highest titre of anti-GAD antibodies using 1 μg/ml of the purified alpha-2-macroglobulin did not reduce the optical density obtained and thus confirmed that no antibody cross-reactivity between GAD and alpha-2-macroglobulin could be demonstrated using our assay. We therefore conclude that most probably autoantibodies against alpha-2-macroglobulin are not present in patients with IDDM despite the fact that there exists a degree of sequence homology between alpha-2-macroglobulin and the major target antigen of this disease and the fact that alpha-2-macroglobulin levels are increased in this condition. Nevertheless, it remains to be established whether the homologous sequence in alpha-2-macroglobulin is a T-cell epitope, since IDDM is a T-cell mediated autoimmune disease [8].

Yours sincerely,
M. N. Norazmi, M. Peakman, D. Vergani, H. Baum

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Reply to Norazmi et al.

Dear Sir,
The letter from Norazmi et al. points to a remarkable homology in amino acid sequence between a normal plasma protein, alpha-2-macroglobulin, and the two isoforms of glutamic acid decarboxylase (GAD), an enzyme which has been proposed as the autoantigen that "drives" the autoimmune attack on islet beta cells. The implication of the sequence homology is based on a variant of the molecular mimicry hypothesis recently elaborated by the authors [1].

No objection can be raised against the sequence homology which is clear both for the hexapeptide proposed and for the flanking sequences. However, to hold this homology responsible for the triggering of autoimmune responses leading to insulin-dependent diabetes mellitus would imply a series of immune deviations which in our opinion are improbable:
1) Development of tolerance to ubiquitous autologous proteins is assumed to be a default mechanism both for B cells in bone marrow and for T cells in the thymus. T-cell autoreactivity to peptides which normally occupy the presenting cavities of HLA molecules in antigen presenting cells (APCs) would be prevented because – being ubiquitous – they should be presented by thymic dendritic cells and T cells capable of recognizing them would be duly deleted. A failure in this process leading to the escape to periphery of T cells reacting to such common antigens can only be due to a central defect in T-cell development and the expected results would be a lupus-like situation as seen in the *lpr* mice who have a defect in apoptosis [2].
2) T cells do not recognize antigenic peptides just because they happen to occupy the presenting cavities of HLA molecules. Peptides have to be presented in the appropriate context; i.e., T cells become activated only when second signals and cytokines are capable of overrunning their dominant trend towards anergy. Passive uptake of extracellular proteins is an inefficient mode of entry into the class II antigen processing pathway. Effective presentation of antigens will depend on endocytosis after binding to a cell surface receptor that is selectively expressed on APCs. It is therefore unlikely than a normal plasma protein would be actively presented in the periphery. Moreover, when presenting cells become activated one would expect that normal peptides present in "quiescent" APC would be displaced by peptides derived from processing of foreign antigens [3].
3) Nevertheless, if alpha-2-macroglobulin reactive T cells would escape to the periphery and they happen to encounter appropriate presentation of the relevant peptide, the most unlikely target cells would then be the pancreatic beta cells. For that to happen we would have to assume a microenvironment which specifically favours the recruitment of enough T cells to the pancreas in addition to providing appropriate antigen presenting conditions in the site.
4) Once we have the peptide-specific T cells in the pancreas, GAD peptide recognition should then lead to destruction of beta cells. However, we have found that GAD is also expressed in the alpha and delta cells of the pancreatic islets [4] which are not destroyed in the diabetic process. In addition to that, we have also observed that GAD remains expressed in the islets of diabetic patients even 10 years after the clinical onset of the disease and that occurs without any persisting sign of inflammation or autoimmune attack [5].

We therefore consider the antigen mimicry between self molecules as an unlikely cause of autoimmunity. In any case a more localized event such as beta cell specific virus infection should happen at the same time for the proposed mechanism to have some probability of occurring. We also tend to believe that immunization to GAD may be, at least in humans, a secondary phenomenon, as are many humoral autoimmune responses.

Sincerely yours,
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Absence of effect of culture duration on glucose-activated alterations in intracellular calcium concentration in mouse pancreatic islets

We would like to comment on the recent report by Gilon et al. [1] regarding their findings with cultured mouse islets.

The rise in intracellular calcium ($[Ca^{2+}]_i$) induced by glucose in islet of Langerhans beta cells, and its' linkage to insulin secretion is firmly established [2]. Based on our own and other studies, a highly reproducible triphasic pattern of changes in $[Ca^{2+}]_i$ occurs in mouse islets in response to raising the extracellular glucose concentration. These comprise an initial reduction in $[Ca^{2+}]_i$, as in "These conprize on initial reduction in $[Ca^{2+}]_i$ [3, 4], which we have termed phase 0, due to the activation of endoplasmic reticulum Ca^{2+}-ATPase-dependent sequestration of Ca^{2+} [5], a transient rise in $[Ca^{2+}]_i$ (phase 1) caused by a combination of Ca^{2+} store mobilization [3, 6] and Ca^{2+} store-depletion activated Ca^{2+} influx [7, 8]) and regular oscillations in $[Ca^{2+}]_i$ (phase 2), that vary in frequency from 0.5–3 min^{-1}, and primarily represent Ca^{2+} influx [3, 7–11]. Recently, Gilon et al., have reported that culturing mouse islets for as little as 3 days causes a gross alteration in this pattern of Ca^{2+} changes; the phase 0 decreases in magnitude and duration and the distinct phase 1 transient and phase 2 oscillations are replaced by a sustained Ca^{2+} rise [1]. No explanation for these changes in responsiveness was offered.

Our studies have failed to reproduce any aspect of these results: in our original description of the triphasic alterations in $[Ca^{2+}]_i$ to glucose [3], we studied islets cultured from periods ranging from 6–12 days and obtained results qualitatively equivalent to the acute/short-term culture (i.e. less than

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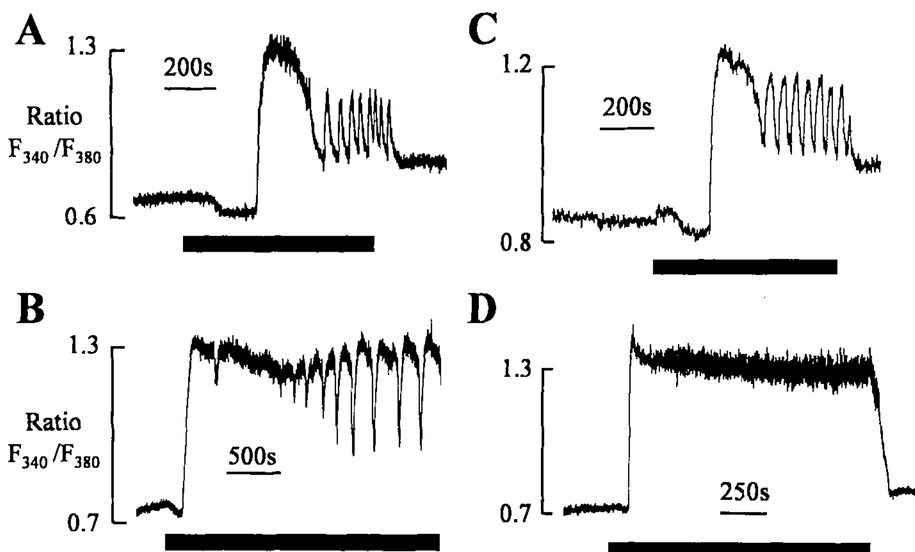

Fig. 1. (A–D) Lack of influence of culture duration on the changes in $[Ca^{2+}]_i$ occurring in mouse islets stimulated by an increase in glucose. The islets were cultured in RPMI 1640 medium containing 11.6 mmol/l glucose, 100 μU/ml penicillin and 100 μg/ml streptomycin for up to 14 days. They were then loaded with fura-2, transferred to the recording chamber, and perifused with a medium containing 2 mmol/l glucose for about 10 min before challenging with 12 mmol/l glucose (filled bar). More complete experimental details appear elsewhere [3, 5, 7, 8, 12, 14]. Shown in the upper panels are the responses following long-term (10 day, **A**) and short-term (2 day, **C**) culture of islets isolated from C57BL/6J and C57BL/KsJ mice, respectively. The lower panels show the responses following long-term (7 day, **B**) and short-term (1 day, **D**) culture of islets isolated from C57BL/6J-ob/ob (ob/ob) and C57BL/KsJ-db/db (db/db), respectively. Note that the only responses that resemble those reported by Gilon et al. [1] are those from the obese hyperglycaemic ob/ob and diabetic db/db islets, and that these responses are independent of culture duration. Control animal islet $[Ca^{2+}]_i$ responses to 12 mmol/l glucose are unaffected by long-term culture