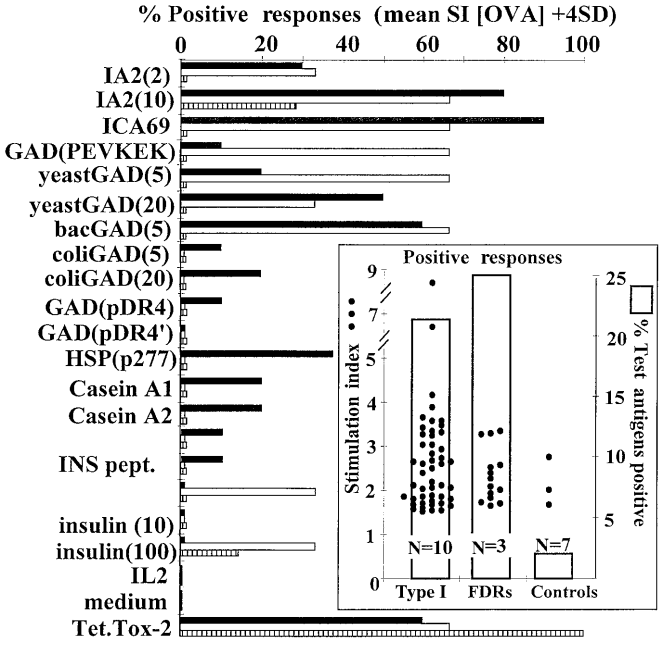

Fig. 1. IL2-supported T cell Responses to Workshop Antigens. Peripheral blood mononuclear cells from children with recent onset Type I diabetes (n = 10, black bars), first-degree relatives (FDRs, n = 3, white bars) and healthy control subjects (n = 7, hatched bars) were cultured for 1 week in serum-free HL/1 medium before measurement of H^3-thymidine incorporation. Cultures contained the workshop antigens (doses are in μg/ml where indicated) and 10 U/ml IL2. GAD, glutamate decarboxylase 65 kD, GAD(PEVKEG), possible mimicry (GAD/Coxsackievirus) peptide, bacGAD, baculovirus-expressed GAD, *coli*GAD, GAD expressed in *E. coli*, pDR4, GAD peptides able to bind to DR4 heterodimers, HSP, heat shock protein peptide 277, INS-pept, overlapping insulin peptides. Insert: individual positive proliferative responses to any of the test antigens (●) and the % of test antigens that evoked positive responses are shown

References

1. Roep BO (1999) Standardization of T-cell assays in Type I diabetes. Immunology of Diabetes Society T-cell Committee [letter]. Diabetologia 42: 636–637
2. Roep BO, Atkinson MA, van Endert PM, Gottlieb PA, Wilson SB, Sachs JA (1999) Autoreactive T cell responses in insulin-dependent (Type 1) diabetes mellitus. Report of the First International Workshop for Standardization of T cell assays. J Autoimmun 13: 267–282
3. Dosch H-M, Cheung RK, Karges W, Pietropaolo M, Becker DJ (1999) Anergic and non-anergic autoreactive T cells persist past onset of autoimmune diabetes. J Immunol 163: 6933–6940
4. Dosch H-M, Cheung RK, Becker DJ (1996) T cell responses in shipped blood samples of newly diabetic children. 15th Immunol Diabetes Workshop, Canberra, Immunology of Diabetes Society, p 76
5. Dosch H-M, Cheung RK, Becker DJ (1998) Are islet self-antigens distinguished by their ability to induce T cell anergy or to recruit ZAP70? Diabetes 47 [Suppl 1]: A 804 (Abstract)
6. Dosch H-M, Cheung RK, Pietropaolo M, Becker DJ (1998) Do proinsulin- & IA-2 specific T cells mark progression of diabetic autoimmunity? Diabetes 47 [Suppl 1]: A 805 (Abstract)
7. Durinovic BI, Hummel M, Ziegler AG (1996) Cellular immune response to diverse islet cell antigens in IDDM. Diabetes 45: 795–800
8. Brooks-Worrell BM, Starkebaum GA, Greenbaum C, Palmer JP (1996) Peripheral blood mononuclear cells of insulin-dependent diabetic patients respond to multiple islet cell proteins. J Immunol 157: 5668–5674

Circulating hyaluronan and hyaluronidase are increased in diabetic rats

Dear Sir,

Hyaluronan (hyaluronic acid) is a highly charged high-molecular-mass polyanion of repeating disaccharide units, found at its most in the extracellular matrix of loose connective tissue. Hyaluronan is implicated in various physiological functions including water and protein homeostasis, cell proliferation, cell locomotion and migration. Serum hyaluronan was found to be a marker of several diseases (hepatitis, primary bilary cirrhosis, rheumatoid arthritis, tumours and other diseases affecting connective tissue) [1]. Previous investigations have shown that patients with Type I (insulin-dependent) diabetes mellitus present a relative and absolute increase in hyaluronan in normal tunica media of arteries compared with non-diabetic subjects [2]. The aim of this study was to determine whether circulating hyaluronan was influenced by Type I diabetes in rats. With this information, one explanation for the enhanced deposition of hyaluronan in the tunica-media of diabetic patients could be provided.

To induce diabetes, a single injection of streptozocin (streptozocin crystallized, Boehringer, Mannheim, Germany; 45 mg/Kg body weight) dissolved in normal saline with sodium citrate (0.02 mol/l, pH 4.5) was carried out through the exposed jugular vein. Control rats received an equivalent volume of the dissolving buffer alone. A group of diabetic rats received (once daily between 1700 and 1800 hours during 6 weeks) a subcutaneous injection of a fixed dose (6 U/rat) of a long-acting insulin (Lente MC, Novo, Paris, France) 7 weeks after injection of streptozocin. The number of rats used was 17 for non diabetics, 14 for non-treated diabetics and 12 for diabetics treated with insulin. Animals were killed by exsanguination and blood was collected for serum glucose, insulin and hyaluronan determinations and further analysis when it was necessary. All experiments were conducted in accordance with institutional guidelines and recommendations for the care and use of laboratory animals established by French Ministry of Agriculture (autorization No. 002245). Serum hyaluronan was quantified with an

Corresponding author: Dr. A. Chajara, Université de Médecine-Pharmacie, Laboratoire DIFEMA-MERCI, 22, Boulevard Gambeta, 76000 Rouen, France

Table 1. Characteristics of rats during the experiment and at time of death. Body weight, serum glucose, insulin, alanine aminotransferase, hyaluronan and hyaluronidase

Group	Control	Diabetes	Diabetes + insulin
n	17	14	12
Start of experiments			
Body weight (g)	288 ± 2.3	288 ± 1.6	287 ± 1.6
7 weeks after streptozocin			
Body weight (g)	468 ± 7.8	265 ± 11[a]	267 ± 15[c]
Death (6 weeks after starting insulin)			
Body weight (g)	515 ± 12	258 ± 13[a]	445 ± 5.2[b,c]
Glucose (mmol/l)	7.7 ± 0.31	40 ± 1.6[a]	12 ± 3.6[b]
Insulin (pmol/l)	327 ± 17	38 ± 6.5[a]	264 ± 54[b]
Alanine aminotransferase (U/l)	28.4 ± 1.01	64.9 ± 8[a]	30.9 ± 2.34[b]
Hyaluronan (μg/l)	53 ± 5.6	389 ± 49[a]	73 ± 12[b,e]
Hyaluronidase (mU/l)	732 ± 11	1132 ± 89[a]	794 ± 22[b,d]

Values are means ± SEM. For each variable, Kruskal-Wallis test was used to compare the 3 experimental groups followed by Mann-Whitney U-test. [a] $2p < 0.0001$ refers to differences between control and diabetes. [b] $2p < 0.0001$ refers to differences between diabetes and diabetes + insulin. [c] $2p < 0.0001$, [d] $2p = 0.017$ and [e] $2p = 0.06$ refers to differences between control and diabetes + insulin

enzyme-linked sorbent assay using alkalin phosphatase conjugated sheep brain hyaluronectin. Phosphatase activity was measured by incubation with substrate (nitrophenyl phosphate). The Kruskal-Wallis test was used for multiple comparison followed by Mann-Whitney U test. Differences were regarded as significant at $2p$ less than 0.05.

Diabetic rats had hyperglycaemia, hypoinsulinemia and retarded body weight. These modifications were corrected by insulin (Table 1). Diabetes induced a significant increase in serum hyaluronan concentration ($2p < 0.00001$). This increase was also abolished by insulin treatment (Table 1). Hyaluronan remained, however, higher than in non-diabetic rats ($2p = 0.06$). Since 90% of circulating hyaluronan is taken up from blood by the liver, we verified whether the increased hyaluronan concentration in diabetic rats was a result of dysfunction of hyaluronan uptake from circulation. To respond to this question we determined serum alanine-aminotransferase in rats. We found that the activity of alanine-aminotransferase in serum of diabetics was about two times higher than that of non diabetics ($2p < 0.00001$) and completely restored by insulin treatment (Table 1). As hyaluronidase, the enzyme which degrades hyaluronan, is also removed from circulation by liver, we wanted to know whether its activity in serum was also modified in diabetic rats. Serum hyaluronidase activity was determined by an indirect assay on plastic microtest plates coated with hyaluronan. Briefly, after incubation with samples and standard (human serum), wells were then incubated with hyaluronectin-phosphatase and phosphatase activity was determined using nitrophenyl phosphate. We observed that circulating hyaluronidase was also increased in diabetic rats ($2p < 0.00001$, Table 1). Hyaluronidase activity was restored by insulin treatment but remained significantly higher than in non diabetic rats ($2p = 0.017$).

Our data showed thus that circulating hyaluronan is increased in Type I diabetes. This finding could help to explain why hyaluronan accumulates in arteries of Type I diabetic patients. This could help furthermore to explain the mechanism of diabetic macroangiopathy, as there is little knowledge about the metabolic change in Type I diabetes responsible for the modifications of extracellular matrix components of the arterial wall. Hyaluronan, which forms a peri-cellular zone around arterial cells (endothelial and smooth muscle cells) is known, when it is present in increased amounts, to perturb arterial cell functions. Hyaluronan and hyaluronan-receptors, receptors for hyaluronan-mediated motility (RHAMM), and CD44 influence arterial smooth muscle cells migration and proliferation, both in vitro and in vivo [3, 4]. On the other hand, hyaluronan-oligosaccharides stimulate proliferation of endothelial cells in culture and high-molecular-mass hyaluronan inhibits formation and disturbs pre-existing endothelial cells monolayers [5]. Insulin treatment reduced, however, circulating hyaluronan but it did not completely abrogat its increase in diabetic rats. It is not, therefore, impossible that poorly controlled Type I diabetic patients have increased circulating hyaluronan. Alanine-aminotransferase has been found to be enhanced in Type I diabetic patients (treated with insulin) suggesting that insulin treatment did not prevent abnormal liver function in these patients [6]. On the other hand it has been reported that serum hyaluronan in diabetic patients (receiving insulin or other hypoglycaemic drugs) was significantly enhanced in comparison with non-diabetic subjects [7].

Our study also showed that serum hyaluronidase was enhanced in Type I diabetic rats. Whether the increased concentration of this hydrolase in serum is associated with some damage in diabetes remains to be determined. Involvement of hyaluronidase, as well as hyaluronan, in physiopathological situations has, however, been intensively investigated in tumour development. Evidence was provided that both hyaluronidase and hyaluronan participate actively in tumour development as well as metastasis. It is of interest to note that diabetic patients have been found to be at excess risk of developing primary liver cancer [8].

Yours sincerely,
A. Chajara, M. Raoudi, B. Delpech, M. Leroy, J. P. Basuyau, H. Levesque

References

1. Laurent TC, Laurent UBG, Fraser JRE (1996) Serum hyaluronan as a disease marker. Ann Med 28: 241–253
2. Heickendorff L, Ledet T, Rasmussen LM (1994) Glycosaminoglycans in the human aorta in diabetes mellitus: a study of tunica media from areas with and without atherosclerosis. Diabetologia 37: 286–292
3. Savani RC, Wang C, Yang B et al. (1995) Migration of bovine aortic smooth muscle cells after wounding injury. The role of hyaluronan and RHAMM. J Clin Invest 95: 1158–1168
4. Jain M, He Q, Lee WS et al. (1995) Role of CD44 in the reaction of vascular smooth muscle cells to arterial wall injury. J Clin Invest 97: 596–603
5. West DC, Kumar S (1989) The effect of hyaluronate and its oligosaccharides on endothelial cells proliferation and monolayer integrity. Exp Cell Res 183: 179–196
6. Meltzer AA, Everhart J-E (1997) Association between diabetes and elevated serum alanine aminotransferase activity among mexican americans. Am J Epidemiol 146: 565–571
7. Ceriello A, Giugliano D, Russo D, Passariello N, Saccomanno F, Sgambato S (1983) Glycosaminoglycans in human diabetes. Diabetes Metab 9: 32–34
8. Adami HO, Chow WH, Nyrén O et al. (1996) Excess risk of primary liver cancer in patients with diabetes mellitus. J Natl Cancer Inst 88: 1472–1477