References

1. Tesfaye S, Malik R, Ward JD (1994) Vascular factors in diabetic neuropathy. Diabetologia 37: 847–854
2. Simpson LO (1994) Essential fatty acid treatment of rats with experimental diabetes: comment. Diabetologia 37: 331
3. Simpson LO (1989) Blood from healthy animals and humans contains nondiscocytic erythrocytes. Br J Haematol 73: 561–564
4. Vracko R (1974) Basal lamina layering in diabetes. Evidence for accelerated rate of cell death and cell regeneration. Diabetes 23: 94–104
5. Schoefl GI (1964) Electron microscopic observations on the regeneration of blood vessels after injury. Ann NY Acad Sci 116: 789–802
6. Jamal GA, Carmichael H (1990) The effect of gammalinolenic acid on human diabetic peripheral neuropathy: a double-blind placebo controlled trial. Diabet Med 7: 319–323
7. Cameron NE, Cotter MA, Robertson S (1991) Essential fatty acid diet supplementation: effects on peripheral nerve and skeletal muscle function and capillarisation in streptozotocin-induced diabetic rats. Diabetes 40: 532–539
8. Kury PG, Ramwell PW, McConnell HM (1974) The effect of prostaglandins E_1 and E_2 on the human erythrocyte as monitored by spin labels. Biochim Biophys Res Commun 56: 478–483
9. Rassmussen H, Lake W, Allen JE (1975) The effect of catecholamines and prostaglandins upon human and rat erythrocytes. Biochim Biophys Acta 411: 63–73
10. Horrobin DF (1992) Nutritional and medical importance of gammalinolenic acid. Progr Lipid Res 31: 163–194
11. Simpson LO (1991) Red cell shape in different anticoagulants. Br J Haematol 79: 136–137 (Letter)
12. Simpson LO (1993) The effects of saline solutions on red cell shape: a scanning electron microscope based study. Br J Haematol 85: 832–834

Response from the authors

Dear Sir,

We are most interested in the comments of Dr. L. O. Simpson regarding the importance of intracapillary blood constituents and endothelial cells in the damaging effects of capillary disease in diabetic nerve. We would agree with the opinion that this is probably just as important as the abnormal haemodynamics in the vessels supplying the nerve. In the original studies of sural nerve biopsies where capillary endothelial changes were noted [1–3] many of the vessels showed debris which was undoubtedly of cellular nature with fibrin and in some instances small plugs of deactivated platelets. However, we would agree that the haemodynamic factors within the nerve are extremely difficult to assess in vivo. We would suggest that there is an urgent need for relatively non-invasive methods of measuring nerve blood flow, perhaps as simple as nerve oxygen tension to study the varying effects of the metabolic state and the influence of drugs known to affect nerve blood flow in animals.

Yours sincerely,

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References

1. Timperly WR, Ward JD, Preston FE, Duckworth T, O'Malley BC (1976) Clinical and histological studies in diabetic neuropathy. Diabetologia 12: 237–243
2. Williams E, Timperly WR, Ward JD, Duckworth T (1980) Electronmicroscopical studies of vessels in diabetic peripheral neuropathy. J Clin Pathol 33: 462–470
3. Timperly WR, Boulton AJM, Davies Jones GAB, Jarrat JA, Ward JD (1985) Small vessel disease in progressive diabetic neuropathy associated with good metabolic control. J Clin Pathol 38: 1030–1038

The cumulative incidence of childhood diabetes mellitus in Sweden unaffected by BCG-vaccination

Dear Sir,

Several studies in the diabetes-prone nonobese diabetic (NOD) mouse have shown that even one single injection of either complete Freund's adjuvant (CFA) [1] or BCG-vaccine [2] given at an early age prevented the development of diabetes in this animal model. The mechanism has been indicated to be due to a non-specific stimulation of natural suppressor activity. Recently, Shehadeh et al. [3] reported that CFA and BCG vaccine modulated the development of diabetes mellitus in NOD mice. Furthermore in an open clinical trial in 17 newly-diagnosed insulin-dependent diabetes mellitus (IDDM) patients intracutaneous administration of 0.1 ml BCG-vaccine (1 mg/ml) led to a clinical remission more frequently when compared to non-treated control subjects. Based on these indications several large-scale placebo-controlled trials have been started in diabetic humans including children with primary prevention in healthy children as a goal.

In Sweden, since 1 July 1977 we have continuously registered all childhood-onset diabetic cases with a level of ascertainment close to 99 % [4]. Before 1975 all newborn babies in Sweden were offered BCG-vaccination (using the dose given above) in the first month of life and the coverage of this vaccination programme was almost complete [5, 6]. Due to side effects, in some cases severe complications, this policy was stopped on 1 April 1975. Since then only high-risk groups such as immigrant children or children with a close relative with tuberculosis have been vaccinated. In 1976 only 0.6 % were vaccinated [5] and between 1976–1980 less than 2 % were vacci-

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nated [6]. When comparing cumulative incidence rates of IDDM up to 15 years of age between birth cohorts 1973, 1974 and 1976, 1977 (Fig. 1) there is clearly no significant difference. As can be seen, the cumulative incidence was slightly lower during 1974 whereas 1973, 1976 and 1977 are almost identical. Since the incidence registration of childhood diabetes started on 1 July 1977 cumulative incidence rates in children with disease onset before the age of 4 years could not be compared. The proportion of diabetic patients in these age-groups is, however, very low, between 10–15 % (e.g. 46/367 in 1977). An effect of BCG vaccination on the incidence of IDDM in very young-onset diabetic patients thus could not be excluded but would not account for a large proportion of cases.

We conclude that, on a population basis, BCG-vaccination in newborn children seems to have no significant effect on the incidence of childhood-onset IDDM and therefore would not offer an effective primary prevention strategy.

Sincerely yours,
G. Dahlquist, L. Gothefors

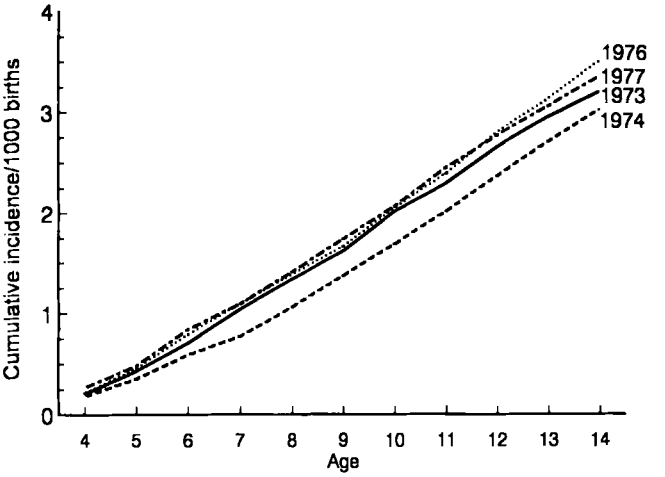

Fig. 1 Cumulative incidence of childhood IDDM in Sweden in children 4–15 years born in 1973 (number of diabetic children = 345/107,582 newborns) 1974 (number of diabetic children 329/108,671 newborns) 1976 (number of diabetic children = 342/97,327 newborns) and 1977 (number of diabetic children 320/95,098 newborns)

References

1. Sadelain MWJ, Qin H, Lanzon J, Singh B (1990) Prevention of type 1 diabetes in NOD mice by adjuvant immunotherapy. Diabetes 39: 583–599
2. Havada M, Kishimoto Y, Makino S (1990) Prevention of overt diabetes and insulitis in NOD mice by a single BCG vaccination. Diabetes Res Clin Pract 8: 85–89
3. Shehadeh N, Calcinaro F, Bradley BJ, Brunchlim J, Vardi P, Lafferty KJ (1994) Effect of adjuvant therapy on development of diabetes in mouse and man. Lancet 343: 706–707
4. Dahlquist G, Blom L, Tuvemo T, Nyström L, Sandström A, Wall S (1989) The Swedish childhood diabetes study – results from a nine-year case register and a one year case-referent study indicating that IDDM is associated with both NIDDM and autoimmune disorders. Diabetologia 32: 2–6
5. Ericson A, Gunnarskog J, Gustavsson D, Källén B, Malker B (1983) BCG vaccination and child cancer in Sweden. In: Crispen RG (ed) Cancer. Elsevier, Amsterdam, pp 411–417
6. Romanov V, Svensson Å, Hallander HO. The impact of changing BCG coverage on tuberculosis incidence in Swedish-born children between 1969 and 1989. Tubercle and Lung Disease 1992; 73: 150–61

Shared amino acid sequences between glutamic acid decarboxylase 65 and 67 and alpha-2-macroglobulin. A focus for cross-reactive autoantibodies?

Dear Sir,

According to the theory of molecular mimicry as an explanation for the development of autoimmune disease, the existence of identical or very similar amino acid sequences between environmental agents (e.g. viruses) and 'self' antigens leads to the immune system cross-reacting against self antigens, hence triggering autoimmunity [1]. In view of the fact that peptides presented by HLA class II molecules are frequently ubiquitous self proteins, we have suggested that mimicry between these and motifs associated with known autoantigens could also trigger autoimmune reactions [2]. In connection with such considerations, we recently noted that a plasma protein, alpha-2-macroglobulin, contains an amino acid sequence bearing a significant homology to the two forms of glutamic acid decarboxylase (GAD_{65} and GAD_{67}) which are well-defined antigens recognised by autoantibodies in insulin-dependent diabetes mellitus (IDDM) [3]. These sequences are PEVKSK on alpha-2-macroglobulin and PEVKEK and PEVKTK on GAD_{65} and GAD_{67}, respectively. Moreover, the immediately succeeding sequences (AIGYL, GMAAL and GMAAV, respectively) would correspond in the main to conservative substitutions. Alpha-2-macroglobulin is an inhibitor of low molecular weight proteases such as trypsin and plasmin [4] and its levels have been reported to be increased in diabetes [5]. These properties of alpha-2-macroglobulin, coupled with the sequence homology, raises an intriguing question of whether T-helper cells, recognising an epitope of alpha-2-macroglobulin presented by HLA class II molecules may also, under certain conditions, respond to GAD, the autoantigen implicated in the autoimmune destruction of beta cells [6]. As an initial test of this hypothesis, we set out to determine whether sera from patients with IDDM have autoantibodies reacting with alpha-2-macroglobulin. We employed an ELISA system established and optimised using polyclonal rabbit anti-human alpha-2-macroglobulin antiserum. Microtitre plates were coated with 200 ng/ml of purified alpha-2-macroglobulin and reacted with five normal sera and ten sera from patients with high titres of anti-GAD antibodies (126 to 212 arbitrary units [7]) followed by reaction with 1 in 6000 dilution of anti-human IgG conjugated to horseradish peroxidase. The substrate used was *o*-phenylenediamine dihydrochloride dissolved in phosphate-citrate buffer containing sodium perborate and the optical density was read in a microplate reader at 490 nm. We found no specific reactivity of these "high titre" sera with alpha-2-macroglobulin as compared to normal sera. Pre-absorption of two sera with the

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