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Soluble immune complexes in the sera of newly diagnosed insulin-dependent diabetics and in treated diabetics

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SUMMARY

Soluble immune complexes were detected by the Raji cell assay in seven out of thirteen newly diagnosed insulin-dependent diabetics, six of whom had islet cell antibodies (ICAb) in the serum. None of the others had ICAb. The titres for a wide range of viral antibodies were similar in these thirteen diabetics as in age- and sex-matched controls, except for antibodies to Coxsackie B4. Six had titres of Coxsackie B4 antibodies greater than or equal to 1:32, but only three of these had evidence of immune complexes in the serum, and these were not correlated with the titres of Coxsackie B4 antibodies. None of these diabetics had antibodies to insulin and none of their age- and sex-matched controls had evidence of immune complexes in the serum.

53% of thirty-two diabetics treated with insulin and 10% of fifty-two diabetics requiring oral hypoglycaemic agents (OHA) or diet had evidence of immune complexes in the serum, compared to 6% of control subjects. High titres of insulin antibodies correlated with evidence for immune complexes. There was a stronger tendency for immune complexes to occur in the presence of moderate titres of insulin antibodies when the age at onset of insulin-dependent diabetes was less than 30 years. Out of sixteen patients treated with heterologous insulin for 13 years or more, and who also had late diabetic complications, twelve had immune complexes in the serum.

INTRODUCTION

Evidence is accumulating that immunological processes may play an integral role in the pathogenesis of type I diabetes that culminates in insulin dependency (Irvine, 1977). In the present study, we have investigated the presence of immune complexes in the sera of newly diagnosed insulin-dependent diabetics and in treated diabetics compared to that in controls, and their association with the occurrence of antibodies to islet cells, insulin and viruses, and with the occurrence of diabetic complications. Their possible role in the pathogenesis of insulin-dependent diabetes and of complications of that form of diabetes is discussed.

MATERIALS AND METHODS

Patients studied. Sera were obtained between October and March from thirteen newly diagnosed diabetics. Control sera for these patients were obtained during the same period from age- and sex-matched healthy subjects, including hospital personnel. In addition, sera were obtained from thirty-two diabetics who had been treated with bovine or porcine insulin for a mean duration of 16 years (range 2–42 years), from thirty-one diabetics who had been treated with oral hypoglycaemic agents (OHA) for a mean duration of 6 years (range 0–21 years), from twenty-one diabetics who had been treated by diet alone for a mean duration of 2 years (range 0–14 years), and from eighty-four control subjects. All sera were stored in 500 μl aliquots at $-40°$C.

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Measurement of immune complexes. The Raji cell radioimmunoassay as described by Theofilopoulos, Wilson & Dixon (1976) was used. The first sample of cultured Raji cells was kindly provided by Dr C. M. Steel (MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh).

Purified human IgG, kindly provided by the Blood Transfusion Service, Edinburgh, was diluted to 40 mg/ml in PBS and then aggregated by heating at 63°C for 15 min. The amount of soluble immune complexes in each test serum was expressed as being equivalent to so many μg aggregated human gamma globulin (AHG) per ml serum. The limit of detection of the assay was equivalent to 8 μg AHG/ml. Since the test serum was used in a dilution of 1:4, this was equivalent to 32 μg AHG/ml test serum.

Autoantibodies. ICAb and other organ-specific autoantibodies were detected by the indirect immunofluorescence test (Irvine *et al.*, 1977). Sera that were positive for ICAb when tested neat were titrated using doubling dilutions.

Viral antibodies. The sera of the thirteen newly diagnosed insulin-dependent diabetics and their age- and sex-matched controls were screened and titrated for the following viral antibodies: to Coxsackie virus B1-5 by a metabolic inhibition test; to Epstein–Barr virus by an indirect immunofluorescence test; and to rubella virus using a haemagglutination inhibition test. Sera were screened and titrated for complement-fixing antibody to the following antigens: influenza A, B and C; parainfluenza type 1; measles; mumps S and V; respiratory syncytial virus; adenovirus; cytomegalovirus, varicella zoster, Herpes simplex, *Coxiella burneti* phase 2; Chlyamydia group B; and *Mycoplasma pneumoniae.*

Insulin antibodies. Antibodies to insulin were estimated by a minor modification of the method of Ortved Andersen, Brunfeldt & Albidgard (1972) according to Mustaffa, Daggett & Nabarro (1977). Insulin-binding of 10 μU/ml serum was regarded as indicative of the presence of insulin antibodies. Insulin-binding between 10 and 300 μU/ml serum was regarded as indicating moderate insulin antibody titres, while binding of > 300 μU/ml was interpreted as being due to high titres of insulin antibodies.

Statistical analysis. All analysis were done using Fisher's exact test, except where indicated.

RESULTS

The occurrence of soluble immune complexes in the sera of seven out of thirteen newly diagnosed insulin-dependent diabetics clearly correlated with the presence of ICAb ($P<0{\cdot}01$), but not with the presence or titres of antibodies to Coxsackie B4 (Table 1). The prevalence and titres of antibodies to the other viruses investigated were similar in the diabetics and in the age- and sex-matched controls. In

TABLE 1. The clinical and serological features in the thirteen newly diagnosed insulin-dependent diabetics

	Clinical features				Serological features			
Diabetics	Sex	Age (years)	Duration of symptoms	Duration of insulin treatment	Immune complexes*	Islet cell antibodies†	Cocksackie B4 antibodies†	Insulin antibodies
1	M	21	3 weeks	0	64	64	neg.	neg.
2	M	13	6 weeks	2 days	64	16	neg.	neg.
3	M	26	2 weeks	0	64	4	neg.	neg.
4	M	16	1 month	0	32	2	32	neg.
5	M	18	2 weeks	5 days	32	2	neg.	neg.
6	F	12	3 weeks	0	32	2	256	neg.
7	M	30	4 months	0	32	neg.	32	neg.
8	F	21	2 months	0	neg.	neg.	256	neg.
9	F	22	3 weeks	0	neg.	neg.	32	neg.
10	M	28	10 weeks	0	neg.	neg.	neg.	neg.
11	F	19	4 weeks	0	neg.	neg.	32	neg.
12	M	24	2 weeks	0	neg.	neg.	neg.	neg.
13	M	21	6 weeks	36 hr	neg.	neg.	neg.	neg.
Controls								
14–26	Age- and sex-matched				neg.	neg.	64 32 remainder neg.	neg.

neg. = Negative.

* μg eq. AHG/ml.

† Reciprocal of titres.

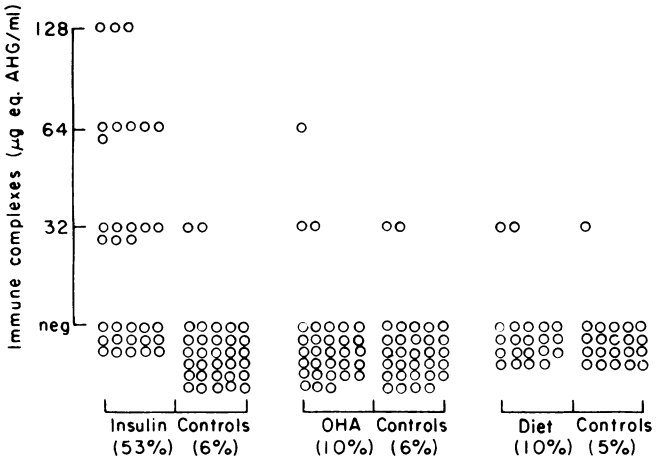

FIG. 1. The prevalence of immune complexes in the thirty-two insulin-treated diabetics and in the fifty-two insulin-independent diabetics compared to that in age- and sex-matched controls. The percentages of patients positive for immune complexes are shown.

view of the absence, or the very short duration, of insulin treatment at the time of this study, no antibodies to insulin were detected. There was no correlation between the occurrence of soluble immune complexes in the serum and the presence of ketosis or the level of the blood sugar. With regard to other autoantibodies one patient was positive for antibodies to thyroid.

Immune complexes were found in seventeen out of thirty-two (53%) insulin-treated diabetics ($P<0{\cdot}001$; χ^2 test), in three out of thirty-one (10%) patients requiring OHA (not significant); and in two out of twenty-one (10%) diabetics requiring diet alone (not significant); while in the eighty-four normal age- and sex-matched control subjects immune complexes were detected in five (6%) of them (Fig. 1).

The sera of all the thirty-two diabetics treated with heterologous insulin had increased binding activity, indicating the presence of antibody to insulin. The titres of insulin antibodies varied in different patients from 26 to 586 μU/ml irrespective of the duration of insulin therapy (between 2 and 42 years) (Fig. 2), dosage of heterologous insulin used or sex. The sera of all the insulin-independent diabetics were negative for insulin antibodies. Immune complexes occurred more commonly in patients treated with insulin for 10 years or more (sixteen out of twenty-three) than in those receiving insulin for less than 10 years (one out of nine) ($P<0{\cdot}001$). There was no correlation between the presence or amounts of insulin antibodies, or of immune complexes, with the patients' insulin requirements. All patients with high titres of insulin antibodies (>300 μU/ml) had soluble immune complexes (Fig. 3).

When the insulin-treated diabetics were subdivided according to whether they developed diabetes before or after the age of 30 years, it is seen that immune complexes occurred more frequently in the presence of moderate titres of insulin antibodies in the early onset, compared to the late onset diabetics ($P<0{\cdot}05$) (Fig. 4). The mean duration of diabetes and of insulin treatment in those with moderate titres who developed diabetes before the age of 30 and in those who developed diabetes after 30 were closely similar. When all the patients in each of these two groups were compared, there were no significant differences in the prevalence of immune complexes. The late onset insulin-treated diabetic, with a low titre of insulin antibodies (28 μU/ml) and immune complexes, had ICAb in the same sample of serum which was obtained 10 years after diagnosis. The only other diabetic among thirty-two insulin-treated patients who had ICAb in the serum had had diabetes for 19 years, and the level of immune complexes was equivalent to 64 μg AHG/ml and of insulin antibodies was 191 μU/ml. The only insulin-independent diabetic with ICAb in the serum was the single patient on OHA (duration: 1 week) who had immune complexes equivalent to 64 μgAHG/ml in the serum (Fig. 1). None of the controls had ICAb in the serum.

Sixteen of the thirty-two insulin-treated diabetics had diabetic complications that were clinically

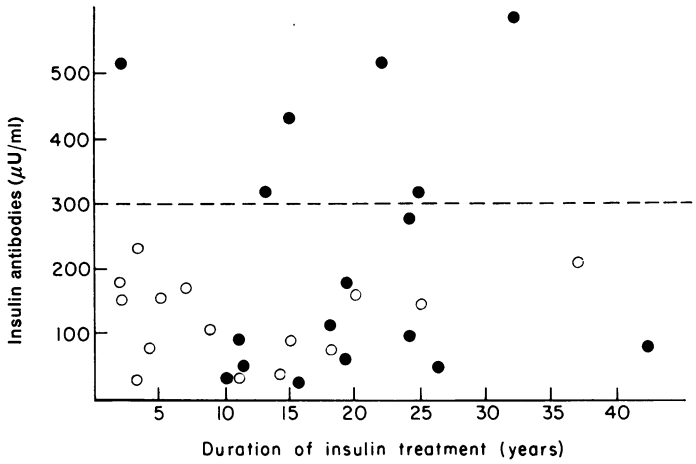

FIG. 2. The occurrence of immune complexes and insulin antibodies in the thirty-two insulin-treated diabetics according to the duration of insulin treatment. (●) Patients positive for immune complexes; (○) patients negative for immune complexes.

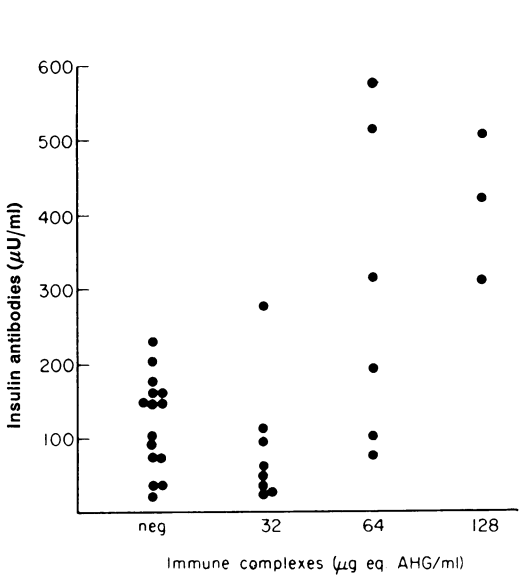

FIG. 3.

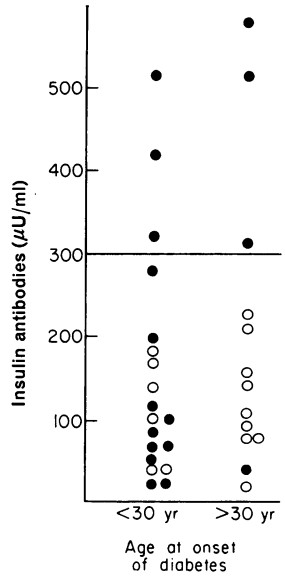

FIG. 4.

FIG. 3. The correlation between the titre of insulin antibodies and the amount of immune complexes in the same samples of sera from the thirty-two insulin-treated diabetics.

FIG. 4. The prevalence of immune complexes and titres of insulin antibodies in insulin-treated diabetics according to the age at onset of the diabetes. The duration of the diabetes and of insulin treatment were closely comparable in the two groups. (●) Patients positive for immune complexes; (○) patients negative for immune complexes.

manifest, including diabetic retinopathy (defined by the presence of more than 3 microaneurisms, retinal haemorrhages, exudates or new blood vessel formation) or diabetic nephropathy (defined by a permanent protenuria with or without abnormal kidney function). Twelve out of the sixteen had soluble immune complexes in the serum while only five of the remaining sixteen patients did so. Fig. 5 shows that the presence of immune complexes in the serum was associated with late diabetic complications only in patients who had had diabetes for more than 13 years. Four of the six patients with insulin antibodies in

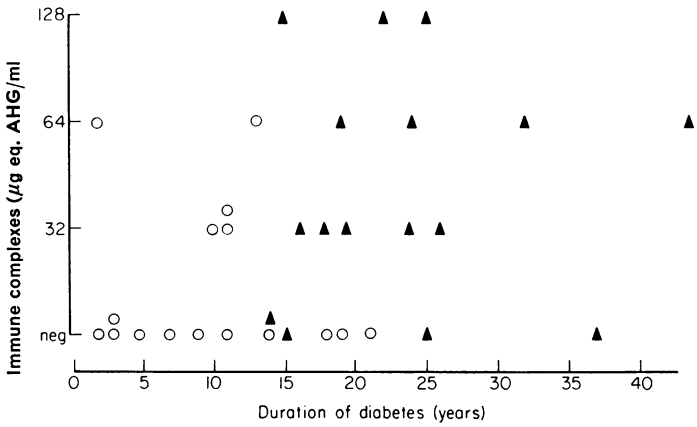

FIG. 5. The correlation between the presence of immune complexes and of late diabetic complications according to the duration of diabetes in the thirty-two insulin-treated patients. (▲) Patients positive for complications; (○) patients negative for complications.

the serum in a titre of $>300\,\mu$U/ml had late diabetic complications and had had diabetes for 15 years or more. In the other two the duration of diabetes was 2 and 13 years, respectively.

DISCUSSION

In the newly diagnosed insulin-dependent diabetics, the close correlation between the presence of soluble immune complexes and ICAb in the serum, and the absence of such a correlation with viral antibodies and other autoantibodies, suggests that the antigens involved in these immune complexes may be derived from islet cells. This would suggest that in such patients there has either been a recent release of islet cell antigens or that such antigens are being continually released after the onset of clinical diabetes of the insulin-dependent type. The fact that the symptoms of diabetes before diagnosis were present for as long as 4 months in patients with immune complexes at diagnosis suggests the latter, and this would accord with the observation that some B cells of the islets persist after the clinical onset of this type of diabetes, as evidenced by their ability (albeit diminished) to produce C peptide (Heding & Rasmussen, 1975). Moreover, ICAb reacts with all cells in the islets, although it is only the B cells that are markedly affected in Type I diabetes (Doniach, 1974; Egeberg *et al.*, 1976).

Soluble immune complexes comprising islet cell antigen and antibody could be important in relation to antibody-dependent cell-mediated cytotoxicity, whereby K cells may be specifically armed by such complexes in antibody excess. If so, the question remains why it is that the B cells are selectively destroyed, unless A and D cells have greater powers of regeneration. The correlation between the presence of ICAb and immune complexes also held in the two ICAb-positive insulin-treated patients and in the one ICAb-positive patient treated with OHA. Alternatively, immune complexes, in relation to islet cell antigens, may simply be a consequence of islet cell damage and not be involved in producing further islet cell damage.

Insulin antibodies were only detected in the insulin-treated subjects, and their titres appeared to be a characteristic of the patient rather than the dosage or duration of insulin therapy. Indeed, it has been shown that patients who are HLA-BW15 tend to have higher titres of insulin antibodies consequent upon insulin treatment (Bertrams *et al.*, 1976). High titres of insulin antibodies have been associated with late diabetic complications (Ortved Andersen, 1976). A correlation was observed in the present study between high titres of insulin antibodies and the presence of immune complexes, suggesting that in these patients the antigen involved in such complexes may be derived from heterologous insulin. The presence of low or moderate titres of insulin antibodies did not show any correlation with the amount of complexes present. This may be due to technical reasons, to the varying affinities that insulin antibodies may have in different patients (Dixon, Exon & Malins, 1975) or the varying rates at which immune complexes of different sizes are removed from the circulation, or it may be due to other antigens

being involved in such complexes. The possibility exists that the late complications of diabetes may be related, at least in part, to the production of immune complexes, and it is therefore noteworthy that juvenile-onset insulin-dependent diabetics tended to have more immune complexes in relation to moderate or low titres of insulin antibodies than did subjects who developed insulin-dependent diabetes after the age of 30 years, even although the duration of diabetes and of insulin treatment was comparable in the two groups. Insulin-dependent diabetes developing in young people is considered to be associated with a greater risk for developing severe late diabetic complications over a given time than diabetes developing later in life (Bradley & Ramos, 1971).

That immune complexes in relation to insulin may be implicated in diabetic complications is suggested by the observation that renal tissue from diabetics shows fine granular deposits on the basement membrane, resembling those found in certain types of glomerulonephritis (Bloodworth, 1968), while the glomeruli in diabetic nephropathy have insulin-binding capacity and also contain immunologically detectable insulin (Berns *et al.*, 1962). The latter features are also seen in the vessels of the diabetic eye (Coleman *et al.*, 1962). However, immunohistopathological studies have shown that the basement membrane in diabetic glomerulosclerosis contains not only insulin, IgG, IgM and components of complement, but also 'non-immunological' plasma proteins deposited in a linear fashion (Westberg & Michael, 1972), which suggests that there may be a non-specific lesion involving the trapping (binding) of serum proteins. These two possible mechanisms for the production of diabetic complications may not be mutually exclusive. While there was no clear correlation between the titres of insulin antibodies and the late complications of diabetes, our preliminary findings suggest that there is a tendency for immune complexes to occur after prolonged insulin therapy and at a time when such complications are likely to become clinically common (i.e. after 10 years).

Clearly a long-term prospective study is required in order to determine whether the development and persistence of soluble immune complexes in insulin-dependent diabetes is correlated with the development of late diabetic complications, and whether the use of monocomponent insulin will avoid the development of immune complexes and whether or not this will be associated with a reduction in late complications.

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