IMMUNOASSAYS FOR ISLET CELL AUTOIMMUNITY

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Immunoassays for islet cell autoimmunity can be classified into two categories : i) assays for antibodies directed against islet cell antigens other than insulin and ii) assays for antibodies directed against beta cell hormone, insulin. The discovery by Bottazzo *et al* in 1974, of the presence of anti-islet cell antibodies reactive against islet cells in diabetic patients with polyendocrinopathies paved the way for the development of anti-islet cell antibody assay as a serological marker for islet cell autoimmunity[1]. Anti-insulin antibodies in insulin treated diabetics were discovered way back in 1959[2]- The reports on the presence of these antibodies in insulin untreated patients have been scanty till the development of a convenient assay system by Palmer *et al* in!983[3].

Nomenclature regarding the antibodies against islet cells depend on the author's 'imagination' of the possible localization of the antigen in the cell and its nature. Hence we find mentions of cell membrane and cytoplasmic antiislet cell antibodies in literature making the scene complicated. In this review these antibodies would be commonly called islet cell antibody (ICAb) for convenience. These antibodies have been found to be of IgG class and disappear usually within an year or two of the clinical diagnosis of IDDM and persists for many more years in some subjects with coexistent autoimmune disorders.

Assays for antibodies directed against islet cell :

Detection and quantitation of these antibodies had been eluding scientists for quite long due to reasons like different variables in the assay, subjective evaluation of the assay result etc. At present, several immnofluroscence and immunoenzymatic methods are available for the assay of these antibodies. Cryostat sections are used as substrate in immunoflourescence and immunoenzymatic methods. In the former, a protein-A or antihuman immunoglobulin conjugated to a flourescent compound is used to probe ICAb whereas in the latter the binder is conjugated to an enzyme. Attempts have also been made to quantitate this assay utilizing fluorescence spectrophotomicroscopy.[4] In this technique, relative difference between the staining of positive islet and the background is measured and the titres expressed in terms of the flourescence intensity difference.

A series of international workshops are being held to co-ordinate different laboratories across the globe working on this assay so as to check the variation in

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the assay result as well as to find out ways to quantitate the same- Standard ICAb positive serum is being supplied to participating laboratories to establish their own reference curves and to report results in JDF (Juvenile Diabetes Foundation) units rather than 1 + , 2 + . . (based on flourescence intensity) or 1/10, 1/20 . . . (final dilution).

Apart from these ICAbs, complement fixing islet cell antibodies, are also being implicated as a marker or even a better marker for islet cell autoimmunity. In search of the surface antibodies, some researchers attempted to use cultured islet cells as substrate and demonstrated cell surface binding antibodies in sera of Type I diabetic patients. A four-layer double immunofluorescence technique was adopted by Pujor-Borrel *et al* to localise the type of islet binding to these antibodies.[5] Biotin-avidin system has also been successfully tried to assay islet cell antibodies.[6] Though it was customary to use blood group O pancreas as substrate Takashaki *et al* demonstrated that pancreas from individuals of any blood group behaved similarly.[7]

Assays for antibodies directed against insulin molecule :

There are isotopic and nonisotopic assays for quantitating insulin autoantibodies in patient serum. The former is more popular as this has been found to have better specificity and sensitivity.

Isotopic assay : 1251 insulin is used as a tracer. This is incubated with patient sera. Another set of samples in duplicates are incubated with the tracer in the presence of excess of non radioactive insulin. This is done to determine the insulin specific binding in the former. The difference between the % binding in the two cases is expressed as % specific binding. In our laboratory we have found that % specific binding (due to undefined factors) in normal human sera to be >1.8% (Mean±3SD). Any specific binding above this is taken as positive. In order to quantitate, the displaced binding is expressed in terms of units of insulin, which could be the "true" amount of insulin bound.

Nonisotopic Assay : ELISA is employed in this technique- Insulin is coated to wells in ELISA plate, incubated with patient serum and with anti-human immunoglobulin conjugated to an enzyme (eg. peroxidase). The enzyme activity is measured and is proportional to the concentration of anti insulin antibody in the serum. Positive sera are serially diluted to generate standard curve-

In the assay procedures mentioned above presence of endogenous unlabelled insulin in patient serum is an interfering factor. Hence attempts have also been made to eliminate this free 'cold' insulin using dextran coated charcoal.

A mathematical relationship also has been lately worked out between the concentration of insulin autoantibody (together with first phase insulin response to intravenous glucose) and the rapidity with which diabetes mellitus develop. The equation being Years to diabetes=l.5 + 0.03 (IVGTT insulin)—0.008 (insulin autoantibodies).[8]

As in the case of islet cell autoantibody assay validation, for insulin antibody assays too, international workshops are being held to evolve ways to do away with the different types of assay result expression in different laboratories and to evolve better quantitation methodologies.

Recently autoimmunity to precursors of insulin molecule have also been suggested, as antiproinsulin autoantibodies noncrossreacting with whole insulin were detected in insulin autoantibody negative sera of patients with IDDM before insulin treatment.' These autoantibodies when bound to proinsulin could be displaced by C-peptide in most of the cases whereas whole insulin did not have any effect.

The quantitation of antibodies directed against islet antigens other than insulin would be greatly facilitated if the target antigens are isolated. Several attempts have been made in this direction. A 64000 Mr beta cell specific autoantigen was isolated from rodent and human islets metabolically labelled with 35-S methionine. This protein, because of its nonreactivity with sera of patients with autoimmune diseases other than Type I diabetes could be a potential antigen for the development of ICAb assay systems.[10] Evidences are also coming up demonstrating a glycolipid as the target antigen involved in islet cell autoimmunity.[11] However charaterisation and isolation of the target antigens involved in autoimmunity is likely to help the researchers make a quantum leap forward in immunodiagnosis and hopefully immunoprophylaxis of autoimmunity related diabetes mellitus.

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