Insulin Antigenicity

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I would like to begin with a brief historical review of the subject of insulin antigenicity before I discuss its clinical significance.

After the discovery of insulin, a little over four decades ago, some of the early preparations of insulin were relatively crude; and, as might be expected, a number of reactions occurred. In one of the first studies on insulin allergy utilizing pure crystalline insulin, Tuft (1928) established that it was the insulin itself which caused the skin reaction and not the protein of the animal from which the insulin came. He further demonstrated that the material causing the skin reaction could be passively transferred to the skin of a normal individual by an intradermal injection of serum from the insulin-allergic individual. He also showed the presence of a precipitin against insulin in the serum of the insulin-allergic patient and demonstrated that the skin-sensitizing antibody remained in serum long after the precipitin was lost. He presented some evidence of an increased insulin requirement in the patient when the precipitin was present and suggested that antibodies to insulin might be associated with insulin resistance. Other workers confirmed the fact that insulin was antigenic (Prout, 1962) and Sir Frederick Banting (1938) made the observation that psychiatric patients receiving insulin for shock therapy required more and more insulin to produce shock as time went on. He demonstrated that serum from these patients protected mice from convulsions when injected with insulin and later showed that the anti-insulin material was located in the serum globulin rather than the albumin.

In 1944 Lowell defined the two kinds of antibodies against insulin, one a reagin which was heat labile and caused skin sensitization, and the other a heat-stable factor that prevented the hypoglycemic effect of insulin in vivo.

Loveless and Cann (1955) showed that the heat-stable precipitin, the material with anti-insulin effect in the intact animal, could in fact act as a blocking antibody for the skin-sensitizing reagin or the heat labile factor. These antibodies traveled in two distinct areas on serum electrophoresis; the blocking antibody was a γ-globulin, while the skin-sensitizing reagin traveled with the β-globulins.

Up to this time insulin was thought to be rarely associated with host reactions except for the occasional local reaction to injection. It was Berson and his associates (1956) who showed that insulin antigenicity was a common phenomenon. In patients who have been treated with intermediate or long-acting insulin, 80% have antibodies in the serum that are measureable by techniques utilizing insulin labelled with iodine 131 (table 1).

Most of the clinical manifestations of the skin-sensitizing reagin are straightforward. Some of the rarer forms of sensitivity reaction attributable to insulin are difficult to substantiate (e.g. thrombocytopenia and gastrointestinal upset), but the skin reactions to insulin are not at all unusual. It is quite common to have local reactions to insulin in the first few weeks after insulin injections are started. In

TABLE 1
Insulin Antigenicity

	Skin Sensitivity	Insulin Resistance
Tuft (1928)	Passive cutaneous transfer	Precipitin in serum
Banting (1938)		Mouse protection by globulin of insulin-resistant patient
Lowell (1944)	Heat-labile	Heat stable. Related to resistance. Possibly species specific
Loveless and Cann (1955)	Separated with β-globulin	Separated with γ-globulin. "Blocking antibody"
Berson (1956)		Insulin-binding antibody

TABLE 2
Saturation in Insulin-binding Antibodies*

Daily Insulin	% Retention in Serum 60 min	% Free Serum Insulin 5 min
45 u/day	83	2.1
90 u/day	72	3.3
190 u/day	48	17.6

*The relationship between daily dose of insulin and saturation of insulin-binding antibodies is shown by these results. Free insulin is increased and becomes available for peripheral use. The increase in daily insulin does not stimulate the over-production of antibodies in this type of patient.

treating the patient with local reactions, it is essential to be certain that the patient is injecting himself properly, that he is using a clean syringe and not injecting alcohol. Some physicians advocate that syringes and sites of injection be changed or that the insulin be warmed or even boiled; after several weeks in which several techniques have been tried, the local reactions subside. I suspect that this is not related to the ingenuity of the therapeutic maneuvers, but to time that has been consumed while waiting for the blocking antibodies to block the skin-sensitizing antibody. The patient has been desensitized in precisely the same way that he has been desensitized for ragweed antigen, and the important part of therapy has been the continuation of injections until the patient is desensitized.

Other phenomena at the injection site are of great interest although it is not proven that they are related to insulin antigenicity. These are the insulin-induced fat atrophy and/or fat hypertrophy. Their cause remains an enigma. I have wondered whether insulin may become fixed to adipose tissue at the injection site and set up a tissue-fixed antigen-antibody reaction with circulating antibodies. The time sequence suggests the validity of this possibility and on occasions a change to insulin of a different species has coincided with the end to fat atrophy. I am currently trying to improve these demonstrations and determine their significance. These phenomena may well relate to the effect of insulin on fat metabolism, but thus far the reasons for both fat atrophy and fat hypertrophy are obscure.

The clinical significance of the heat-stable antibody to insulin found in the γ-globulin is more easily determined. If we study the insulin-binding capacity of the serum of patients attending a diabetes clinic, we find that all patients taking intermediate insulin develop antibodies in six to eight weeks.

In general the titre of antibodies is low, and as a group the patients who have been on insulin the longest period of time have the higher titres. There is, of course, great variability. There are several interesting exceptions to this generalization. Insulin antibodies have been noted to disappear in patients receiving steroid for sarcoidosis. In other patients having the proteinuria of Kimmelstiel-Wilson syndrome, insulin-binding antibodies are often lost along with other proteins. Part of the insulin sensitivity seen in patients with this syndrome is probably related to the loss with the proteinuria of the buffering effect of the antibodies. In such patients the antibody titre seen in the serum is low even through the patient had been on insulin for more than 20 years.

As Berson (1956) demonstrated, the presence of antibodies is readily shown in vivo. In patients with little or no antibody, there is rapid disappearance from the blood of insulin labelled with I^{131} following an intravenous injection. Patients with insulin resistance show a marked prolongation of the half-time of labelled insulin in blood, and it is easy to show that the insulin I^{131} continues to circulate because it is bound to the γ-globulin.

Let me give an illustration of the clinical significance of this. A man previously controlled on 40 units of insulin developed ketoacidosis without obvious cause. He was treated successfully and discharged on his previous dose of 40 units daily. In a short time he returned again with ketoacidosis. An insulin effect had been obtained and the acidosis had been treated successfully, but this effect was promptly dissipated when he returned to his maintenance dose that was less than his daily needs. It was postulated that by saturating the circulating antibodies with larger daily doses of insulin the anti-insulin effect of his antibodies could be neutralized. It was first demonstrated that approximately 90% of the injected insulin I^{131} remained in circulation for more than two hours while he was on an inadequate dose of insulin. We then progressively raised his insulin dose, and he began to come under better control. Now we found that injected insulin I^{131} disappeared from the blood more quickly, presumably because some of the binding sites of the insulin-binding antibody were being occupied by the daily dose of insulin he was receiving. As his insulin dose was increased during the next week, he showed progressive shortening of the half-time of insulin in his blood and reciprocally a greater amount of the insulin was free to be utilized in the periphery. Insulin bound to antibody is preserved not only from peripheral use but also from peripheral degradation. The results of these studies of insulin-I^{131} over this period are shown in table 2. As the increased daily dose of insulin occupied more of the binding sites of the insulin-binding globulin, more of the daily insulin dose was free to exert its physiological effects, and the control of his diabetes was thereby improved (Prout and Katims, 1959).

Insulin resistance is not always related to insulin-binding antibodies, of course, and in the differential diagnosis of this problem a number of other conditions must be considered (table 3). Obesity is usually associated with relative insulin resistance. Obese patients who have never received insulin may require three or four times as much insulin to produce the same fall in blood glucose on an insulin tolerance test as their colleagues of normal weight. Obese diabetic subjects who have not followed their prescribed diet and who hence are not controlled may require 50 to 60 units daily at the onset to achieve control, although they are not dependent on insulin to prevent ketoacidosis.

Moderate degrees of insulin resistance are seen in acromegaly, Cushing's syndrome, hyperthyroidism, and with steroid treatment.

Cyclic resistance associated with the menstrual cycle has been reported. None of these states are related to antibodies and are all relatively mild forms of resistance for the most part.

Infection, of course, is the most common cause of insulin resistance and resistance quickly disappears as the infection is brought under control. Although an increase in insulin-binding antibodies with infection would appear likely, we have not found a significant rise in circulating antibodies of any of these patients' studies, the most remarkable of whom has been reported by Knowles and his colleagues (*see* Tucker et al., 1964).

Peripheral or tissue resistance to the effects of insulin has been postulated and there are remarkable instances reported of severe resistance on this basis that are unrelated to insulin antibodies (Field, 1962). Another cause of apparent insulin resistance is the so-called Somogyi effect or paradoxical hyperglycemia (Somogyi, 1959). This refers to the patient who appears to need more insulin but in whom control becomes more difficult despite the increase in his insulin dose. Hypoglycemia that often goes undetected is followed by rebound hyperglycemia and the patient and the physician are likely to increase the dose further unless the paradoxical hyperglycemia is recognized. The proper treatment is to decrease rather than increase the insulin dose after which the apparent insulin resistance disappears.

Evidence that the antibodies are not the result of high insulin dosage used in insulin resistance rather than a cause is difficult to find. I had an opportunity to study one patient who has helped to answer this question. A woman had had diabetes for a number of years and had developed insulin resistance with requirements of insulin exceeding 1,500 units per day. Her physician had told her that insulin was useless for her and the patient had discontinued her injections. When seen, she had been off all treatment for four years. During this time, she had had constant glycosuria and had developed severe peripheral neuropathy. We measured her insulin-binding capacity after four years on no insulin and found an insignificant amount of binding still present, about 50 microunits per ml. We began insulin in relatively low doses and at the dose of 60 units of insulin per day, an insulin effect was seen. Her urine became free of glucose for the first time in four years. On the eighth day after beginning therapy she was again found to be extremely resistant to insulin and impossible to control; her insulin-binding antibodies had increased ten fold. An attempt was made immediately to saturate the high level of circulating antibodies by giving her 500 units of insulin intravenously which, under these specific circumstances, was quite safe and no effect of this insulin was seen on the blood glucose. Insulins from different species were tried as well as steroids; but in spite of this, on insulin in doses up to 1,500 units daily, she did not respond. After oral agents became available, hyperglycemia was controlled to some degree. This patient was found responsive to insulin in moderate doses before the recurrence of insulin resistance. Antibodies developed before massive doses of insulin were used and resistance became clinically significant with the rapid rise of antibodies.

What steps do we take when confronted with an insulin-resistant patient? Let us assume that the obvious causes of insulin resistance, such as obesity or infection, have been ruled out and that the insulin requirements are in excess of 200 units per day. First we should determine whether the patient is really insulin dependent. The two phenomena do not necessarily go hand in hand. Insulin dependency is related to the presence or absence of retrievable insulin from the pancreas. Insulin resistance is related to response to injected insulin and may develop in patients still capable of responding to oral agents. The patient just described illustrates this. If the patient is not insulin dependent, an oral hypoglycemic agent may be effective. If this fails, and insulin therapy is mandatory, one may attempt to saturate the antibodies by rapidly increasing the insulin dose as I have described above. This can sometimes be best accomplished by using crystalline insulin in multiple doses throughout the day; this is always more effective in patients with a large amount of insulin-binding antibody than is intermediate or long-acting insulin. If this does not succeed, insulin from different species, usually pork insulin, can be tried.

TABLE 3
Differential Diagnosis of Insulin Resistance

1) Obesity	Relative resistance easily overcome.
2) Endocrine states:	
a) acromegaly b) Cushing's syndrome c) hyperthyroidism d) menstrual cycle	Mild resistance. Usually less than 100 units/day of insulin required unless other complications are present.
3) Peripheral resistance	Unusual condition difficult to prove. Very high quantities of insulin used with little effect.
4) Infection	Commonest form of resistance. Rapidly reversed with treatment of infection.
5) Antibodies to insulin	Demonstration by in vivo or in vitro techniques.

TABLE 4
Treatment of Insulin Resistance Related to Insulin-binding Antibodies

1) Determine insulin dependency
2) Antibody saturation
3) Change to insulin of another species (pork, fish)
4) Adrenal steroids or ACTH
5) Anti-metabolites not recommended
6) Time and continued treatment with insulin in crystalline form

Antimetabolites such as 6-mercaptopurine theoretically might help these patients by suppressing formation of antibodies. This has been tried on several occasions without any great success (Merimee, 1965).

Adrenal steroids are relatively low on the treatment list for insulin resistance. It is better to have a patient maintained on 300 units of insulin a day than to have him on steroids for life. Steroids can sometimes be used to decrease insulin resistance but the resistance frequently returns when steroids are tapered and stopped, and long-term steroid treatment may be required. Steroids must be used, as elsewhere in medicine, with circumspection.

Not the least important in the treatment of insulin resistance due to insulin binding antibodies is the use of time. If the resistant patient can be maintained on a high dose of insulin required for control, eventually the resistance may disappear in the same way it came, indeed, with less explanation. Continuation of insulin, especially as crystalline or regular insulin, is essential in such patients (table 4). Interruption of insulin therapy may in fact be one of the settings in which relative insulin resistance occurs when insulin is restarted.

Thus we have seen that evidence of insulin antigenicity in one or more forms is present in most individuals receiving intermediate insulins for six weeks or longer. A number of clinical manifestations of insulin antigenicity and their treatment have been discussed.

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