by tubular epithelial cell in tubulointerstitial damages (unpublished observation) were noted.

From above observations, we concluded that in DN, both glomerulosclerosis and tubulointerstitial damage were partly caused as a result of increased synthesis of interstitial type III collagen and type IV basement membrane collagen.

Sincerely yours,
M. S. Razzaque, T. Taguchi

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Assignment of islet cell antibody titres and selection of subjects for IDDM prevention trials

Dear Sir,
Despite the substantial improvement achieved in the interlaboratory standardization of islet cell antibody (ICA) detection by international quality control programmes, problems may arise in the assignment of ICA titres depending on the time of incubation used.

We studied different ICA-positive samples in order to assess possible discordances in ICA titres between long and short incubation assays. Nineteen samples previously considered as positive and validated in International Diabetes Workshops (IDW) [1], were used in the study. Seven samples were previously assigned to 10 Juvenile Diabetes Foundation units (JDF), 6 to 20 JDF units and 6 to 80 JDF units. ICA were determined by indirect immunofluorescence using human pancreas group 0. Long incubation was performed for 18 h using samples diluted with phosphate buffered saline (PBS) plus aprotinin at 4 °C [2]; short incubation lasted 30 min and was performed at room temperature with undiluted samples as described [3]. After incubation, the procedure was the same for both methods, including two washings with PBS and the use of goat anti-human IgG (Kallestad, TX USA). Three positive controls (5, 10 and 20 JDF units) and one negative control were included in each experiment. Samples were evaluated by two independent readers. Results are shown in Figure 1 and indicate that while no discordance was observed for samples showing high titres (80 JDF units), in about 60 % of those samples with titres assigned to 10–20 JDF units by the long incubation procedure, a decrease in the subsequent dilution endpoint or even a negativization was observed when assayed with the short incubation protocol. Although the reason for this phenomenon is unknown, it is possible that it may be due to the heterogeneous nature of the ICA, with differing affinities of the IgG for their antigens. At short incubation, a serum with low titre may interact with less avidity with islet antigens, leading to a decreased immunofluorescent pattern when compared to the long incubation procedure; longer incubation time allows sufficient interaction for a more stable ICA-islet antigen binding resulting in a higher immunofluorescent pattern when compared to short incubation [4]. It is interesting to note that this decrease in titre assignment is seen in a substantial percentage of samples around 20 JDF units, but not in all of them, confirming the aforementioned heterogeneous and policlonal nature of ICA. The relevance of this phenomenon is that ICA titres are considered to be an important parameter for insulin-dependent diabetes (IDDM) prediction [5]. Furthermore, cut-off titres at 20 JDF units may be critical in order to consider the inclusion of ICA-positive individuals in trials aiming to prevent IDDM development. Careful evaluation of the chosen ICA method may be taken into account at the time of consideration of ICA at low titres.

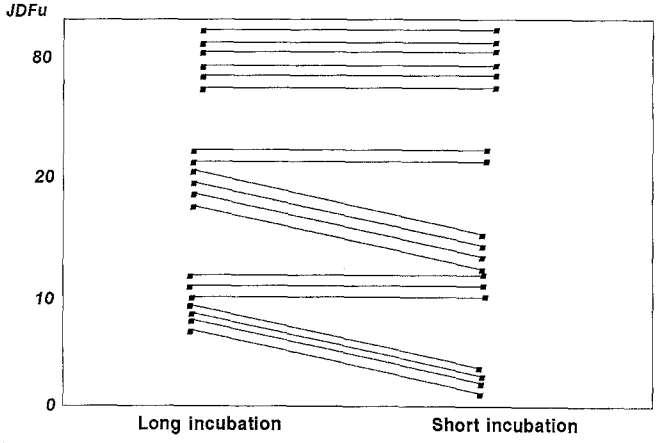

Fig. 1. Differences in ICA titre assignmenitin reference to incubation time

Sincerely yours,
J. Morales, M. Puig-Domingo, D. Mauricio, A. De Leiva

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