Workshop report

First International Workshop on the Standardisation of Insulin Autoantibodies

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Report prepared by: T. Wilkin, J. Palmer, E. Bonifacio, J.-L. Diaz and V. Kruse on behalf of the Immunology and Diabetes Workshops and participating laboratories*

Insulin autoantibodies (IAA) occur spontaneously in the circulation of insulin-naïve individuals. The possibility that IAA are a marker of the immunologic B cell destructive process of Type 1 (insulin-dependent) diabetes has led to prolific, but confusing, literature in which the relationships between IAA and islet cell antibody, HLA haplotype and diabetes itself are variously reported. It is unclear to what extent this variation in published data is due to differences in the methodology used to measure IAA. The long-term aim of the IAA workshops is to achieve a consensus between participating centres in the measurement of IAA. The immediate aims of the first workshop were: (1) to determine the current degree of consensus when each centre measured the same panel of sera blindly, and (2) to compare the assays currently in use in terms of several parameters of assay behaviour. Twenty-four centres took part, though not all in both studies, and all data was analysed anonymously. This is a workshop report; a definitive analysis of the data will appear subsequently.

Part 1

Nine centres contributed a total of 49 coded sera. The codes were sent to one of us, E.B., a non-participant. The sera were recoded centrally, and sets of 49 lyophilised samples were distributed to the 21 participating centres. The sera were described by the individual contributing laboratories as ten insulin antibody (IA) positive (from insulin-treated diabetic patients), 13 IAA positive (from newly diagnosed Type 1 diabetic patients and relatives of Type 1 diabetic patients) and seven normal sera. Of the remaining 19 sera, 14 were purported to contain other antibodies and five to be unspecified "problem" sera. Participants assayed the sera according to their own methodology and reported their data in a variety of units. Accordingly, all data were normalised on a continuous scale based on the highest value obtained from the six normal samples which all laboratories reported as negative (0), and the value for sample 7, which all except one laboratory (which withdrew) found to be most strongly positive (100). The normalised value, y , was derived from the formula

$$y = \frac{x - (\text{highest signal from six normal samples})}{(\text{signal from serum 7}) - \text{highest signal from six normals}} \times 100$$

where x is the raw signal.

The data is summarised in Figure 1, which details the mean value and standard deviation for each sample measured in the 21 laboratories. As presented, the data suggest wide variance between laboratories, particularly in the measurement of IAA. Closer analysis, however, revealed that much of the variance was systematic, and

attributable to the use of thermodynamically different assays (Radio-binding assay and enzyme-linked immunosorbent assay) (ELISA) and antigenically different insulins (human and non-human). Thus, radiobinding assays obtained a systematically higher signal than ELISA's from some samples (sample 48, Fig. 2), though not all (sample 32, Fig. 2). Similarly, ELISA's obtained a systemically higher signal than radiobinding assays from some samples (e.g. sample 28), though not all (e.g. sample 24). Furthermore, for some sera both methods obtained higher signals against human insulin compared with non-human insulin, indicating the human insulin specificity of some IAA (e.g. samples 16 and 34). Finally, the presence of particularly high variance for low mean signals seen in the samples presented as "other autoantibodies", indicates that one or two laboratories obtained a high insulin-binding signal for samples which most found to be negative. Much of the remaining variance resulted from sometimes major differences in individual assay behaviour, examined in Part 2.

Part 2

Seventy samples and a calibration serum from an insulin-treated diabetic patient were lyophilised, coded and distributed by one centre (Southampton GB) to 17 participating centres. The 70 samples comprised seven groups of ten, prepared so as to compare the assays currently in use in terms of: the shape of the calibration curve, the detection threshold, the result of diluting an IAA positive serum in normal human serum and in phosphate buffered saline, the non-specific background, reproducibility, the response to dose-dependent inhibition with unlabelled insulin, the assay noise and the ability to distinguish human insulin-specific from cross-reactive IAA.

Although the limited data did not allow statistical comparison of assays, some general observations were possible. All centres produced satisfactory dilution curves from the calibration serum (insulin-treated). There was considerable inter-laboratory variation, but individual examples of both ELISA and radiobinding assays could approximate to the "ideal" of what was expected from the IAA samples provided. The signals in ELISA's were frequently higher, but the level of non-specific binding frequently greater, than in the radiobinding assay. IAA specific for human insulin were detectable in some examples of both ELISA and radiobinding assays. Attempts to produce a dose-dependent inhibition curve with ELISA frequently resulted in a biphasic curve. Some centres were unable to establish a smooth dilution curve in normal serum, irrespective of assay used. This suggests that their dilution curves in buffer were at least in part recording non-specific binding.

Conclusions

1. Although certain features distinguished ELISA and radiobinding systems, there was considerable inter-laboratory variation in both. As long as such variation persists, assays should be considered individually rather than grouped according to type.

* D.J. Becker, Pittsburgh, K. Behbahani, Kuwait, C. Binder, Copenhagen, B.M. Dean, London, U. Di Mario, Rome, R.B. Elliott, Auckland, R. Gomis, Barcelona, A. Hübinger, Düsseldorf, K. Helmke, Gießen, R. Kientsch-Engel, Tutzing, H. Kolb, Düsseldorf, V. Kruse, Copenhagen, A. Kurtz, London, J. Ludvigsson, Linköping, N.K. Maclaren, Gainesville, J.L. Molenaar, Delft, L. Nell, Houston, J. Palmer, Seattle, W.G. Reeves, Nottingham, J.C. Sotoyoz, Liege, J.S. Soeldner, Boston, M. Wolk, Jerusalem, T.J. Wilkin, Southampton, R. Zanchetta, Padova

Figure 1. Mean normalised signal (see text) and standard deviation based on 30 data sets from 20 laboratories using a variety of methods and ligands to measure insulin binding in 49 sera detailed in text

Figure 2. Scattergrams revealing factors which contributed to the variance of data in Figure 1.
○ = ELISA; ● = Radiobinding assay; □ = human insulin ligand

2. The assays showed less concordance when measuring insulin autoantibodies than insulin antibodies from insulin treated diabetic patients.
3. Non-specific binding was a very important source of variation in some assays (ELISA as well as radiobinding assays).
4. Specificity for human insulin was clearly demonstrated in some IAA sera.
5. The observed variation in IAA assays could account for the differences in published reports on the frequency and associations of IAA.

Comment

The study was limited by a number of factors. First, the categorisation of sera distributed in Part 1 was based on the findings of single laboratories, that is the laboratories which contributed the sera. Second, the samples used in Part 2 were prepared by one laboratory and, because of availability, comprised a predominance of human insulin-specific IAA sera. Although all centres were asked to use human insulin ligands, some were unable to do so, and were therefore

not able to analyse adequately the human insulin specific IAA sera. Third, assay results from the different laboratories were reported in markedly varying units, making comparison of results difficult. The overriding needs are for standard calibration and reference sera to be made available, for a uniform system of binding units to be developed, for a single ligand to be employed, and for signal specificity, assay precision and detection thresholds to be established. These are the aims of phase 2.

Those wishing to participate in phase 2 should contact Dr. Helga Gleichmann if they are not already registered.

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Dr. Helga Gleichmann, IDW Sekretariat,
Diabetes-Forschungsinstitut, Auf'm Hennekamp 65,
D-4000 Düsseldorf 1, FRG