Diabetologia (1993) 36: 682–685

**Diabetologia**


# *Letters to the editor*

## Early insulitis and the islet vascular system

Dear Sir,
De Paepe et al. [1], using a technique based on high resolution protein-A gold cytochemistry, found increased vascular permeability in diabetic Bio Breeding rat islets. In their introduction it was stated with reference to Papaccio et al. [2] that "The vascular damage noted in the islets of animals with streptozocin-induced diabetes is most likely secondary to the islet cell necrosis caused by the beta cytotoxic agent and may, therefore, not be related to diabetes per se". We must disagree with this and we think that an explanation is necessary.

Firstly in our experiments we used a morphological and not a functional approach as stated in their article. Moreover, we have clarified the exact time-points when a significant decrease of the total vascular bed occurs in low-dose streptozotocin treated mice [2, 3]; we stressed that this decrease is concurrent to margination of phagocytic monocytes followed by diapedesis across the vascular wall and transformation into tissue macrophages [4, 5]. At this time islet beta cells were still intact or only initially damaged by the direct streptozotocin cytotoxic action [6]. These data are not consistent with the thought that vascular damage follows islet beta-cell necrosis and loss in this animal model.

Vascular damage occurs later and could be ascribed, apart from beta-cell necrosis, to the formation of extended areas of oedema [3], which may be responsible for further vascular derangement and beta-cell destruction, in a vicious circle.

The islet vascular system and its involvement in early insulitis and beta-cell lysis is an area of increasing interest in Type 1 (insulin-dependent) diabetes research and could help the understanding of the mechanisms responsible for selective islet beta-cell destruction.

Yours sincerely,
G. Papaccio and G. Chieffi Baccari

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## Response from the authors

Dear Sir,
We read with great interest the comments made by Pappaccio and Chieffi Baccari. As we did not have the benefit of the authors' recent or unpublished results at the time, we indeed suggested in our manuscript that the vascular alterations noted in streptozotocin-treated animals might be a consequence of the often extensive islet inflammation caused by this beta-toxic agent [1]. Recent findings, however, shed more light on the chronology of histologic changes witnessed in low-dose streptozotocin-induced diabetes [2, 3].

The early stages of disease of the two animal models of human Type 1 (insulin-dependent) diabetes mellitus under discussion – the spontaneously diabetic BioBreeding rat and the low-dose streptozotocin-treated mouse – seem to have many features in common. In both models, macrophage infiltration of the islets is one of the earliest, and presumably essential events in the course of the disease which occurs prior to the development of insulitis and/or beta-cell damage [2, 4]. Furthermore, around that same time the microvasculature in and around the pancreatic islets in both animals undergoes various alterations, such as vasoconstriction ([3], streptozotocin) and increased vascular permeability ([1], BB).

In our report [1], which is to be seen primarily as the introduction of a novel, high resolution method with which to study the islet microvasculature, we did not elaborate on the possible mechanisms of the observed increase in vascular permeability in pre-diabetic BB rats. Although conclusive evidence is lacking, several observations made during this study suggest, however, the existence of a functional relationship between the islet microvasculature and macrophages in the early stage of disease. In the pre-diabetic BB rats mononuclear phagocytes were frequently observed within the microvasculature and parenchyma of the islets and the surrounding acinar tissue. In extravascular locations, the mononuclear cells often displayed the ultrastructural features of activated macrophages (cellular protrusions, expanded endoplasmic reticulum, endocytic vesicles and a large euchromatic nucleus). With pre-embedding immunostaining, we were able to demonstrate strong immunoreactivity for Ia (class II MHC) antigens on macrophages in the acinar

tissue surrounding the islets, which again reflects the state of activation of the macrophages. Finally, the vascular leakage was most prominent in the post-capillary venules, site of action of vasoactive monokines.

The microvascular changes observed early in the disease process may have important clinical implications. Indeed, it has been demonstrated that administration of serotonin and histamine inhibitors suppresses the development of diabetes in low-dose streptozotocin-treated mice [5]. These and other findings suggest the role of the pancreatic microvasculature, and possibly its interaction with mononuclear phagocytes, in the pathogenesis of diabetes, and we hope that our contribution may prove useful for further investigations in this field.

Yours sincerely,
M. E. De Paepe and E. Colle

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# Follow-up of anti-beta-lactoglobulin antibodies in children with Type 1 (insulin-dependent) diabetes mellitus

Dear Sir,
Recently Dahlquist et al. [1] reported that early exposure to cow's milk formula is related to an increased risk of early-onset Type 1 (insulin-dependent) diabetes mellitus. The authors found increased beta-lactoglobulin IgA antibodies and cow's milk protein IgA antibodies in children with early-onset Type 1 diabetes and suggest that early exposure to beta-lactoglobulin in genetically susceptible children might be one trigger in the autoimmune process leading to development of Type 1 diabetes. We studied 26 children (14 males and 12 females) aged 1.4–16.2 years, with recently-diagnosed Type 1 diabetes followed-up for 2–5 years. Nine patients had been breast-fed and 17 bottle-fed. In all patients we evaluated IgA and IgG antibodies to beta-lactoglobulin by ELISA. The control group comprised 59 healthy age- and sex-matched subjects. At diagnosis, 13 patients (50%) had elevated beta-lactoglobulin IgA (> +2SD) and 2 (7.7%) elevated beta-lactoglobulin IgG antibodies (> +2SD). ICA were present in 19 of 26 (73%) and anti-insulin antibodies in 6 of 26 (23%) patients.

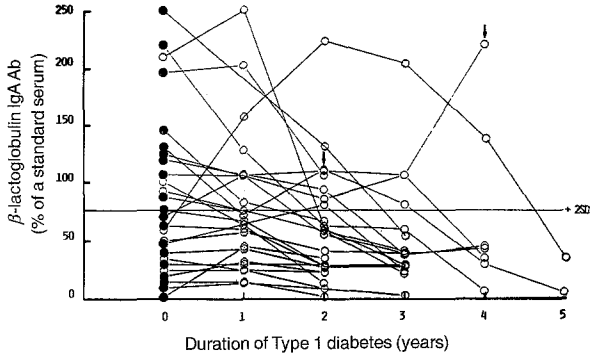


**Fig. 1.** Beta-lactoglobulin IgA antibody (IgA-ab) levels in 26 children with Type 1 diabetes at diagnosis and during 2–5-year follow-up. ● ICA-positive patients; ○ ICA-negative patients; ↓ the two patients with elevated beta-lactoglobulin IgA-antibody levels at the end of the follow-up. The line at 75% indicates 2SD

Over the 2–5-year follow-up we observed a transient increase in beta-lactoglobulin IgA antibodies in two other patients. At the end of follow-up beta-lactoglobulin IgA antibodies decreased to normal levels in all but two females (Fig. 1) and beta-lactoglobulin IgG antibodies in all patients. No correlation was found between beta-lactoglobulin IgA or IgG antibodies and age, breast-feeding duration, islet-cell, anti-insulin or other organ and non-organ specific antibodies and HLA types. No difference was found in beta-lactoglobulin antibody levels between breast- or bottle-fed patients.

Some investigators have suggested that breast-feeding may protect against the risk of Type 1 diabetes in later life [2–4], but the results of more recent studies are conflicting [5]. It has been hypothesized that intestinal permeability is increased in Type 1 diabetes [6]. On the other hand a strong antigenic similarity has been observed between human beta-casein and bovine beta-lactoglobulin [7] and the presence in human milk of beta-lactoglobulin from cow's milk in the mother's diet has been confirmed [8]. These observations together with our findings that anti-beta-lactoglobulin antibodies tend to disappear during follow-up, suggest that they represent only a transient abnormal immunological response. These antibodies may reflect increased production of antibodies against different antigens by B-lymphocyte clones [9].

Yours sincerely,
R. Lorini, M. A. Avanzini and L. Vitali

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