 was positive in glial cells and neuronal structures. In rodents, the monkey and man the CD 15 positivity could be attributed to cerebellar Bergmann fibers of the Golgi cell. A typical topographic distribution in parasagittal bands was demonstrated in the mouse cerebellum and the developing human cerebellar cortex (22).

In the development of the rat, CD 15 is not expressed in dorsal root ganglion cells. The contrary is true for the expression of CD 15 in a subset of dorsal root ganglion cells during chick embryonic development (23), indicating species variability in the expression of CD15 (see also 22).

In the adult guinea pig inner ear, CD 15 was demonstrated in the tectorial membrane of the cochlea and the endolymphatic sac. The upper part of the main body of the tectorial membrane stained abundantly, while in the rugosal and distal part of the endolymphatic sac several unequally distributed cells showed strong intra- and extracellular presence of this epitope (24).

Figure 4 shows CD15-positive areas in the cat brain.

**CD15 AMD CULTURED NEURAL CELLS**

H Astrocytes in culture will express CD 15 (25). 20% of immortalized astrocytes derived from embryonic rat brain (E19/20) will express CD 15 located at specific morphological features. In the presence of retinoic acid the proportion of CD 15-positive astrocytes increased in a time dependent manner, reaching about 90% within four days, again related to typical morphological features like perinuclear granula, tips of astrocyte processes and contract sites between astrocytes. After treatment with neuraminidase all astrocytes showed CD 15 positivity, revealing the epitope partially masked by sialylation (25).

The expression of CD 15 in dissociated cultured rat dorsal root ganglia was studied in five experimental
Figure 4. Half sections stained for CD15 (LeuM1) though cortex, hippocampus and mesencephalon (a) ami though the frontal cortex (b). Note the differential staining in cortex, the strong positivity for CD15 in the hippocampus and absence of CD15 positivity in the mesencephalon (cat brain fixated in 4% formaldehyde, sections 40 jdm, nr C 1176)
situations: chemically defined medium and the same medium with added nerve growth factor, retinoic acid or antibodies against insulin or tyrosine phosphate. Using astrocyte cultures as a positive control for exclusion of newly developed monoclonal antibodies that did not react with CD 15, it was shown that masking of the CD 15 antibody occurs, which influence the detection capacity of the monoclonal antibodies used. Moreover, in contrast to the vivo situation, two populations of dorsal root ganglion cells could be discerned: a CD15-positive and a CD 15-negative population. The CD 15 expression is not involved in the outgrowth of protrusions or the ensheathing by non-neuronal cells of dorsal root ganglion cells (26).

CD15 AMD NEUTROPHILS

Polymorphonuclear neutrophils play an important role in the defence against invading pathogens. Neutrophils are also the major cells of the acute inflammatory response. They also express CD 15 antigens. CD 15 antibodies affect a series of neutrophil functions including adhesion to endothelium, phagocytosis, stimulation of degranulation and the respiratory burst (27). The addition of low concentration of antibodies to CD 15 to neutrophils enhances the adherence of neutrophils to substrates, possibly mediated by selectins. It therefore seems possible that CD 15 plays a role in the activation of integrin-dependent adhesion.

THE FUNCTION OF CD15

The function of CD 15 is not well characterized and is most likely multifactorial.

- **Brain**

Till now several functions were proposed for the CD 15 antigen in the brain. However its function in the brain will be different from that in the periphery. Within the brain, adhesion is expected to be the main function of CD 15, being both receptor and ligand. Transient expression could be an argument in favour of this function. Since the neural turnover of astrocytes and the morphology of neurons is changing during aging and CD 15 patterns are not constant either during the same process, a continuously shifting adhesion situation during aging is to be expected.

Neutrophils

In neutrophils, CD 15 clearly seems to contribute to their adhesion to substrates enhancing their function in defence against pathogenic organisms and in the inflammatory response.

Tumors

The function of CD 15 in tumors is presumably related to the dedifferentiating process.

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