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Short Communication

Soluble adhesion molecules in pre-clinical Type 1 diabetes: a prospective study

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Abstract

Aims. This prospective case-control study aimed at evaluating the time course of serum concentrations of soluble adhesion molecules; intercellular adhesion molecule-1 and L-selectin in siblings with signs of pre-clinical Type 1 diabetes in order to relate these concentrations to autoantibody status and to assess whether these markers could discriminate between those siblings who progressed to clinical diabetes and those who remained non-diabetic.
Methods. Serum levels of soluble adhesion molecules were measured with enzyme-linked immunosorbent assays in autoantibody-positive initially healthy siblings of diabetic children who progressed to clinical disease during the observation period of 10 years and in sex- and age-matched autoantibody-positive siblings who have remained unaffected.
Results. The intraindividual and interindividual variability in the concentrations of soluble adhesion molecules was conspicuous both among the progressors and non-progressors. Integrated concentrations (area-under-the curve) of intercellular adhesion molecule-1 over a period of 6 to 48 months before the diagnosis was higher in the progressors (p=0.035), the difference being most evident 18 to 24 months before diagnosis (p=0.015). The integrated concentrations of soluble L-selectin were similar in progressors and non-progressors over the total pre-clinical period. There were no differences in the integrated concentrations of soluble adhesion molecules in relation to the initial or maximal number of autoantibodies detected during the follow-up.
Conclusion/interpretation. These observations suggest that the process of destructive insulitis could be initiated approximately 4 years before the manifestation of clinical diabetes, being most active about 1.5 years before diagnosis. Peripheral concentrations of soluble intercellular adhesion molecule-1 or L-selectin are not helpful in the identification of those prediabetic subjects who will progress to clinical disease over the next 10 years, since there is substantial overlapping in these concentrations between progressors and non-progressors. [Diabetologia (2003) 46:492–495]

Keywords ICAM-1, L-selectin, autoantibodies, prediction, prospective.

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Abbreviations: GADA, antibodies to the 65 kD isoform of glutamic acid decarboxylase; IAA, insulin autoantibodies; IA-2A, antibodies to the protein tyrosine phosphatase-related IA-2 antigen; ICA, islet cell antibodies; ICAM-1, intercellular adhesion molecule-1; JDF, juvenile diabetes foundation; NOD mouse, non-obese diabetic mouse.

The pre-clinical phase of Type 1 diabetes seems to cover a wide spectrum making it difficult to predict with autoantibodies and genetic markers on an individual basis whether the disease process will be mild, possibly reflecting a stable, non-progressing, or very slowly progressive subclinical beta-cell dysfunction, or whether it will run a severe course rapidly resulting in clinical diabetes [1]. The reliable identification of individuals en route to clinical diabetes would make it possible to avoid the exposure of low-risk individuals to potential adverse effects of any intervention modality. This consideration is more important,

the more aggressive the preventive therapy planned to be used is.

The physiological role of soluble adhesion molecules is unknown but considering the crucial impact of cell adhesion molecules in lymphoid-endothelial interactions increased concentrations in circulation, shed from the cell surface, could be an epiphenomenon of immune activation and thus might provide a useful monitor of disease activity in inflammatory disorders. So far the studies on the role of soluble adhesion molecules in Type 1 diabetes have been cross-sectional [2, 3, 4, 5], and there are no data available on the dynamics of these molecules during pre-clinical diabetes. In this current work we evaluated whether serum concentrations of soluble ICAM-1 and L-selectin in autoantibody-positive siblings of diabetic children, when measured serially over a relatively long period of time, differed between those who progressed to clinical diabetes and those who remained non-diabetic, and assessed accordingly whether these biomarkers could be used to discriminate between disease progressors and non-progressors.

Subjects and methods

Subjects. The study subjects comprised of 39 initially unaffected siblings, positive for at least one out of four diabetes-associated autoantibodies on at least one occasion during the prospective 4-year follow-up period, who progressed to clinical Type 1 diabetes during the observation period from September 1986 to the end of 1998 in the nation-wide, population-based Childhood Diabetes in Finland (DiMe) Study [6]. Of the autoantibody-positive siblings, who remained non-diabetic, matched for sex, age (±2 years in 90% of the subjects) and the initial number of autoantibodies, 39 were chosen as a control group. The median age of the siblings at recruitment was 7.4 years among the progressors (range 1.5–16.4 years) and 7.6 years among the non-progressors (range 1.1–16.5 years). The median duration of follow-up before diagnosis from the initial blood sampling was 4.4 years (range: 9 days–11.7 years). A subgroup of the initial study group comprised of 30 sibling-pairs matched for the duration of follow-up in addition to matching for age, sex, and initial number of antibodies. Among the progressors the mean follow-up time was 3.0 years (range 0.2–5.7 years) and in the non-progressors 2.9 years (range 0.2–6.2 years) in this subgroup. The study protocol was approved by the ethical committees of the participating hospitals, and informed consent was obtained from the subjects and/or their parents.

A total of 345 serum samples were analysed for the sICAM-1 levels and 344 samples for the sL-selectin levels, the number of samples per sibling varying from one to nine (mean for progressors 4.6 and non-progressors 4.3).

Soluble adhesion molecules. The concentrations of soluble adhesion molecules were determined with commercially available sICAM-1 and sL-selectin ELISA kits (Bender Medsystems Diagnostics, Vienna, Austria). The assays are based on two monoclonal antibodies directed against different epitopes on the soluble adhesion molecules. Briefly, diluted serum samples (1:10 for sICAM-1 and 1:200 for sL-selectin) were applied to the microwells, precoated with murine monoclonal antibody to human ICAM-1 or L-selectin. A horseradish peroxidase-conjugated anti-mouse monoclonal antibody that binds to the sICAM-1 or sL-selectin captured by the primary antibody was then added. Following incubation and thorough washing to remove the non-reactive component, 3,3,5,5-tetramethylbenzidine was added to form a coloured end product. The enzyme reaction was terminated with phosphoric acid and the absorbances were measured at a wavelength of 450 nm with a Multiscan MS photometer (Labsystems, Helsinki, Finland). The concentrations were read from a standard curve and expressed in μg/l for sICAM-1 and in mg/l for sL-selectin. All samples from each individual were run on the same assay plate. The coefficient of intra-assay variation in the sICAM-1 assay was less than 5% and in the sL-selectin assay less than 4%.

Autoantibody assays. The ICA, GADA, IA-2A and IAA were quantified as described previously [1]. The detection limit for ICA was 2.5 JDF units. The cut-off limits for GADA, IA-2A and IAA-positivity were set as 6.6 relative units (RU), 0.43 RU and 55 nU/ml which correspond to the 99th percentiles in non-diabetic children.

Data handling. In addition to visual inspection of the soluble adhesion molecule profiles for each individual, a summary measure approach was used to compare the integrated concentrations over time between various groups. For each individual sICAM-1 and sL-selectin concentrations were also characterised by the lowest (nadir) and highest measured (peak) value. The siblings were divided into autoantibody-positive and negative subgroups based on the antibody status in the first available sample (integrated concentrations) or during the follow-up (nadir and peak concentrations).

Statistical analysis. The Mann-Whitney U-test was used to analyse continuous variables with skewed distribution and to analyse serial measurements we used the AUC as a summary measure. A p value of less than 0.05 was considered statistically significant. All data were analysed using the SPSS 9.0 or 10.0 software (SPSS, Chicago, Ill., USA).

Results

No conspicuous general pattern existed in the course of either sICAM-1 (Fig. 1) or sL-selectin (not shown) among the progressors or the non-progressors. There was no difference in the total integrated concentrations of sICAM-1 between progressors and non-progressors, but $AUC_{ICAM\text{-}1}$ was higher in the progressors than in the non-progressors 6 to 48 months before the diagnosis of Type 1 diabetes (p=0.035) (Table 1, Fig. 1). The difference in the levels of sICAM-1 between the groups was most conspicuous 18 to 24 months before diagnosis (p=0.015). The integrated concentrations of sL-selectin were comparable in both groups over the total observation period, also when analysed according to the autoantibody status (data not shown). When the analyses were carried out separately in two age groups;less than 10 years, and greater than or equal to 10 years or less than 5 years, and greater than or equal to 5 years, no differences were observed for the total integrated or peak concentrations of soluble adhesion molecules between the groups (data not shown).

Table 1. Circulating concentrations of sICAM-1 in siblings (n=4–39) of affected children who progressed to clinical Type 1 diabetes during observation for a median period of 10 years, and in unaffected siblings (n=11–39)

	Progressors	Non-progressors	p-value
Total AUC (µg months/l)	10464; 7214, 13177 (n=30)	9110; 5807, 12370 (n=30)	0.287
AUC 48–6 months before diagnosis (µg months/l)	7634; 4420, 9316 (n=24)	4527; 2890, 7435 (n=24)	0.035
Nadir concentration (µg/l)	256; 212, 303 (n=39)	213; 185, 274 (n=39)	0.058
– ICA-positive	262; 218, 303 (n=35)	212; 180,268 (n=28)	0.009
– ICA-negative	211; 197, 329 (n=4)	258; 199, 358 (n=11)	0.794
– GADA-positive	247; 210, 300 (n=34)	210; 189, 265 (n=21)	0.066
– GADA-negative	263; 227, 338 (n=5)	239; 183, 287 (n=18)	0.205
– IAA-positive	250; 211, 304 (n=30)	212; 178, 262 (n=26)	0.071
– IAA-negative	256; 214, 312 (n=9)	239; 194, 288 (n=13)	0.404
– IA-2A-positive	243; 210, 299 (n=33)	210; 181, 257 (n=13)	0.059
– IA-2A-negative	283; 216, 329 (n=6)	239; 191, 284 (n=26)	0.148
– ≥2 abs in the initial sample	262; 210, 299 (n=29)	207; 180, 252 (n=12)	0.039
– ≥2 abs during the follow-up	249; 212, 302 (n=36)	207; 177, 239 (n=20)	0.003
Peak concentration (µg/l)	355; 280, 432 (n=39)	347; 290, 403 (n=39)	0.374
– ICA-positive	355; 319, 434 (n=35)	344; 298, 395 (n=28)	0.162
– ICA-negative	303; 206, 366 (n=4)	361; 280, 406 (n=11)	0.267
– GADA-positive	348; 277, 434 (n=34)	345; 298, 404 (n=21)	0.591
– GADA-negative	361; 299, 394 (n=5)	355; 251, 380 (n=18)	0.628
– IAA-positive	360; 336, 434 (n=30)	350; 282, 405 (n=26)	0.158
– IAA-negative	280; 249, 367 (n=9)	343; 293, 371 (n=13)	0.217
– IA-2A-positive	346; 265, 428 (n=33)	329; 307, 409 (n=13)	0.583
– IA-2A-negative	367; 341, 434 (n=6)	350; 285, 371 (n=26)	0.201
– ≥2 abs in the initial sample	346; 261, 421 (n=29)	346; 316, 405 (n=12)	1.000
– ≥2 abs during the follow-up	353; 272, 433 (n=36)	337; 298, 391 (n=20)	0.194

The values are medians; 25th, 75th percentiles

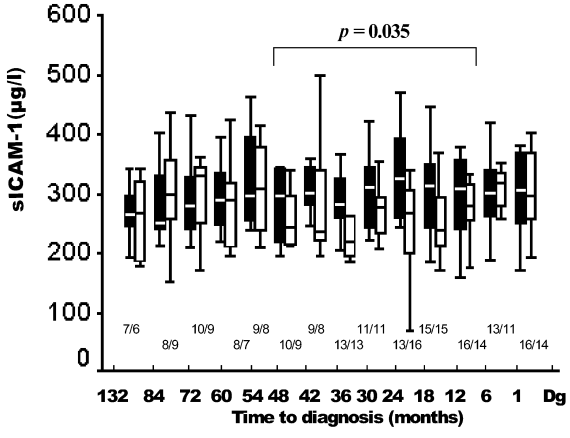

Fig. 1. Concentrations of soluble ICAM-1 in 39 autoantibody-positive progressors compared with non-progressors in pre-clinical Type 1 diabetes. Each box-plot represents the median, and the 25th and 75th centiles. The bars represent the minimum and maximum values except for the outliers. The figures above the x-axis refer to the number of siblings available in the two groups for each time interval. Closed bars represent the progressors and open bars the non-progressors

ICA-positive progressors had higher nadir sICAM-1 concentrations than the ICA-positive non-progressors (Table 1). Among the siblings with two or more autoantibodies initially or during the follow-up the progressors had higher nadir concentrations of sICAM-1 than the non-progressors (Table 1). Otherwise the total, peak or nadir concentrations of sICAM-1 or sL-selectin did not differ between the progressors and non-progressors when the siblings were categorised according to the initial or maximal number of detectable autoantibodies during the follow-up.

Discussion

Although the source and physiological function of sICAM-1 is largely unknown, its release in vitro by at least mononuclear [7] and endothelial cells [8] has been shown. Owing to the broad tissue distribution of ICAM-1 increased concentrations of sICAM-1 in progressors over a 3.5 year period in the pre-clinical phase could reflect endothelial and leukocyte activation due to insulitis being most prominent about 1.5 years before the diagnosis. Alternatively, since soluble ICAM-1 proteins have been shown to reduce destructive insulitis in NOD mice [9] and suppress antigen-specific T-cell proliferation [10] we cannot rule out that sICAM-1 could be increased due to an attempt of the immune system to reduce aggressive insulitis.

The conspicuous intra- and interindividual variation in the concentrations suggests, however, there are likely other factors affecting these levels. Viral infections could be important modifiers of circulating concentrations of soluble adhesion molecules, and e.g. the peak concentrations observed in this study might reflect on-

going viral infections. We suggest, however, that the impact of common viral infections cannot be remarkable in our study cohort, since the concentrations were alike in younger and older children. One would also expect that the impact of viral infections would be about the same both in the progressors and non-progressors, although there are some indications that immunological abnormalities related to the autoimmune process preceding the onset of Type 1 diabetes, such as depletion of memory CD4+ cells and defective killer cell activity could transiently impair host defences against viral diseases in high-risk first-degree relatives [11].

No clear associations could be seen between the peripheral concentrations of soluble adhesion molecules and diabetes-related autoantibodies in this prospective study targeting autoantibody-positive siblings of affected children. No differences were observed in the integrated concentrations of sICAM-1 or sL-selectin in relation to various autoantibody specificities. This could be due to the limited number of cases in each group, however. In our previous study we found an association between high titres of ICA and sICAM-1, and between IA-2A positivity and high concentrations of sICAM-1 and sL-selectin [5], whereas another survey did not show any association [4]. Despite contradictory findings it is still possible that the soluble adhesion molecules could reflect other characteristics of the autoimmmune process than conventional humoral immune markers.

In this study we did not analyse the relationship between the concentrations of soluble adhesion molecules and HLA class II alleles, since the study cohort was highly selective. In our previous cross-sectional study we did not find any association between the levels of sICAM-1 and sL-selectin and HLA-DR-status [5]. Accordingly HLA-conferred predisposition to Type 1 diabetes hardly explains the differences in the concentrations of sICAM-1 between progressors and non-progressors observed among these autoantibody-positive siblings.

In conclusion, the increased concentrations of sICAM-1 seen in the progressors 6 to 48 months, and especially 1 to 2 years, before the diagnosis of clinical diabetes compared to the levels in non-progressors is likely to reflect either immune activation /ongoing tissue destruction or alternatively a defensive mechanism against progressive beta-cell destruction. Due to a substantial overlapping in the concentrations on all occasions during the pre-diabetic period, however, peripheral sICAM-1 levels can not be used for discrimination between the progressors and non-progressors.

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