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SHORT COMMUNICATIONS

Insulin-Precipitating Antibodies in Insulin-treated and Untreated Diabetic Patients

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Summary. Although the existence of precipitating insulin antibodies (PIA) has been questioned by many authors, others have shown that such antibodies can really be found in insulin-treated animals. The authors have studied the problem in insulin-treated and untreated diabetic patients, using the agar-precipitation test of Oudin. It has been possible to demonstrate PIA in 20% of the 276 unselected diabetics studied. All control examinations were negative. Unexpectedly, the precipitation reaction was more often positive in the group of diabetics not treated with insulin. — The data of the authors disclose systematically for the first time the existence of PIA in man, and the prevalence of such antibodies in diabetic patients that have never been treated with insulin and in prediabetic subjects. These findings raise the question of an autoimmune pathogenesis of some types of diabetes.

Anticorps précipitant l'insuline chez des sujets diabétiques traités et non traités par l'insuline

Résumé. Bien que l'existence d'anticorps précipitant l'insuline (PIA) ait été discutée par beaucoup d'auteurs, d'autres ont montré que de tels anticorps peuvent réellement être trouvés chez des animaux traités par l'insuline. Les auteurs ont étudié ce problème chez des diabétiques traités et non-traités par l'insuline, en utilisant le test de précipitation à l'agar de Oudin. Il a été possible de démontrer l'existence d'anticorps précipitant l'insuline chez 20% des 276 diabétiques non-sélectionnés étudiés. Tous les examens de contrôle étaient négatifs. Fait inattendu, la réaction de précipitation était plus souvent positive dans le groupe des diabétiques non traités par l'insuline. — Les données des auteurs démontrent systématiquement pour la première fois l'existence de PIA chez l'homme, et la prévalence de tels anticorps chez des patients diabétiques qui n'ont jamais été traités par l'insuline et chez les sujets prédiabétiques. Ces résultats soulèvent la question d'une pathogenèse autoimmune de certains types de diabète.

Insulin-präzipitierende Antikörper bei Insulin-behandelten und unbehandelten Diabetikern

Zusammenfassung. Obwohl das Vorhandensein von Insulin-präzipitierenden Antikörpern (PIA) von manchen Autoren erörtert worden ist, haben andere gezeigt, daß solche Antikörper in Wirklichkeit nur bei Insulin-behandelten Tieren gefunden werden können. Die Autoren haben diese Frage bei Insulin-behandelten und unbehandelten diabetischen Patienten untersucht, wobei sie den Agar-Präzipitationstest von Oudin verwendeten. Dabei konnten sie PIA in 20% von 276 unausgewählten untersuchten Diabetikern nachweisen. Alle Kontrolluntersuchungen waren negativ. Unerwarteterweise war die Präzipitationsreaktion in der Gruppe von Diabetikern, die nicht mit Insulin behandelt worden waren, öfter positiv. — Die Ergebnisse der Autoren weisen erstmal systematisch die Existenz von PIA am Menschen nach und das besonders häufige Vorkommen solcher Antikörper bei Diabetikern, die niemals mit Insulin behandelt worden sind und bei prädiabetischen Personen. Angesichts dieser Befunde ergibt sich die Frage, ob es eine auto-immunologische Pathogenese für manche Diabetes-Typen gibt.

Key-words: Diabetes, antibodies by, insulin-precipitating antibodies by, insulin-antibodies by insulin-treated diabetics, by insulin-untreated diabetics, by prediabetes, autoimmunity to insulin.

Although insulin antibodies of the "reacting" or "blocking" type certainly exist [6, 13, 15, 17, 23], the existence of "precipitating" insulin antibodies (PIA) is disputed. Many authors [1, 2, 3, 8, 11, 13] have found no evidence of their presence, and conclude that insulin cannot form large antigen-antibody complexes; on the other hand, others have reported evidence for the presence of PIA in experimental animals treated with an insulin-adjuvant mixture [10, 11, 12, 14, 16, 18, 19, 21], the precipitation being positive even with animals injected with homologous insulin preparations [20, 21]. It was of interest therefore to examine the problem of PIA in the sera of diabetic patients.

Methods and Material

PIA was determined by means of the precipitation test of Oudin [8, 11]. Recrystallized bovine insulin (Hoechst and Novo preparations) was used. The agar-agar was processed before use to free it of any contaminants. The precipitation reaction was carried out in the following manner. Three media were layered in succession in glass tubes (diameter, 5 mm): at the bottom, 0.3 ml of a mixture of equal parts of the test serum and of 0.8% agar ("Difko") in saline (9 g/litre), pH 5.6—6.0, cooled to. 46—48° C; in the middle layer 0.4% agar in saline (9 g/litre); and finally, in the top layer, 0.3 ml of an agar-insulin mixture containing 2 U of insulin per ml of a 0.4% solution of agar in saline (9 g/litre). The tubes were sealed with wax or paraffin, and then incubated by sealing in larger tubes. The whole array was placed in a refrigerator at 3—5° C. The tubes were examined after the second day. In the positive cases the precipitation ring appeard in the middle layer and was clearly visible in transverse light.

In some cases the specificity of the precipitate was checked by dissolving the precipitation ring in excess of the antigen (insulin), and by staining for lipids with Sudan-Schwarz B.

The sera from 276 unselected diabetic patients (129 males) and 15 patients with both diabetes and tuberculosis were examined. The sera from 50 healthy subjects and from 15 patients with tuberculosis but without diabetes were used as controls. The diabetics were grouped according to the type of diabetes as follows: 120 with growth-onset, 148 with maturity-onset diabetes and 8 with prediabetes, the last category being defined by a high familial incidence of diabetes. Of the patients examined, 180 had been treated with insulin, and 96 had never received the hormone. None of the control subjects had ever received insulin. In terms of the severity of their diabetes, evaluated by the mode of treatment and the dose of insulin used, the patients were grouped as follows: mild diabetes (treatment by diet or oral hypoglycaemic agents) 101; medium-severe diabetes (insulin dosage up to 60 U per day) 121; and severe diabetes (insulin dosage above 60 U per day) 54. Five patients with a positive insulin-precipitation reaction were examined repeatedly over the course of a year using human insulin (a gift of Novo Research Institute) as the antigen.

Results

IPA were found in 55 ($20 \pm 5\%$) out of 276 diabetic patients studied; none of the control sera showed positive precipitation. The incidence of positive precipitation in the different patient groups (Table 1) revealed the following; a greater frequency in patients with maturity-onset diabetes than in patients with growth-onset diabetes ($22 \pm 7\%$ and $13 \pm 6\%$ respectively); most frequent in cases of prediabetes ($75 \pm 3\%$); considerably more frequent in patients not treated with insulin than in those treated ($33 \pm 10\%$ and $13 \pm 6\%$ respectively, the difference being highly significant); more frequent in patients with mild diabetes than in those with medium-severe or severe diabetes ($32 \pm 9\%$, $12 \pm 6\%$ and $15 \pm 10\%$ respectively); and a difference between males and females in respect to positive precipitation, which was not statistically significant (19 and 21% respectively). It was also found that the incidence of positive precipitation was doubled for patients over 40 years of age, and showed a slight tendency to decrease after 10 years duration of diabetes. The patients with tuberculosis and diabetes revealed positive reactions more often than the diabetics without this disease. All 5 patients repeatedly examined with human insulin as an antigen showed a positive precipitation reaction.

Table 1

Material	Number of cases	% positive precipitations
1. Total number of the cases examined	276	19.9
2. Maturity-onset diabetes	148	22.3
3. Growth-onset diabetes	120	13.3
4. Prediabetes	8	75.0
5. Insulin-treated patients	180	12.8
6. Insulin-untreated patients	96	33.3
7. Mild diabetes	101	31.6
8. Medium-severe diabetes	121	12.4
9. Severe diabetes	54	16.6
10. Males	129	18.4
11. Females	147	21.0
12. Diabetes and tuberculosis	15	33.3

Discussion

These results demonstrate, for the first time to our knowledge, the presence of PIA in the serum of diabetics. Moreover they reveal a greater incidence of precipitation reactions in those subjects never treated with insulin. It has been objected [24] that such precipitation reactions are nonspecific, based upon experiments using bacterial or tissue extracts as the antigen. Such criticisms do not apply to our experiments because: 1. a highly purified protein (insulin), and more especially human insulin, was used as the antigen; 2. there was an absence of positive precipitation in all the control subjects; 3. the precipitation rings dissolved completely in excess of the antigen; 4. the absence of lipids was shown by staining with Sudan-Schwarz B.

Thus it would appear that the precipitation complex really is a product of the antigen-antibody reaction. The failure to establish PIA using the method of BERSON and YALOW [2, 3], that is using paper electrophoresis with immune-serum to which ^{131}I-insulin has been added, could be explained by the precipitate remaining at the origin and thus not separated from the free ^{131}I-insulin [10].

The fact that PIA were found not only in the sera of diabetics treated with insulin but also in those not treated, introduces the subject of insulin autoantibodies. Until 1963 most authors rejected such a possibility [3, 5, 7, 15, 22, 25], but recently there have been several communications that indicate the possible existence of autoimmunity to insulin under certain conditions (in cows, [20, 21]; in guinea pigs and rabbits, [9]). These authors showed that animals would produce antibodies when immunized with homologous insulin, that would bind their own insulin. PAV et al. [17] and later CHETTY and WATSON [5] showed antibody-like activity by the complement-consumption method in diabetic patients not treated with insulin. They presumed that either there was the production of such antibodies in connection with inflammatory processes in the pancreas [17] or complement was fixed to a complex formed by insulin and its carrier-protein [5]. Our results add to these observations and raise the

question whether diabetes, or at least certain forms of the disorder, could be an autoimmune disease. A number of arguments in favour of this view have already been advanced [4, 9, 21].

The higher frequency of positive precipitation in cases of mild maturity-onset diabetes, and especially in prediabetes, together with the occurrence of the reaction with diabetics never treated with insulin could be explained by the hypothesis that the production of the higher titre of PIA represents a peculiarity of the early phases of diabetes. Such an autoimmunity could arise because of the production and secretion by the pancreas of an immunologically-different insulin or insulin-complex, which not being "recognized" by the organism, acts as an antigen. This might bring about an increase in the peripheral insulin resistance, raising the functional requirement of the B-cells, and later the development of insulin deficiency. The existence of PIA in the early stage of diabetes would indicate that at this stage the pancreas is secreting "antigenically-active" insulin; later on, the secretion of the antigenic form diminishes, possibly in parallel with the diminution in the secretion of non-antigenic insulin, leading to a discontinuation of the production of PIA. Thus the lower incidence of precipitation reaction in long-standing diabetes could be accounted for.

Although this hypothesis is only a speculation, stimulated by the results of our investigation of PIA in diabetic patients, both treated and nontreated with insulin, it may be fruitful in suggesting areas for further investigation.

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