Association of acute local reactions to insulin with an insulin-binding gamma I M antibody

J. G. DEVLIN¹ AND D. K. O'DONOVAN

From the Department of Medicine and Therapeutics, University College, Dublin

SYNOPSIS Sera from 15 diabetic patients treated with insulin for periods varying from four weeks to 19 years and from two control subjects were investigated for the presence of insulin I¹³¹-binding antibody. It was found in all patients even as early as four weeks after the initiation of therapy, but the antibody in the gamma I M region was found in only one, the only patient who had severe local reactions to insulin at the time when the serum was being collected. It is suggested that the acute local reactions to insulin are due to the development of a transient gamma I M insulin-binding antibody.

Twenty-five per cent of all diabetics receiving insulin develop at one stage or another local skin reactions to insulin (Joslin, Root, White, and Marble, 1959; Arkins, Engbring, and Lennon, 1962). One of the most frequently occurring local reactions develops within a few days after insulin therapy being initiated and subsequently subsides (Paley and Tunbridge, 1952; Marble and Cahill, 1962). The reaction consists of local tenderness, redness, swelling, and pain and suggests an allergic response. There is still controversy as to whether this is due to an allergic response to an impurity in the commercial insulin used or to the crystalline insulin itself. The local reaction can be controlled by the addition of small amounts of hydrocortisone to the insulin but antihistamine drugs are ineffective whether given locally or orally (O'Donovan and MacCormac, 1961).

This investigation shows that the acute local reaction is associated with the presence of an insulin-binding gamma I M antibody which is not present in the general diabetic population receiving insulin.

MATERIALS, METHODS, AND CLINICAL DETAILS

Sera from 15 diabetic patients and two healthy control subjects were studied. The age, sex, insulin dosage, and duration of insulin therapy at time of venupuncture of each patient are set out in Table I. Patient no. 6 is of special interest.

C.R., aged 47, weight 129 lb., was admitted for investigation of diabetes of recent onset. The fasting blood sugar level was 225 mg.%. Acute local reactions developed two weeks after the commencement of insulin

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TABLE I
AGE, SEX, INSULIN DOSAGE, DURATION OF THERAPY,
AND HAEMAGGLUTINATION ANTIBODY TITRES OF
15 PATIENTS STUDIED

Patient No.	Age (yr.)	Sex	Insulin Dosage (units)	Duration of Therapy	Haemag- glutination Antibody Titres
1 C.S.	69	F	56	12 yr.	1/4
2 S.C.	36	M	80	5 yr.	1/64
3 T.M.	52	M	30	8 yr.	0
4 M.N.	66	M	60	19 yr.	1/32
5 T.E.	50	F	40	7 yr.	1/16
6 C.R. (acute local reactions)	45	F	40	6 wk.	1/2
7 E.C.	47	F	40	14 yr.	0
8 M.A.	55	F	40	3 yr.	0
9 M.G.	68	F	20	4 wk.	0
10 M.B.	66	F	42	10 yr.	0
11 M.C.	79	F	60	19 yr.	1/16
12 J.B.	73	M	20	11 yr.	1/8
13 A.C.	23	F	60	3 yr.	1/16
14 J.C.	45	F	90	4 yr.	0
15 R.A.	55	M	28	14 yr.	1/4

therapy and subsided after a further three weeks. She had never had any evidence of a generalized allergic reaction such as urticaria, eosinophilia, fever, or asthma. The patient gained 4 lb. while in hospital. She was re-admitted eight months later with an ovarian carcinoma from which she died within two months.

In all the cases insulin antibody titres were determined by the tanned red-cell method of Boyden (1951) as modified by Moinat (1958). Insulin immuno-globulin patterns were studied by radioimmuno-electrophoresis (Morse and Heremans, 1962; Yagi, Maier, and Pressman, 1962). Antihuman serum was obtained from Hyland Laboratories, L.A., and specific anti gamma I M and anti gamma I A antisera were donated by Dr. J. Fahey, N.I.H., Bethesda. Insulin I¹³¹ was obtained from Abbot

Laboratories (specific activity 36.7 mc./mg.). This was further purified according to the method of Banerjee and Gibson (1962) and the subsequent chromatogram was scanned in a Baird and Tatlock radioactive chromatogram counter. The insulin I^{131} used contained only 3.9% impurities. Sera and insulin I^{131} were incubated at 4°C. for four days before agar-gel electrophoresis.

All sera were tested for precipitin antibodies to insulin in Agar Noble and in Ionagar.

RESULTS

Insulin I^{131} -binding antibody was present in all the diabetic sera tested. The pattern of the autoradio-

graphic lines obtained is demonstrated in Figure 1. The 7S gamma globulin line is reproduced in its full extent. There is also some non-specific staining of the alpha 2 macroglobulin precipitin line which also occurs in the control sera using total antihuman serum (Fig. 2). There was no non-specific staining of the control sera with the specific antisera.

The autoradiographic line obtained from the serum of patient C.R. shows a well-marked curve and an increased density in the region of the starting well (Fig. 3). Good precipitin lines were obtained with the specific anti-beta 2a antiserum but no insulin binding could be demonstrated (Fig. 4).

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 1. Pattern of autoradiographic line obtained in diabetic sera. Radioactivity is found in the 7S gamma globulin precipitin lines and in the alpha 2 macroglobulin precipitin line.

FIG. 2. Non-specific staining of the alpha 2 macroglobulin precipitin lines in control sera.

FIG. 3. Autoradiographic lines obtained from patient's (C.R.) serum.
Upper line = whole antihuman serum.
Bottom line = specific anti-gamma I M antiserum.

FIG. 4. Anti-beta 2A precipitin lines and negative testing for radioactivity.

FIG. 5. Gamma I M precipitin lines.

The precipitin lines obtained with the specific antigamma I M were much fainter (Fig. 5), but were present in all sera tested. Insulin binding was demonstrated in the gamma I M immunoglobulin in the serum from the patient C.R. only (Fig. 3).

There was no relationship between haemagglutination antibody titre and the presence of this gamma I M antibody (Table I).

No demonstrable precipitin antibodies to insulin were present in any of the sera tested.

DISCUSSION

The natural history of the acute local reaction suggests that it is an allergic phenomenon. The fact that it could be partly or completely controlled in some cases by using heated insulin (Dolger, 1952) suggested that some impurity in the insulin was responsible although partial degradation of the insulin molecule with alteration of immunological response could not be ruled out. The presence of a gamma I M insulin-binding antibody in the serum of the patient C.R. strongly suggests that this antibody was responsible for the local reaction and is supported by the absence of an insulin-binding gamma I M antibody in the serum of patient no. 9 who did not have an acute local reaction at a time when a macromolecular antibody could be expected (Svehag and Mandel, 1964). Also two patients (cases 2 and 3), who had acute local reactions when insulin therapy was initiated, now have no local reactions and screening for gamma I M antibody was negative.

The nature of skin-sensitizing antibodies has not been fully elucidated although the subject has been reviewed by Schon (1963) and by Stanworth (1963). There is some evidence that the three classical immunoglobulins, viz., gamma I M, gamma I A, and 7S gamma globulin, may play a part. It is accepted, however, that passive cutaneous transfer reactions are not readily demonstrable with macromolecular antibodies. So far in this study all Prausnitz-Küstner reactions have been negative. Previous studies of insulin immunoglobulin patterns have not included specifically patients with acute

local reactions. Loveless and Cann (1955) found an antibody in the beta region in one allergic diabetic patient. Yagi, Maier, Pressman, Arbesman, Reisman, and Lenzner (1963) found an insulin-binding antibody in the gamma I M and gamma I A region in one out of 23 diabetic sera investigated. Their patient had generalized insulin allergy with eosinophilia, urticaria, and a positive Prausnitz-Küstner reaction. Miller and Owen (1960) and Harris-Jones, Miller, and Owen (1963) found insulin-binding antibody in the beta globulin region but clinical details relating to the presence or absence of acute local reactions were not stated. Morse and Heremans (1962) failed to find other than 7S gamma globulin insulin-binding antibody in the sera of eight diabetic patients. The present study confirms the presence of 7S gamma antibody to insulin in diabetic sera and records the association of a gamma I M insulin-binding antibody with acute local reactions to insulin.

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