Islet Cell Antibodies

Light at the end of the tube?

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The study by Chaillous et al. (1) in this issue analyzes the relationships between antibodies to several islet antigens, measured by different assays, in sera from 95 newly diagnosed insulin-dependent diabetes mellitus (IDDM) patients with a median age of 23 years. Most of the findings have previously been published by others, and there are few surprises for the cognoscenti of islet autoantibodies. Nevertheless, this is a combined study on a sizable group of newly diagnosed IDDM subjects, and as such, it is a useful vehicle for delivering the message to a wider diabetes audience, which may justifiably be confused by the burgeoning literature in this area.

Conventional islet cell antibodies (ICAs) detected on frozen sections of a human group O pancreas were compared with ICAs on frozen sections of Balb/c mouse pancreas and with antibodies to the molecular weight 64,000 (64K) islet antigen and antibodies to glutamic acid decarboxylase (GAD). 64K antibodies were detected by their ability to precipitate a specific 64K protein from a Triton X–114 extract of [^{35}S]methionine-labeled 7-day-old Wistar rat islets. GAD antibodies were detected by their ability to precipitate GAD enzymatic activity from an aqueous extract of adult female Wistar rat brain.

Chaillous et al. (1) substantiate previous evidence that ICAs are heterogeneous (2,3) and comprise at least two subtypes, namely, GAD and non-GAD antibodies (4–6). ICAs reactive with human pancreas differed from ICAs reactive with mouse pancreas in that they correlated with 64K antibodies and GAD antibodies, were blocked (six sera) by adsorption with rat brain homogenate, and remained elevated in the first year after clinical diagnosis of IDDM. Of the 63 of 95 (66%) sera that were ICA positive on human pancreas, 61% were reactive with mouse pancreas. Intriguingly, there was no difference between ICA titers on human pancreas of sera that did or did not react with mouse pancreas. Six sera negative on human pancreas reacted with mouse pancreas. Thus, the ICA assay on human pancreas was more sensitive, presumably in part because it also detected antibodies to GAD (see below). However, the degree to which GAD antibodies contributed to ICAs on human pancreas was not clearly defined.

Despite the plethora of antibodies to different islet antigens, ICAs remain the "gold standard" in terms of sensitivity, specificity, and predictive value for IDDM (7). Yet, paradoxically, the chemical and physical nature of the ICA antigen(s) remains an enigma. The view that GAD accounts for some of the ICA reactivity on human but not mouse pancreas is upheld by recent data on the tissue distribution and immunoreactivity of the two major GAD forms. Human islets express predominantly the molecular weight 65,000 form or GAD_{65}, mouse islets the molecular weight 67,000 form or GAD_{67}, and rat islets both forms (5,6,8,9). Antibodies to GAD in humans with or "at-risk" of IDDM recognize predominantly GAD_{65} (10–12). The fact that GAD_{65} detects 64K antibodies (8) no doubt accounts for the significant association found by Chaillous et al. (1) between GAD antibodies and 64K antibodies. Indeed, the rather serendipitous discovery that the 64K antigen is GAD (13) came about because people with the rare neurological disorder stiff-man syndrome were found to have GAD antibodies, an increased risk of IDDM, and strong positive ICAs on human pancreas (14).

Although they found that ICAs on human pancreas correlated with GAD and 64K antibodies, direct proof that GAD_{65} is one of the target antigens of ICAs on human pancreas was limited to showing that titers of ICAs on human but not mouse pancreas were reduced after six IDDM sera were incubated with rat brain homogenate. The specificity of this effect with crude brain homogenate must be questioned. Brain shares a variety of protein and non-protein molecules with islets, some of which in addition to GAD might be targets of autoantibodies in IDDM. Blocking or adsorption with purified native GAD is required to prove that GAD accounts for the positivity, albeit in part, of ICAs on human pancreas. On the other hand, the finding that crude rat brain homogenate did not reduce ICA titers on mouse pancreas suggests that the target antigen(s) in mouse pancreas is unlikely to be either GAD_{65} or GAD_{67} or any other cross-reactive antigen in rat brain.

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Received for publication 6 May 1994 and accepted 12 May 1994.

IDDM, insulin-dependent diabetes mellitus; ICA, islet cell antibody; GAD, glutamic acid decarboxylase.

The ICA antigen in human pancreas is widely accepted as being cytoplasmic and not restricted to β-cells within the islet, characteristics consistent with the known distribution of GAD_{65}. If ICA staining on mouse pancreas is not contributed to by GAD antibodies, then it would have been interesting to know about the pattern of staining compared with human pancreas. Was it or was it not restricted to β-cells?

Perhaps the most interesting finding of Chaillous et al. (1), with possible implications for pathogenesis and prediction, was the decrease in ICAs on mouse pancreas in the first year after clinical diagnosis, in contrast to the stable level of either ICAs on human pancreas or GAD antibodies. The stability of GAD antibodies has been demonstrated before as well as after diagnosis (15), but the traditional view is that ICAs on human pancreas decrease after diagnosis (16), although surprisingly few studies have reexamined this. That the immune response to GAD remains elevated when the majority of β-cells is lost is not that surprising even if GAD autoimmunity is antigen-driven, because GAD_{65} expression is not restricted to β-cells in human islets and GAD is also present in several other peripheral tissues as well as in the brain. A key question concerns the nature of the non-GAD target antigen of ICA in mouse islets, presumably also present in human islets. In the pre-GAD era, it was proposed to be a sialic acid-containing glycolipid (ganglioside) (17). We are still none the wiser, and the advent of GAD has only distracted our attention from the real ICA.

Chaillous et al. (1) do not address the predictive value of antibodies to islet cell antigens analyzed singly or in combination. Heterogeneity of ICAs might be expected to reflect clinico-pathological heterogeneity. They did not find relationships between age and sex and ICA subtype. This is somewhat surprising because there is evidence (2–4,15) that high levels of GAD antibodies are more frequent in older females in whom the rate of β-cell destruction appears to be slower. They had equal numbers of males and females but with a median age of 23 years. It would be interesting to know if the ICA results would be different in children with IDDM. It would be important, also, to know whether heterogeneity of ICA reflects differences in HLA alleles, because higher levels of GAD antibodies have been associated with the B8-DR3 haplotype (18).

There is no doubt that GAD antibodies are a sensitive and specific marker of islet cell autoimmunity in IDDM, but the jury is still out on the predictive value of GAD antibodies, alone or in combination with conventional ICAs or insulin autoantibodies. Most investigators have found a close association between conventional ICAs and GAD antibodies (11,12,15), but there is as yet no convincing evidence that GAD antibodies can replace ICAs as a marker for the prediction of IDDM (19,20). Indeed, if GAD antibodies are a subset of ICAs, it will be important to determine how they modify the predictive value of ICAs, given that the latter are heterogeneous. Analysis of sera from ICA positive relatives in the Bart's-Windsor-Oxford prospective family studies shows that combination of antibodies to islet antigens improves prediction (20). This important finding is not unexpected, if one takes the quantitative view that the more antibodies detected, the stronger the autoimmune response. Further analysis of multiple antibodies in at-risk individuals is necessary to define which are independent predictors of risk.

In conclusion, it is worth emphasizing that the pathology of IDDM and the data from animal models demonstrate that β-cell destruction is mediated primarily by T-cells, not antibodies. We can hypothesize, therefore, that islet antigen-specific T-cells ought to be the most relevant markers for pathology and prediction, and may not necessarily mirror antibodies (18). Convenient and reliable clinical assays for antigen-specific T-cells have now been developed (21) and may eventually supplant traditional serology, not only for prediction but for monitoring the response to immunotherapy. The time has arrived for islet autoantigens to prove that they are more than signposts of autoimmunity. Do they elicit and/or drive autoimmune disease and meet criteria for pathogenicity analogous to Koch's postulates for infectious pathogens?

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