Measurement of Soluble Adhesion Molecules: Can It Improve Diabetes Prediction?

Commentary on the article by Toivonen *et al.* on page 24

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Type 1 diabetes is an autoimmune disease mediated by T lymphocytes (1). Progressive loss of β-cells occurs over months to years, without any clinical presentation until overt hyperglycemia develops. Recent work in this field has focused on understanding the pathogenesis of β-cell destruction and on prediction of disease before symptoms occur. A number of preventative therapies have been successful in animal models and in human pilot studies. Two large, multicenter trials of diabetes prevention in high-risk subjects are in progress (2, 3). Both trials have screened large numbers of relatives of individuals with type 1 diabetes for the presence of islet cell antibodies (ICA), a marker of increased diabetes risk. Many other factors correlated with diabetes development have been described, most importantly, the presence of antibodies to other islet proteins, such as insulin, glutamic acid decarboxylase (GAD) and a protein tyrosine phosphatase, IA-2. There are also significant human leukocyte antigen (HLA) associations, including DR3/or 4. A recent analysis of combining risk markers to predict diabetes reported a risk of developing diabetes of 65% over 10 y in individuals with autoantibodies to IA-2 or GAD on initial screening followed by positive ICA or insulin autoantibodies (IAA) on subsequent testing (4). Identification of other factors during the prediabetic state, which are strongly associated with the development of diabetes, will aid the goal of finding reliable methods of predicting diabetes.

One area of advancement in understanding of the immune response has been the characterization of adhesion molecules involved in binding of cells to each other or to the extracellular matrix. The interactions of adhesion molecules with their ligands are important for increasing avidity of cell-cell interactions, aiding in lymphocyte activation and for traffic of cells from the circulation to sites of inflammation (5). In the immune

response, interaction of intercellular adhesion molecule-1 (ICAM-1) on antigen presenting cells with its ligand, lymphocyte function-associated antigen-1 (LFA-1), on T lymphocytes helps to activate T cells. ICAM-1 expression on endothelial cells contributes to migration of activated T cells through venules. L-selectin, expressed on lymphocytes, is important for lymphocyte adhesion to endothelial cells of peripheral lymph nodes. Both L-selectin and ICAM-1 are shed from the cell membrane and are found in the circulation in soluble form. Nanogram/mL concentrations of these and other adhesion molecules can be measured in the sera of healthy persons and increased levels have been found in many inflammatory and infectious conditions (6). Some studies in rheumatic diseases have found positive correlations with other markers of disease activity while others have not. In these conditions, soluble adhesion molecule levels have generally been 1.5- to 3-fold higher than in healthy controls (6). Studies have been hampered by differences in monoclonal Ab used to measure adhesion molecule levels, making it difficult to directly compare studies. Universal standards have not been used. Interestingly, interference with the immune response by high levels of soluble adhesion molecules has been reported, suggesting that they could be protective (7, 8). However, the functional role of soluble adhesion molecules is not clearly understood.

The paper in this issue of Pediatric Research from the Childhood Diabetes in Finland Study Group adds to the evidence of increased levels of soluble adhesion molecules in type 1 diabetes (9). The authors present data on soluble ICAM-1 and L-selectin in a large and well-characterized group of subjects at high risk of developing diabetes. These subjects (mean age 9.8 y) are siblings of patients with type 1 diabetes who have at least one of four risk markers for diabetes, ICA, antibodies to GAD, IA-2 or insulin, and are compared with age-matched siblings without antibodies. There are only two previous publications of soluble adhesion molecules in subjects at high risk of diabetes. The first, measured soluble ICAM-1 and L-selectin in 6 high risk ICA positive (>5 JDF (Juvenile Diabetes Foundation) units) first-degree relatives, 27 lower risk ICA negative relatives and 100 nonrelated healthy blood donor controls (10). Levels of ICAM-1 were higher in relatives than in controls but did not differ by ICA status. L-selectin levels were also higher in relatives than controls and were higher in ICA positive relatives. The mean age of ICA positive subjects (8.3 y) was significantly lower than in ICA negatives or controls (24.5 and 25.7 y, respectively). Higher levels of both adhesion molecules were found in the high risk HLA DR3 or DR 4 ICA negative subjects. No follow up on diabetes development was available. The second report examined soluble ICAM-1 levels in 26 first-degree relatives with high titer ICA (>20 JDF units) compared with an age-matched and sex-matched control group (11). Mean ICAM-1 levels were 1.9-fold higher in subjects than controls. Positive correlations were also found between soluble ICAM-1 levels and ICA, GAD antibodies, and the number of antibodies. Again, no data on diabetes development was presented.

The study by Toivonen *et al.* examines the ICAM-1 and L-selectin levels in a large group of subjects followed for a minimum of 7 y, therefore providing the first opportunity to correlate soluble adhesion molecules with diabetes development. The subjects include 95 siblings of a child with diabetes with at least one autoantibody (ICA, GAD Ab, IA-2 Ab, or IAA) and 95 age-matched and sex-matched siblings who remained antibody negative throughout the follow up period. ICAM-1 and L-selectin levels were then correlated with age, HLA, ICA titer, other antibody status, first phase insulin response (FPIR), a measure of insulin secretory reserve, and progression to diabetes. Unlike the two previous studies, this study did not find significant differences in ICAM-1 or L-selectin levels between autoantibody positive and autoantibody negative siblings. However, both previous studies compared levels with healthy unrelated controls. In the study by Lampeter *et al.*, ICA positive and negative relatives are both investigated, but the mean levels are not described or compared (10). Another important finding in the study of Toivonen *et al.* was the inverse correlation with ICAM-1 and L-selectin levels and age, emphasizing the need for age-matched controls in such studies. Some other statistically significant correlations were found. ICAM-1 levels were significantly higher (1.2-fold) in subjects with higher ICA titers, a factor that is associated with higher diabetes risk. Higher L-selectin and ICAM-1 levels were also found in IA-2 Ab positive siblings. Subjects positive for 3 or more antibodies, who are known to be at higher risk of diabetes, had higher ICAM-1 levels than those positive for only 1 or 2 antibodies. However, the ICAM-1 levels in subjects with 1 or 2 antibodies were not higher than those in antibody negative siblings. Surprisingly, the ICAM-1 and L-selectin of relatives at highest risk of diabetes, *i.e.* individuals who had loss of first phase insulin release, did not differ from siblings with normal FPIR. The final analysis showed that subjects who progressed to diabetes ($n = 29$) had significantly higher (1.2-fold) ICAM-1 levels than the nonprogressors. Using a cut off level of soluble ICAM-1 of 1 or 2 standard deviations (SD) above the level in nonprogressors, gave a positive predictive value of diabetes of 50%.

This study identifies some significant associations between soluble adhesion molecules and diabetes development. It is limited because adhesion molecule levels were measured only at the time of entry into the study, 7 to 11 y before the end of the follow up period. It is possible, therefore, that some children were studied at an early stage of disease before serum levels of adhesion molecules were elevated. This might have decreased the differences between subjects and controls. The sensitivity of elevated ICAM-1 levels is 10% to 38%, depending on whether a level of 1 or 2 SD above nonprogressors is used; suggesting that this assay would not be sufficiently sensitive for screening high-risk relatives. A cut off of 2 SD above nonprogressors, gives 95% specificity, making it potentially useful in refining prediction in those identified as being at risk of diabetes through a sensitive screening test. No information is yet available about the evolution of these levels as disease progresses. As there are no good markers of disease progression or regression, apart from FPIR, a longitudinal study will be necessary to determine whether the measurement of soluble adhesion molecules really adds value to the "diabetes prediction profile" currently in use.

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