Target antigens in autoimmune diabetes: pancreatic gangliosides

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Summary. - Type 1 diabetes mellitus is a disease caused by the autoimmune destruction of insulin-producing pancreatic β-cells that takes place in genetically predisposed individuals. Autoantibodies and autoreactive T lymphocytes reacting with islet target molecules or protein of glycolipid nature have been shown in the circulation of individuals and of animal models of type 1 diabetes (NOD mouse and BB rat) before and at the onset of the disease. As far as autoantigens of glycolipid nature is concerned, gangliosides such as GT3, GD3 and especially GM-1, have been shown to be target of autoantibodies associated to autoimmune diabetes. Of particular interest is the islet-specific monosialo-ganglioside GM2-1, which is target of an autoimmune response highly associated to future progression to diabetes development in first degree relatives of type 1 diabetic individuals. This molecule is recognized by IgG autoantibodies which have been detected before the appearance of clinical diabetes both in man and in the NOD mouse, representing a novel marker of β-cell autoimmunity.

Key words: type 1 diabetes, gangliosides, autoantibodies.

Riassunto (Antigeni bersaglio nel diabete autoimmune: gangliosidi pancreateici). - Il diabete mellito di tipo 1 è una malattia causata dalla distruzione autoimmune delle cellule β pancreatiche che si manifesta in individui geneticamente predisposti. Autoanticorpi e linfociti T autoreattivi diretti contro molecole insuline proteiche e glicolipidiche sono stati osservati in pazienti sia prima che al momento dell’esordio clinico della malattia. Per quanto riguarda gli autoantigeni di natura glicolipidica, i gangliosidi GT3, GD3 e specialmente GM2-1 sono bersaglio di autoanticorpi associati alla malattia. Di particolare interesse è il GM2-1, che si è dimostrato essere il bersaglio di una risposta autoimmune altamente associata a una futura comparsa della malattia in parenti di soggetti con diabete di tipo 1. Tale molecola è infatti riconosciuta da autoanticorpi di classe IgG, che precedono l’esordio clinico del diabete sia nell’uomo che in un modello animale di diabete autoimmune quale il topo NOD.

Parole chiave: diabete tipo 1, gangliosidi, autoanticorpi.

Gangliosides

Glycosphingolipids are amphiphilic molecules which are usually located in the outer leaflet of the plasma-membrane bilayer, with the hydrophilic portion exposed extracellularly and the hydrophobic tail (the ceramide) inserted into the lipid bilayer. The ceramide can be both glycosylated (originating the family of glycosphingolipids) or phosphorilated. Furthermore, sialylated glycosphingolipids form the family of gangliosides, while sulphated glycolipids form sulphatides.

Glycosphingolipid expression and distribution have been shown to be species- and tissue-specific and are influenced by events such as cell growth, differentiation, metabolic activity and oncogenesis [1].

Gangliosides are sialic acid containing glycolipids which can be found in virtually all vertebrate tissues. Initially described in central and in peripheral nervous system, gangliosides have been shown in extraneural tissues as well.

Immunochemical studies with monoclonal antibodies have indicated the involvement of gangliosides in the chemical basis of blood group antigens (Ii, A and H). In addition, the ganglioside nature of the heterophile H-D antigen in tumor cells has been established.

Modifications in glycolipid metabolism have been observed in a large variety of transformed cells as well as in tumor cells in vivo. Changes in ganglioside pattern may account for some of the abnormal interactions between tumor cells and their environment.

Furthermore, it has been demonstrated that the antigenicity of gangliosides on cell membranes is due not only to their oligosaccharide sequence, but also to their localization and distribution in the membrane. Tumor associated gangliosides or cell surface gangliosides acting as antigens, often contain an aberrant
ceramide composition that may contribute to change the glycolipid organization within the plasma membrane.

Several studies have indicated that autoantibodies to many glycolipids including gangliosides can be found in a number of in autoimmune disorders, pathologic conditions. Circulating anti-ganglioside antibodies occur in neurological disorders such as Guillain-Barré syndrome [2], multiple sclerosis, amyotrophic lateral sclerosis, lower motor neuron syndromes, Alzheimer disease as well as in immune mediated disorders like lupus erythematosus, Behcet’s disease, AIDS, Hashimoto’s thyroiditis and Graves’ disease.

**Type 1 diabetes mellitus**

Insulin-dependent (type 1) diabetes mellitus is a genetically determined chronic autoimmune disease caused by the selective destruction of pancreatic β-cells by the immune system [3]. To date, the only well-established genetic marker is represented by genes within the human leukocyte antigens (HLA) system, both in man and in animal models of autoimmune diabetes like the non obese diabetic (NOD) mouse and the BB rat. Important insights into the genetics of type 1 diabetes have been gained through its association with the HLA-DQβ1 gene, and the presence of the disease has been shown to be associated with an aminoacid other than Asp at position 57 of the DQβ-chain (non-Asp57). Furthermore, a correlation with the disease has been found with those alleles of the DQA1 gene that carry an Arg at residue 52 (Arg52) of the DQα-chain.

Since the initial demonstration of islet cell autoantibodies (ICA) in type 1 diabetic patients, accumulated evidence has shown that this disease has a prodromal phase during which antibodies against islet cell antigens can be demonstrated in circulation, representing important predictive tools of future disease [4]. An increasing number of autoantibodies in β-cell autoimmunity has been identified and characterized: insulin, carboxypeptidase H and glutamic acid decarboxylase (GAD) have been demonstrated to be target of autoantibodies. In addition, it has been shown that one of the ICA target antigens has properties of a sialic acid containing glycolipid (ganglioside) since an islet monosialo-ganglioside (GM2-1) is a potent inhibitor of the binding of a subset of ICA on pancreatic frozen sections [5]. This molecule is specifically expressed in human and rodent islets, is hyperepressed in NOD mouse islets, is metabolically regulable and biochemical studies indicate that it has a structure very similar to a major neuronal autoantigen. The most practical markers of β-cell autoimmunity currently available are circulating antibodies against islet antigens. Among these, anti-insulin autoantibodies (IAA), anti-64kD protein (recently identified as GAD) antibodies and cytoplasmic islet cell autoantibodies (ICA) appear to have a sufficient disease-specificity to be used for the identification of subjects at risk to develop type 1 diabetes. Recent studies have indicated that ICA are heterogeneous, with the description of at least two classes of these antibodies: ‘restricted ICA’ that bind to human and rat but not to mouse islets, appear to be β-cell specific, are directed against GAD and are not associated with the future development of the disease; “non restricted ICA” that bind to human, rat and mouse pancreatic islets, are not β-cell specific, are strongly predictive of future disease and appear to be directed against an islet ganglioside. Specifically, ICA reactivity of the “non restricted” type on pancreatic frozen sections can be abolished by treatment with neuraminidase, periodate, chloroform and methanol while it is resistant to pronase digestion.

**Islet ganglioside expression**

Immunohistochemical studies with monoclonal antibodies to gangliosides showed that islet-specific ganglioside expression can be found within the pancreas; consequently ganglioside expression was studied in human, rat and mouse whole pancreas and isolated islets. Interestingly, we showed that the GM2-1 ganglioside, suggested to be one of the ICA target antigens, is the major islet ganglioside in all three species while it is minimally expressed in whole pancreas glycolipid extracts. Quantitative analysis showed that GM2-1 is present in isolated islets at a concentration approximately 100-fold higher than in whole pancreas [6], suggesting the islet-specificity of this molecule. In addition, by comparing islet ganglioside expression in NOD and C57 mice, we found an hyperexpression of GM2-1 in young NOD islets compared to age matched C57 islets. Of note, GM2-1 content in NOD but not in C57 mice clearly decreased with age, and diabetes onset in the NOD is associated to a virtual disappearance of this ganglioside from pancreatic islets. This phenomenon (hyperexpression of GM2-1 in NOD islets followed by decrease with β-cell destruction) may reflect a genetic predisposition to autoimmune islet destruction of this mouse strain and the specific involvement of that molecular structure in diabetes-related events [7].

**Biochemical characterization of GM2-1 ganglioside**

We have biochemically characterized the GM2-1 ganglioside. Gangliosides have an hydrophobic portion (ceramide) formed by a fatty acid and a sphingosine and an hydrophilic part: the sialo-oligosaccharide chain. Through the ceramide, they are anchored to cell membranes; the length of the fatty acid determines the level of exposure of the hydrophilic portion. Through the oligosaccharide chain, gangliosides can bind to hormones, toxins, antibodies, etc. Therefore, in order to
characterize a ganglioside structure it is necessary to analyze: the fatty acids in the ceramide; the number, type and position of sialic acid residues; the type and sequence of monosaccharides forming the oligosaccharide chain. We have identified the fatty acids and the type, number and position of sialic acid residues on the GM2-1 molecule, employing gas chromatography and enzymatic digestions. Fatty acids identified ranged from C16:0 to C24:1; experiments on the sialic acid have shown that GM2-1 has a single sialic acid residue in terminal position identified as N-acetyl-neuramic acid (NANA) by gas chromatography. The terminal position of the sialic acid has been established from the sensitivity of the GM2-1 molecule to both endo- and esogalactosaminidase digestion; the presence of a single sialic acid residue has been determined by anion exchange column chromatography. In addition, by sequential treatment with esogalactosidases followed by gas chromatographic identification of the liberated sugar, the oligosaccharide chain has been shown to contain the following sugar sequence: NANA-galactose-galactosamine-galactosamine-glucose- ceramide [8].

In conclusion, these data indicate that this molecule is an hexosamine-containing monosialo-ganglioside with a sialic acid in terminal position. Interestingly, these data show a striking similarity between GM2-1 and LM1, a major neuronal autoantigen in demyelinating autoimmune syndromes.

Anti-GM2-1 antibodies

Employing an indirect immunoperoxidase technique performed on aluminum-backed HPTLC plates, we have started to analyze the presence of autoantibodies directed against pancreatic gangliosides in human subjects and in an animal model of type 1 diabetes, the NOD mouse. The presence of autoantibodies directed against the GM2-1 ganglioside was detected in a percentage (65%) of ICA+ first degree relatives of type 1 diabetes and in all NOD mice. Interestingly, these autoantibodies appear in circulation well before the disease onset (up to 7 years), and occurred in all relatives developing type 1 diabetes within 5 years, thus identifying a cohort of subjects with markedly increased diabetes risk [9]. Anti-GM2-1 antibodies were not found in normal subjects nor in ICA-relatives nor in mice from normal strains (no. 20). The life-table analysis of the progression to diabetes in ICA+ relatives of type 1 diabetes showed the following data: thirty-one ICA+ first degree relatives were prospectively evaluated for a period of up to ten years. At recruitment, eleven were GM2-1 autoantibody negative and 20 were positive. Fourteen subjects subsequently developed type 1 diabetes. Thirteen out of 14 individuals who became diabetic were GM2-1 positive during the subclinical latency period. The sensitivity of the GM2-1 autoantibody test to the presence of diabetes was 93% and the specificity 80% out of the subjects in this study. The risk for diabetes in GM2-1 autoantibody positive ICA+ relatives of type 1 diabetic patients was statistically evaluated by log-rank test applied to the survival analysis (p < 0.001). The survival estimate for GM2-1 autoantibody positive subjects at 7 years was 8% (95% confidence interval (CI): 0-24) compared to 83% (95% CI: 54-100) for GM2-1 negative subjects. The positive predictive value of GM2-1 autoantibodies for diabetes, calculated according to Bayes’ theorem taking values at the longest follow up intervals usable for comparison (7 years), was 93% in this cohort of patients. Gangliosides have been shown to be autoantigens in other autoimmune diseases: autoimmunity to this class of molecules may therefore be central to β-cell destruction in diabetes, providing the basis for the prediction and possibly the prevention of the disease.

Submitted on invitation. Accepted on 14 February 1997.

REFERENCES


