Short Communication

Circulating anti-pericyte autoantibodies are present in Type 2 diabetic patients and are associated with non-proliferative retinopathy

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Abstract

Aims/Hypothesis. This study aims to determine the prevalence of anti-pericyte autoantibodies in Type 2 diabetes and to characterize these autoantibodies as new markers of disease activity in diabetic retinopathy.

Methods. A total of 299 patients with Type 2 diabetes participated in this study. Retinopathy was assessed by 7-field stereo fundus photography and was graded according to the ETDRS scale. Serum anti-pericyte autoantibodies were detected by immunofluorescence on tissue cultured bovine retinal pericytes.

Results. The prevalence of anti-pericyte autoantibodies in Type 2 diabetic patients was 54% and was approximately equal in men and women. The prevalence was approximately 55% with retinopathy at grades

from 10 to 53. At grades above 53 the prevalence declined to 23% ($p < 0.0001$). The highest prevalence by duration of diabetes, 70%, was found at 0 to 5 years and the lowest, 25% at more than 25 years duration ($p < 0.0001$).

Conclusion/interpretation. Anti-pericyte autoantibodies are present at high prevalence in Type 2 diabetes. Their presence during earlier stages of retinopathy could be due to a reaction with antigens expressed by “activated” pericytes. The decline in antibody prevalence in advanced retinopathy could mark pericyte loss and progression to an angiogenic retinal milieu. [Diabetologia (2003) 46:511–513]

Keywords Pericytes, diabetes mellitus, diabetic retinopathy, autoimmunity, retinal diseases, capillaries.

Although it has been shown that tight metabolic control has beneficial effects on the development and progression of diabetic retinopathy in Type 1 and Type 2 diabetes, the increase of blood glucose concentrations does not account for all the risk for development of and progression to sight threatening retinopathy. It is therefore

possible, in subjects with a certain permissive genetic background, that mechanisms in addition to glucotoxicity can be induced by the initial glucotoxic insult.

We have previously found anti-pericyte autoantibodies at high prevalence in sera of Type 1 diabetic patients [1]. In this study we report on the existence of anti-pericyte antibodies in sera from a cohort of Type 2 diabetic patients.

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Abbreviations: ETDRS, Early treatment of diabetic retinopathy study.

Subjects and methods

Subjects. A total of 299 Type 2 diabetic patients attending the Department of Ophthalmology, Malmö University Hospital, Sweden, gave their informed consent to participate in the study, which was approved by the Ethics Committee of Malmö, Lund. The investigations reported were carried out in accordance with the principles of the Declaration of Helsinki as revised in 1996 [2].

Data on age at diagnosis of diabetes mellitus and duration of diabetes were collected. Two patients were excluded due to known HIV or Hepatitis C positivity. Patients on insulin treatment who were younger than 30 years of age at diabetes diagnosis were considered as having Type 1 diabetes, others as having Type 2 diabetes.

Control sera from 24 donors from the Red Cross, reporting no family history of diabetes, were used as negative controls. The characteristics of the donors were reported previously [1].

Ophthalmological evaluation. Visual acuity was tested using ETDRS charts. After dilation of the pupils, stereo photographs were taken from seven standard fields in each eye, using a 30° fundus camera (Topcon, TRC-50).

Grading was done in a masked fashion by an experienced ophthalmologist (EA). The retinopathy stage was graded according to the ETDRS scale [3]. Eyes treated with pan-retinal photocoagulation without signs of neovascularization were included with those graded as grade 61. The patients were characterised according to the retinopathy grade in the worst affected eye. Due to cataract or insufficient pupil dilation, the quality of the photographs permitted grading of one eye in 16 patients only. For the same reasons, grading of any eye was not possible in seven patients.

Indirect immunofluorescence assay. Anti-pericyte autoantibodies were detected by immunofluorescence as described previously [1] with minor modifications. Bovine retinal microvascular pericytes were isolated, tissue cultured and characterized as described previously [1, 4]. Near confluent early passage pericytes (passage number 1–3) were stained by indirect immunofluorescence after cells were grown in poly L-Lysine coated 16-well Lab-Tek chamber slides (Nalge Nunc, Naperville, Ill., USA). Pericyte cultures were fixed in phosphate buffered formalin (3.7%, 200 µl/well) (Sigma-Aldrich, St. Louis, Mo., USA) containing 2 mmol/l CaCl₂ at pH 7.2 for 1 h. The fixed cells were then washed with PBS three times before incubation with sera. Sera were diluted 1:100 in DMEM containing 1% BSA and 100 µl overlaid onto the cells and incubated for 30 min at room temperature. The cells were then washed 3x with DMEM and 100 µl of rhodamine conjugated goat anti-human IgG(Fc) (diluted 1:20) was overlaid onto the cells for 30 min. The cells were washed as before and fixed again in 3.7% buffered formalin before mounting in Gel Mount (Biomed Corp, Foster City, Calif., USA) under a cover slip which was then sealed with nail polish and viewed with a fluorescence microscope. Staining was visually scored as reported previously [1].

Statistical analysis of the anti-pericyte autoantibody prevalence stratified by ETDRS grade was done with 2x2 contingency tables analysed using Fisher's exact test with Yates' correction. The *p* values reported are two tailed. The statistical analysis was carried out using Instat software (GraphPad Software, San Diego, Calif. USA). A *p* value of less than 0.05 was considered to be statistically significant.

Results

Sera from patients with varying degrees of diabetic retinopathy were screened for the presence of anti-pericyte autoantibodies by immunofluorescence microscopy. In addition to the staining patterns that we have reported [1], we found additional patterns that had the appearance of staining of forward moving membrane processes and cellular processes that were forming inter-cellular membrane contacts (Fig. 1).

Fig. 1A, B. Fluorescence photomicrographs of immunofluorescent detection of serum anti-pericyte autoantibody binding to bovine retinal pericytes in vitro. **A** Immunostaining with control donor serum. **B** Positive immunostaining with a diabetic patient's serum

The prevalence of anti-pericyte autoantibodies in men with Type 2 diabetes was 52% (90/173) and in women it was 57% (68/120), *p*=0.4753. Two patients were excluded and no autoantibody assay result was obtained for four patients.

The anti-pericyte autoantibody prevalence according to retinopathy grade in Type 2 diabetic patients was as follows (Fig. 2): grade 10–15, 57% (69/122); grade 20–43, 64% (47/73); grade 47–53, 58% (23/40); grade 61–65, 35% (15/43) and grades above 65, 25% (2/8). Of the 299 patients seven were not gradable, four had no autoantibody assay result and two were excluded. Anti-pericyte autoantibodies were not detected in any of 24 control sera screened. Statistical comparisons that achieved significance were as follows: ETDRS grade 10–15 vs 61–65, *p*=0.0206; 20–43 vs 61–65, *p*=0.0036; 47–53 vs 61–65, *p*=0.0487.

Fig. 2. Anti-pericyte autoantibody prevalence stratified by severity of retinopathy

Antibody prevalence was also stratified by duration of diabetes in 5-year blocks. At 0 to 5 years of diabetes duration, the antibody prevalence was 71% (58/82), at 6 to 10 years it was 40% (18/45), at 11 to 15 years it was 57% (36/63), at 16 to 20 years the prevalence was 51% (21/41), at 21 to 25 years duration the prevalence was 50% (16/32) and at a duration of 25 years or longer the prevalence was 29% (8/28). Two patients were excluded, four had no autoantibody assay result and two were of unknown duration of diabetes. Statistical comparisons of anti-pericyte autoantibody prevalence stratified by a duration of diabetes that reached significance were as follows: 0–5 vs 6–10, $p=0.0012$; 0–5 vs 16–20, $p=0.0457$; 0–5 vs 21–25, $p=0.0495$; 0–5 vs >25, $p=0.0001$; 11–15 vs >25, $p=0.0136$. The difference in autoantibody prevalence between the 0 to 5 year duration group and all the other groups was, in each case, statistically significant except 0–5 vs 11–15 years.

Discussion

The prevalence of anti-pericyte autoantibodies in Type 2 diabetic patients in this study was similar to the prevalence reported previously in Type 1 diabetic patients, i.e. approximately 50 to 60% [1]. This high prevalence was found among patients with diabetic retinopathy up to ETDRS grade 53. At retinopathy levels above ETDRS grade 53 the prevalence of autoantibody declined precipitously suggesting that conversion from an autoantibody positive state to an autoantibody negative state could indicate impending proliferative disease. Sero-conversion could be a marker or a consequence of changes in the retinal microenvi-

ronment that cause down-regulation of autoantibody production (i.e. change in cytokine levels or profiles).

Because pericytes have been shown to negatively regulate endothelial cell proliferation through a paracrine mechanism that depends on cell to cell contact between pericytes and endothelial cells, it is widely thought that pericyte dysfunction or loss is a necessary prerequisite for the future development of capillary proliferation [5]. Consequently, the presence of circulating anti-pericyte autoantibodies in earlier stages of diabetic retinopathy is consistent with autoantibody binding to antigens that are preferentially expressed by “activated” pericytes. Thus, the presence of these autoantibodies could indicate an alteration of pericyte phenotype due to the effects of diabetes on the retinal capillary.

Stratification of pericyte autoantibody prevalence by duration of diabetes showed peak prevalence at a duration of 0 to 5 years. This is different from our previous finding of a peak at 6 to 10 years duration in Type 1 diabetic patients [1]. This difference is most probably an artifact generated by imprecision in determining the onset of Type 2 diabetes. At the time of diagnosis, many Type 2 diabetic patients have had the disease for several years [6], this leads to inaccurate estimates of diabetes duration and skew the peak of anti-pericyte autoantibody prevalence to an apparently earlier appearance in the time course of the disease. Consequently, the effect of diabetes duration on the prevalence of anti-pericyte autoantibodies is likely to be similar in Type 1 and Type 2 diabetes.

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