Characterization of Antibodies to the Insulin Receptor

A CAUSE OF INSULIN-RESISTANT DIABETES IN MAN

JEFFREY S. FLIER, C. RONALD KAHN, DAVID B. JARRETT, and JESSE ROTH

From the Diabetes Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT We have characterized the circulating inhibitor of insulin receptor binding found in several patients with a new syndrome of extreme insulin resistance. The inhibitor is an immunoglobulin by multiple criteria, including precipitation by 33% ammonium sulfate, migration on G-200 Sephadex gel filtration and DEAE chromatography, and immunoprecipitation with specific anti-human immunoglobulins. Although predominantly IgG, some activity is found in the IgM fraction of the immunoglobulins in one patient. The inhibitory immunoglobulins reacted with antisera to both κ and λ light chain determinants and are therefore polyclonal. In addition, activity is retained in the $F(ab')_2$ fraction of pepsin-digested IgG. Evidence suggests that these antibodies are directed at determinants on or near the insulin receptor, and that they are responsible for the observed clinical insulin resistance.

INTRODUCTION

We have previously reported six patients with an unusual syndrome of extreme insulin resistance in whom none of the previously known causes of insulin resistance could be identified (1). Most of the patients have been female, ranging in age from 11 to 49 yr, and have suffered to variable degrees from the skin condition acanthosis nigricans. Glucose tolerance ranged from normal to severely impaired. Insulin levels, both basal and stimulated, were elevated 10 to 100-fold, and insulin resistance was marked when directly assessed by insulin tolerance testing. In each of the patients, insulin binding to its specific membrane receptors on circulating monocytes was markedly reduced (1).

The clinical features shared by these patients and several similar patients previously reported (2–7) suggested two distinct clinical syndromes, which we have designated types A and B (1). Type A patients have been younger females with signs of virilization or accelerated growth, in whom the receptor defect may be primary. Type B patients have been older, and have had a variety of clinical features suggesting an autoimmune disease, including leukopenia, hyperglobulinemia, arthralgias, and antinuclear and anti-DNA antibodies. This prompted a search for a circulating antibody that might alter insulin receptor function. In a previous communication we demonstrated that plasma of these three patients contained a factor or factors that could specifically reduce insulin binding after brief exposure to normal cells or membranes (8). In this report we demonstrate the immunoglobulin nature of this inhibitor, and present its preliminary characterization.

METHODS

All sera were obtained after an overnight fast and were stored at −20°C until use. A 33% ammonium sulfate precipitate was prepared by addition of drops of a saturated solution of ammonium sulfate to serum at 4°C with constant shaking. After 1 h, the precipitate was sedimented at 10,000 *g* for 60 min. The precipitate was resuspended in 0.15 M phosphate-buffered saline (PBS),[1] and both supernatant and resuspended precipitate were dialyzed for 48 h against 200 vol of PBS at 4°C. 95% of the IgG was found within the precipitate as determined by quantitative immunoelectrophoresis. Sera were fractionated by gel filtration and DEAE chromatography as described in the legends.

Preparation of $F(ab')_2$ fraction of IgG. 4 mg of IgG (DEAE peak 1, see Fig. 1) in 0.4 ml of 0.01 M phosphate buffer, pH 8.0, was incubated for 24 h at 37°C with 0.6 ml of

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[1] *Abbreviation used in this paper*: PBS, phosphate-buffered saline.

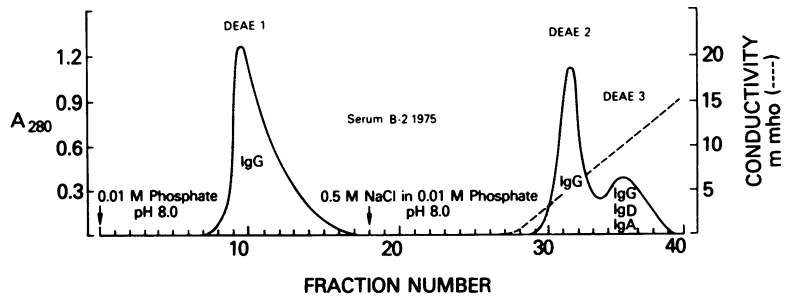

FIGURE 1 DEAE fractionation of 33% ammonium sulfate fraction of serum B-2. The 33% ammonium sulfate precipitate of serum B-2 was resuspended in 3 ml of 0.01 M K_2HPO_4:KH_2PO_4 (potassium phosphate buffer) at pH 8 and dialyzed against 1 liter of the buffer at 4°C. The sample was then applied to a DEAE cellulose (DE52, Whatman) column (1.5 × 30 cm) that had been equilibrated in the same buffer, and 3-ml samples were collected. Most of the IgG molecules eluted with 60 ml of this buffer (DEAE peak 1). The remaining immunoglobulins (DEAE peaks 2 and 3) were eluted with a linear salt gradient developed with 0.5 M NaCl. The immunoglobulins in the various fractions were characterized by immunodiffusion in agar with specific rabbit anti-human immunoglobulin antibodies. The first peak appeared to be mainly IgG with a yield of 6 mg/ml of serum.

0.2 M sodium acetate buffer with 0.5 N NaCl, pH 4.5, containing 80 μg of pepsin (9). After 24 h, the pH was adjusted to 8.0 with 1 N NaOH, and the solution was dialyzed against a solution containing 0.1 M Tris HCl and 0.5 M NaCl, pH 7.4, for 24 h. The $F(ab')_2$ fraction was separated from undigested IgG by gel filtration on G-200 Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The $F(ab')_2$ nature of the fragment was confirmed by gel immunodiffusion and immunoprecipitation with specific anti-human IgG antibodies directed at the Fab and Fc fractions of the IgG molecule. Protein concentrations of the ammonium sulfate fraction, purified IgG, and $F(ab')_2$ were determined by the method of Lowry et al. (10) with a human immunoglobulin standard.

Immunoprecipitation. Rabbit antisera to human IgG (γ chain, Fab or Fc-fragment specific), IgM (μ-chain specific), IgA (α chain-specific), and light chain (κ and λ-specific) were purchased from Behring Diagnostics (American Hoechst Corp., Somerville, N. J.). Immunoprecipitation curves were obtained by mixing increasing amounts of anti-human immunoglobulin of indicated specificity with a constant amount of an appropriate dilution of serum or column eluate, with the total volume kept constant by addition of PBS. An incubation at 37°C for 60 min was followed by 24 h at 4°C, after which the immune precipitate was sedimented at 4°C at 2,000 rpm for 20 min in an International model PR-1000 centrifuge (Damon/IEC Div., Damon Corp., Needham Heights, Mass.). A sample of the supernatant from each tube was assayed for inhibition of insulin binding. The precipitate was washed twice at 4°C with PBS, pH 7.6, and the absorbance at 280 nm of the resuspended precipitate was determined.

Insulin binding studies. Porcine insulin (Eli Lilly & Company, Indianapolis, Ind.) was iodinated to a sp act of 100–200 μCi/μg by a modification of the chloramine-T method (11). Human lymphoblastoid cells (IM-9), maintained in continuous culture in plastic flasks, were grown to stationary phase at 37°C in Eagle's minimum essential medium (NIH Media Unit) supplemented with 10% fetal calf serum, penicillin, and streptomycin. Glutamine (0.29 mg/ml) was added to the medium just before transfer of cells on the day of feeding. Cells were split twice a week and fresh medium was added. Viability of cells was monitored by trypan blue dye exclusion and was always greater than 90%. We have previously shown that insulin receptors on these cells (12) have binding properties essentially identical to those of insulin receptors on circulating human monocytes (13), as well as more classical targets of insulin action such as liver (14) and fat (15). For this assay, IM-9 cells were sedimented and resuspended at a concentration of 15×10^6 cells/ml in an assay buffer containing 100 mM Hepes, 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, and 1% bovine serum albumin, pH 7.6. A 0.2-ml cell suspension was incubated for 1 h at 4° or 22°C with 0.2-ml samples of either serum or partially purified immunoglobulin at dilutions indicated in the figure legends. After 1 h, cells were washed three times with 1 ml of PBS at 4°C. The cell pellet was resuspended in 0.5 ml of assay buffer containing 100 pg of ^{125}I-insulin and incubated for 90 min at 15°C. In every case, duplicate sets of tubes were prepared, one set of which also contained unlabeled insulin to give a final concentration of 10 μg/ml. At the end of the incubation, duplicate 200-μl aliquots were layered onto 100 μl of cold buffer in microfuge tubes and centrifuged in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 1 min. The supernatant was aspirated and discarded and the radioactivity in the pellet was determined. Specific ^{125}I-binding was considered to be the difference between tracer binding in the absence and presence of 10 μg/ml of unlabeled insulin.

RESULTS

Serum from three patients with insulin resistance and evidence for a circulating inhibitor of insulin receptor binding were studied. The titers of the circulating inhibitor in these sera varied over a wide range. Titers, expressed as that dilution of serum which depressed by 50% the binding of ^{125}I-insulin to 3×10^6 cells, ranged from 1/2 to 1/4,000 (Fig. 2) and correlated well with the degree of insulin resistance in these patients, as assessed by the intravenous insulin tolerance test (1). In one patient studied on two occasions 1 yr apart (B-2 1974 and 1975), the titer increased 10-fold.

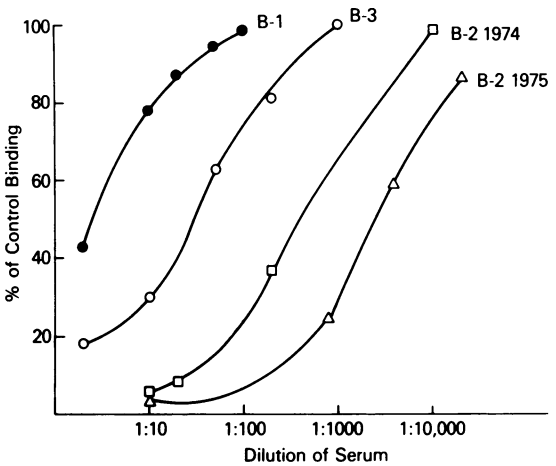

FIGURE 2 Titers of insulin-binding inhibitor in patient sera. 3×10^6 IM-9 lymphocytes were incubated for 60 min at 22°C in a volume of 0.4 ml with serum dilutions as indicated or with buffer. Cells were then washed three times with 1 ml of PBS, pH 7.6, at 4°C, and then resuspended in two sets of tubes containing 0.5 ml of assay buffer, to which was added 100 pg of ^{125}I-insulin. One set of tubes contained in addition 10 μg/ml unlabeled insulin. Cells and insulin were incubated for 90 min at 15°C, after which duplicate 200-μl samples were centrifuged through 100 μl of cold buffer in a Beckman microfuge for 1 min, the supernatant was aspirated, and the ^{125}I-insulin bound to the pellet was determined. Specific ^{125}I-insulin binding was considered to be the difference between tracer binding in the absence and presence of 10 μg/ml of unlabeled insulin. The percent of control binding was calculated by dividing the percent bound to antibody-treated cells by the percent bound to buffer-treated cells and multiplying by 100.

The circulating inhibitor appeared to be an immunoglobulin by multiple criteria. First, the inhibitor was quantitatively precipitated by 33% $(NH_4)_2SO_4$ in each of these three sera. Studies of inhibition of insulin binding with the reconstituted 33% $(NH_4)_2SO_4$ fraction (which contained more than 95% of the immunolgobulins) were superimposable with those obtained with whole serum (Fig. 3). When calculated per microgram of protein, the inhibitory activity was concentrated three-to fivefold in the reconstituted precipitates. No significant inhibitory activity remained in the supernatant fraction (data not shown).

To further localize the inhibitory activity, sera were fractionated on columns of G-200 Sephadex. Three main protein peaks were seen. The first is known to contain primarily IgM with some α_2 macroglobulins, the second peak is mainly IgG (plus some IgA and IgE), and the third peak is primarily albumin and some globulins with mol wts less than 100,000 (16). With serum B-2, the entire activity coeluted with the "IgG" peak. No activity was present in the prominent IgM fraction or in the peak albumin tubes (Fig. 4). With serum B-3, the main inhibitory activity again corresponded to the IgG peak; however, some activity clearly coeluted with the macroglobulin peak as well (Fig. 4). Immunoprecipitation experiments carried out on fractions from the G-200 separation of serum B-3 suggested that the inhibitory activity of the macroglobulin peak was due to IgM immunoglobulins (Fig. 5). Thus, the inhibition of binding caused by a sample from the macroglobulin peak (fraction 18) was almost entirely abolished by immunoprecipitation with increasing amounts of anti-human IgM antisera, while addition of anti-human IgG had no effect. Conversely, the inhibitory activity of the IgG peak (fraction 23) was entirely eliminated by immunoprecipitation with anti-IgG, but not at all by anti-IgM. The inhibitory activity of serum B-1 was too low to permit an accurate evaluation of column fractions.

The immunoglobulin nature of the inhibitor was further suggested by the results of the DEAE fractionation. Insulin binding was inhibited by peaks 1, 2, and 3 from the DEAE fraction of the ammonium sulfate precipitate of serum from patient B-2 (Fig. 6). All three peaks contained IgG by immunodiffusion, although the concentration of IgG and inhibitory activity was much higher in peak 1 than in the later peaks. The inhibitory activity again could be neu-

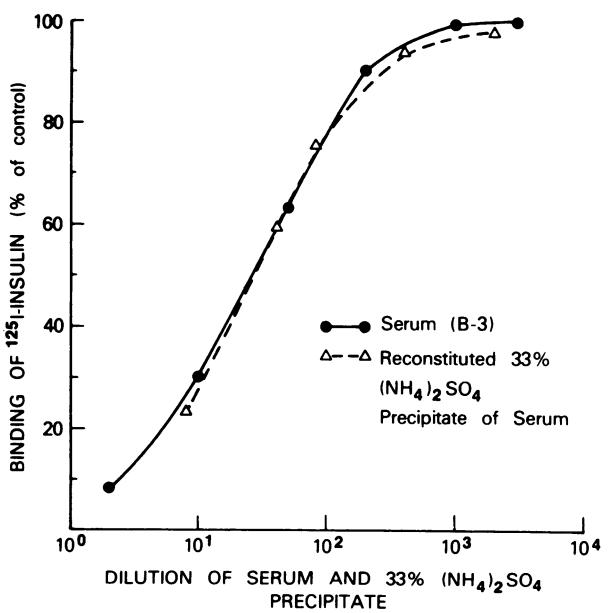

FIGURE 3 Effect of whole serum and 33% ammonium sulfate precipitate of serum on ^{125}I-insulin binding to IM-9 lymphocytes. 2 ml of serum B-3 was precipitated by 33% ammonium sulfate, as described in the Methods, resuspended in 2 ml of PBS, and dialyzed against 200 vol of PBS at 4°C. 3×10^6 IM-9 lymphocytes were then incubated for 60 min at 4°C in 0.4 ml of assay buffer with final dilutions of whole serum or reconstituted 33% ammonium sulfate precipitate as indicated. Cells were then washed three times in 1 ml of PBS at 4°C, after which an insulin-binding assay was performed as in legend to Fig. 2.

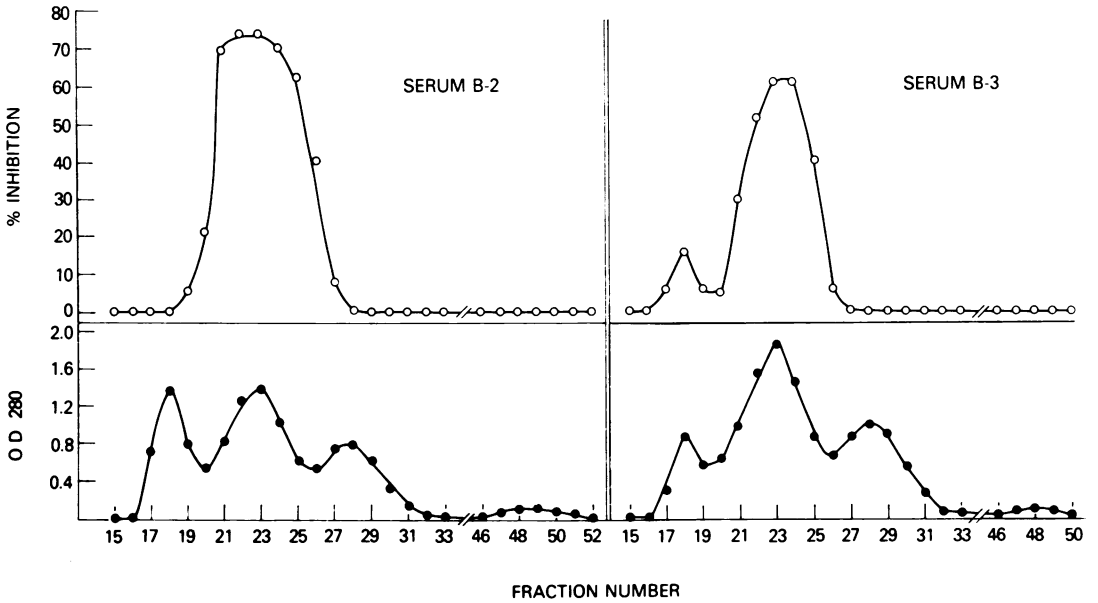

FIGURE 4 Effect of G-200 Sephadex column fractions on ^{125}I-insulin binding to IM-9 lymphocytes. 2 ml of each serum (B-2, 1975, and B-3) was fractionated on a 2.5 × 100 cm G-200 Sephadex column equilibrated with a 0.1 M Tris, 0.1 M NaCl buffer at pH 7.6. 10-ml fractions were collected, and absorbance at 280 nm was determined. 3×10^6 IM-9 lymphocytes were incubated in a final volume of 0.4 ml of assay buffer with samples of each column fraction or with buffer. With serum B-2 fractions 10-μl samples were used, while 200-μl samples were used for serum B-3. After 60 min at 22°C, cells were washed twice in 1 ml of PBS at 4°C, and then resuspended in 0.5 ml of assay buffer for insulin binding assay as described in legend to Fig. 2. Percent inhibition of binding was calculated as: [1 − (% specifically bound to antibody-treated cells/% specifically bound to buffer-treated cells)] × 100.

tralized by adding to the IgG containing fraction anti-IgG with specificity for either the Fab or Fc portion of the molecule (Fig. 7). When the specific inhibitory activities of the various preparations of immunoglobulin from serum B-2 were compared (Fig. 6), a progressive increase in activity during the purification was evident. Thus, 50% inhibition of binding occurred with 110 μg of plasma protein, with 20 μg of the ammonium sulfate precipitate, and with 18 μg of the IgG of DEAE peak 1. The second DEAE peak, shown by gel diffusion to contain some IgG, produced a 50% inhibition of binding with 46 μg of protein. For comparison, in the same assay, porcine insulin produced 50% inhibition of tracer binding at a concentration of 15 ng/ml.

Further evidence that the entire inhibitory activity of the patients serum was immunoglobulin in nature was provided by immunoprecipitation studies with whole serum. Specific immunoprecipitation of serum B-2 with rabbit anti-human IgG completely depleted the serum of inhibitory activity, while anti-human IgA and IgM were without effect (Fig. 8). These findings correlate well with the pattern observed on G-200 Sephadex and DEAE fractionation (*vide supra*). In addition, precipitation with both specific anti-κ and anti-λ antisera removed inhibitory activity from serum (Fig. 8). Anti-κ antisera removed 40% of the activity, and anti-λ removed 65%. Immunoprecipitation of serum B-3 with anti-human IgG removed a maximum of 85% of the inhibitory activity (Fig. 9). Anti-IgA and anti-IgM antisera had no significant effect on binding inhibition produced by whole serum, although the amounts of these immunoglobulins precipitated were not great in these experiments. Further, the gel filtration experiments suggested that only a small fraction of the activity detected in serum was due to IgM and it may well be impossible to detect the depletion of this amount of the activity under these experimental conditions.

To prove that the IgG-mediated inhibition of insulin binding is mediated by the $F(ab')_2$ portion of the molecule, and not through Fc binding, $F(ab')_2$ fragments were obtained by peptic digestion of IgG. The $F(ab')_2$ fragments clearly inhibited insulin binding. This inhibition was completely prevented by prior immunoprecipitation with anti-IgG with $F(ab')_2$ specificity but not with anti-IgG directed at the Fc region (Fig. 7).

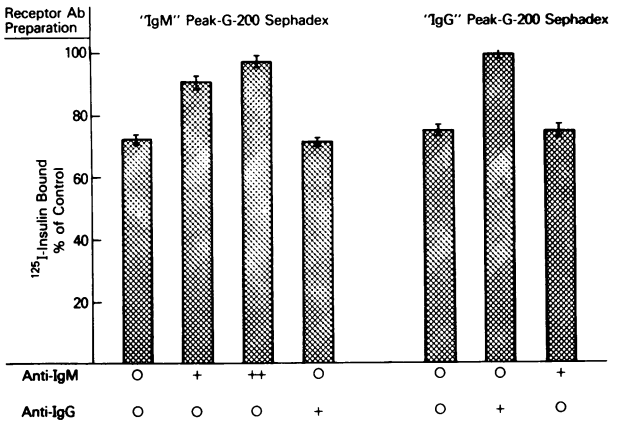

FIGURE 5 Effect of specific immunoprecipitation on binding inhibitory activity of G-200 column fractions of serum B-3. 200-μl samples of fraction 18 of the Sephadex G-200 fractionation of serum B-3 (described in the legend to Fig. 4) were incubated in duplicate with 200 μl PBS, 200 or 400 μl anti-human IgM, or 200 μl of anti-human IgG, with final volumes brought to 800 μl with PBS. Similarly, 200-μl samples of fraction 23 were each incubated in duplicate with 200-μl of PBS, anti-IgG, or anti-IgM. The mixtures were incubated at 37°C for 60 min and then at 4°C for 24 h, after which the immune precipitates were sedimented at 2,000 rpm for 20 min. 100-μl samples of the supernatants from each tube were then incubated with 3×10^6 IM-9 lymphocytes in 0.4 ml of assay buffer at 22°C for 60 min, washed twice with 1 ml of PBS, and then resuspended in 0.5 ml of assay buffer for insulin-binding assay. Percent of control binding was calculated as described in legend to Fig. 2.

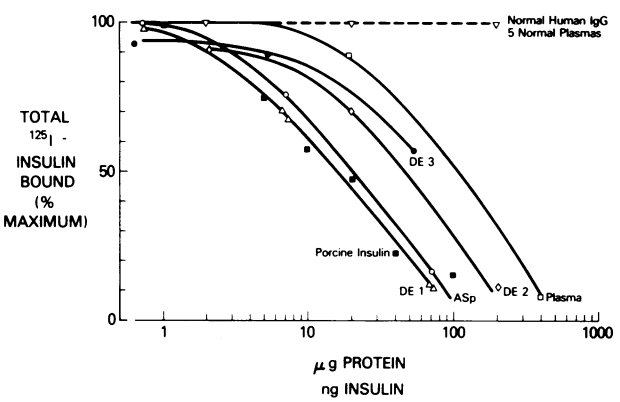

FIGURE 6 Inhibition of ^{125}I-insulin binding by various serum preparations. 5×10^6 IM-9 lymphocytes were incubated with 100 pg of ^{125}I-insulin in a total volume of 0.5 ml of assay buffer, in the presence of increasing amounts of several preparations of serum B-2, normal IgG, or porcine insulin. The preparations included whole plasma, 33% ammonium sulfate precipitate (ASp), DEAE peaks 1, 2, and 3, and porcine insulin. After 90 min at 15°C, duplicate 200-μl samples were centrifuged through 100 μl of cold buffer, the supernats were aspirated, and ^{125}I-insulin bound to the pellets was determined. Percent of maximal binding was calculated as: (% bound to cells in presence of serum preparation or insulin)/(%bound to cells in presence of buffer alone).

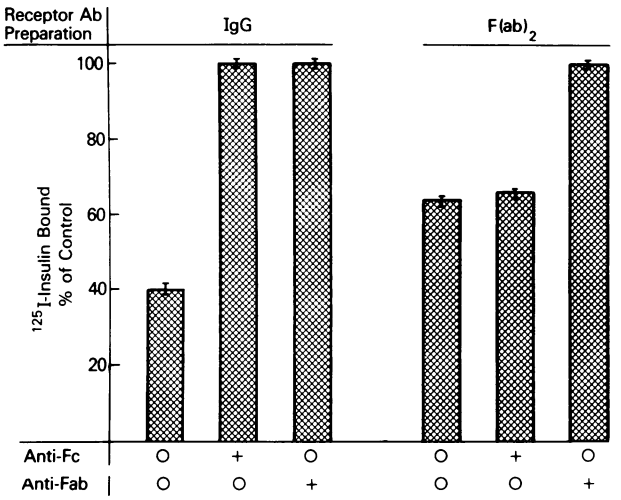

FIGURE 7 Effect of IgG and $F(ab)_2$ fractions of IgG on ^{125}I-insulin binding to IM-9 lymphocytes. (left) 100 μl of DEAE peak 1 (obtained as in legend to Fig. 1) was incubated in duplicate with 100 μl of anti-human IgG directed at the Fc or the Fab portion of the molecule, or with PBS alone. After 1 h at 37°C, the immune precipitates were sedimented in a Beckman Microfuge, and duplicate 50-μl samples of the supernate were assayed for inhibition of insulin binding. Assay tubes contained 5×10^6 IM-9 lymphocytes, 100 pg of ^{125}I-insulin, and 50 μl of the above supernates or PBS alone, with a final volume of 700 μl/tube. One set of tubes contained, in addition, 10 μg/ml of unlabeled insulin. The assay was completed and data were calculated as in the legend to Fig. 2. (right) An $F(ab)_2$ fraction of IgG was prepared as described in Methods. 50-μl samples were incubated in duplicate with 100 μl of anti-human IgG with specificity for the $F(ab)_2$ or Fc regions, or with PBS alone for 1 h at 37°C. Immune precipitates were sedimented, and duplicate 50-μl samples of the supernatants were assayed for inhibition of insulin binding as described above.

DISCUSSION

Immunologically mediated insulin resistance has generally been equated with the development of antibodies to exogenously administered insulin (17). Such anti-insulin antibodies may cause insulin resistance in a small percentage of insulin-treated diabetics, but in the vast majority of diabetics the antibodies are too low in affinity or capacity to have any clinical effect.

We have recently described a group of diabetic patients with severe insulin resistance (1, 8). The patients had several features suggestive of an autoimmune illness. However, anti-insulin antibodies were undetectable or of sufficiently low titer to exclude an etiologic role in insulin resistance. In addition, direct studies of insulin binding to receptors on circulating monocytes of these patients suggested a defect at the level of the insulin receptor (1, 8). We previously demonstrated that the decrease in insulin binding was due to a circulating factor that could reproduce the binding defect with insulin receptors on normal

cells or membranes (8). Using a variety of techniques, we have now demonstrated that the circulating inhibitor of insulin receptor binding in these patients is an immunoglobulin.

In each serum, the inhibitory activity was quantitatively recovered in the 33% ammonium sulfate precipitate, a fraction known to contain most of the immunoglobulins of serum. The inhibitory activity of this fraction diluted superimposably with the activity of whole serum, suggesting identity of the activity in these two fractions.

In the two sera with the highest titers, characterization of the activity was confirmed by gel filtration and immunoprecipitation techniques. In serum B-2, essentially all of the activity co-migrated in the "IgG" region on a G-200 Sephadex column, with no activity coincident with the macroglobulin peak.

Immunoprecipitation of this serum with specific anti-human immunoglobulins confirmed these findings, since all activity was removed by anti-IgG and none by anti-IgM or IgA antisera. The inhibitory activity of this serum was depleted by immunoprecipitation with antisera directed at either κ or λ light chain determinants, showing that each of these antisera will remove a fraction of the inhibitory activity and suggesting that the antibody in this case is polyclonal.

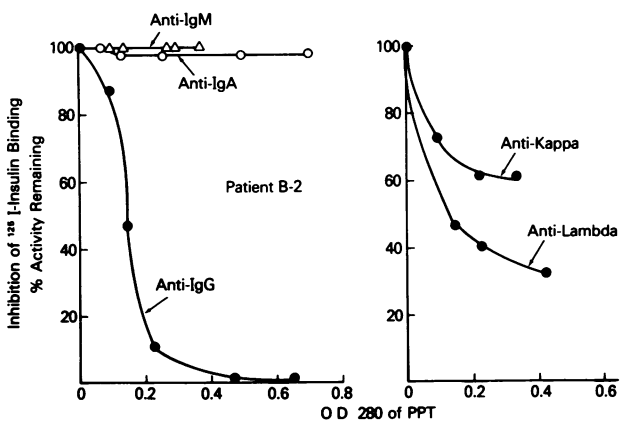

FIGURE 8 Effect of specific immunoprecipitation on binding-inhibitory activity of whole serum. 10 μl of serum B-2, 1975, were incubated with various amounts of anti-human immunoglobulin antisera of indicated specificity in a final volume of 500 μl. The mixture was incubated at 37°C for 60 min and then at 4°C for 24 h, after which the immune precipitate was sedimented at 2,000 rpm for 20 min. The precipitate was washed twice with PBS, pH 7.6, and the OD at 280 nM of the resuspended precipitate was determined. A 100-μl sample of the supernatant from each tube was then incubated with 3×10^6 IM-9 lymphocytes in 0.4 ml of assay buffer at 22°C for 60 min. after which cells were washed twice with PBS, and then resuspended in 0.5 ml of assay buffer for insulin binding assay. Percent inhibition of binding was calculated as described in the legend to Fig. 4. The percent activity remaining was calculated as: (% inhibition after immunoprecipitation/% inhibition before immunoprecipitation) $\times$ 100.

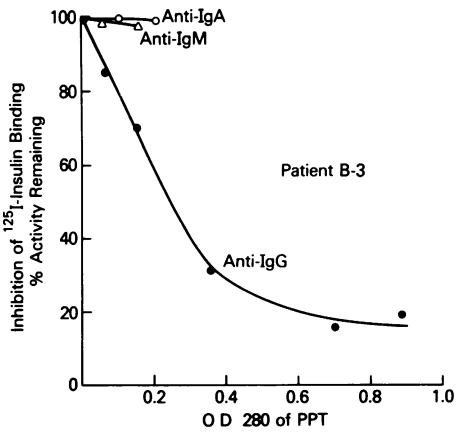

FIGURE 9 Effect of specific immunoprecipitation on binding-inhibitory activity of serum B-3. 200-μl samples of serum B-3 were incubated with various amounts of anti-human immunoglobulin antisera of indicated specificity in a final volume of 500 μl. Incubation and assay were carried out as described in the legend to Fig. 8.

Serum B-3 differed from serum B-2 in that inhibitory activity with this serum was found in both IgG and IgM immunoglobulins, as demonstrated by G-200 Sephadex fractionation, as well as by specific immunoprecipitation of relevant column fractions.

Finally, since immunoglobulins can bind through sites on the Fc region as well as through specific antibody-combining sites on the $F(ab)_2$ portion of the molecule, we prepared an $F(ab')_2$ fraction of IgG of patient B-2 by pepsin digestion. The purified $F(ab')_2$ fragment retained the ability to block insulin binding, further demonstrating that binding inhibition is mediated by the antibody-combining site of immunoglobulin molecules.

The circulating antibodies that block insulin receptor binding most likely act by binding directly to the insulin receptor. Evidence for this assertion comes from both indirect and direct studies. Firstly, these antibodies competitively inhibit insulin binding to receptors on a wide variety of tissues from several species, including cultured human lymphocytes and circulating human monocytes, rat adipocytes and hepatocytes, and avian erythrocytes (8). Such an effect might be caused by binding to the insulin receptor itself or to a closely linked membrane structure. Although no evidence exists for the latter possibility, it cannot be ruled out until the receptor is isolated in pure form. In addition, the binding inhibition is quite specific for the insulin receptor. Human growth hormone binding to its receptor on IM-9 lymphocytes is unaffected by serum incubations that markedly impair insulin binding to these cells (8). Similarly, the binding of labeled

nonsuppressible insulin-like activity (soluble), a peptide isolated from human serum, to its receptor on liver membranes is unaffected by sera that markedly impair insulin binding to its receptor on these same membranes.[2]

Studies of antibody interaction with isolated adipocytes also suggest binding determinants closely related to the insulin receptor (18). All three antibody-containing sera and their partially purified immunoglobulin fractions specifically impair insulin binding to its receptors on isolated rat adipocytes. These antibodies also show a variable ability to stimulate glucose oxidation and to block insulin stimulation of this biological process (18). This suggests that the antibody and insulin interact with the receptor similarly.

A previous report by Field et al. (2) describes a patient with a clinical syndrome similar to that of our patients. In this case, insulin-like biological activity was found in the immunoglobulin fraction of serum, but could at least in part be attributed to insulin bound to anti-insulin antibodies. Several lines of evidence in the present study make it extremely unlikely that insulin, free or bound to anti-insulin antibodies, could account for the inhibition of insulin binding produced by these sera or immunoglobulin fractions. Firstly, addition of very high concentrations of insulin to normal sera, or to sera with high titers of anti-insulin antibodies, caused no inhibition of labeled insulin binding under the conditions of preincubation and washes employed in these experiments. In addition, the species specificity of the inhibitory effect produced by these sera (8) is clearly different from the inhibitory effect of unlabeled insulin on ^{125}I-insulin binding to these cells or membranes. Further, the inhibition by patients' immunoglobulins of insulin binding and the stimulation of glucose oxidation in isolated adipocytes are both blocked by anti-IgG, and not by anti-insulin antibodies (18). Finally, the inhibitory effect persisted in several thousand-fold dilutions of serum,[3] even after depletion of insulin and anti-insulin antibodies by sequential affinity chromatography on insulin Sepharose columns, followed by ammonium sulfate precipitation.[4]

Recent work from our laboratory using ^{125}I-labeled immunoglobulin from these patients that has been affinity-purified on tissues rich in insulin receptors has been used to assess directly the site of antibody binding (19). These studies indicate that ^{125}I-insulin and ^{125}I-immunoglobulin bind in a parallel manner to a variety of tissues containing insulin receptors, and that labeled antibody binding is specifically competed for by insulin and insulin analogues in direct proportion to their affinity for the insulin receptor. Thus, insulin and antibody directly compete with each other for binding to a closely related membrane site.

Precedents exist for the development of autoantibodies directed at membrane receptors in human disease. Patients with myasthenia gravis have antibodies that bind to soluble preparations of human and rat muscle that contain receptors for the neurotransmitter acetylcholine (20–23). In some cases, these antibodies block the binding of labeled α-bungarotoxin (an irreversible cholinergic antagonist) to the receptor (20, 21). With most sera, however, the antibodies leave the cholinergic binding site intact, but in some way alter receptor function (23).

Other recent studies have also demonstrated that immunoglobulins from many patients with Graves' disease inhibit the binding of labeled thyroid-stimulating hormone (TSH) to human thyroid membranes (24). These same immunoglobulin preparations stimulate adenylate cyclase activity in this tissue (25–28), and when administered to test animals, stimulate thyroid hormone release (29, 30). Thus, the anti-TSH receptor antibodies in Graves' disease not only block the binding of TSH but also mimic its action.

The growing evidence that anti-receptor antibodies are important in myasthenia gravis, Graves' disease, and now in one variety of insulin-resistant diabetes raises two interesting questions. Firstly, by what mechanism do these unusual antibodies develop? Is the receptor a victim of a state of altered immunologic reactivity, or did some primary or induced receptor defect directly stimulate the observed antibody response? Second, we might wonder, as suggested by Lennon and Carnegie (31), whether some property of membrane receptors makes them particularly likely to be the objects of an immune response. If this were true, we would expect to find additional examples of receptor dysfunction mediated by anti-receptor antibodies.

The incidence of anti-insulin receptor antibodies among insulin-resistant patients is not yet established. In preliminary studies (32), we have found such antibodies in 7 of 16 cases of extreme insulin resistance, in whom no other causes could be demonstrated, but not in 70 additional sera from patients with diabetes, autoimmune diseases, and conditions in which other anti-receptor antibodies have been described. Whatever their incidence, these antibodies suggest new models for the pathogenesis of other states of hormone resistance and should serve as a powerful probe in further studies of the insulin receptor.

[2] Megyesi, K. Unpublished data.

[3] Direct determination of insulin levels in whole serum of patients B-2 and B-3 was made impossible by the presence of low titers of anti-insulin antibodies, which cause spuriously high values in the double antibody radioimmunoassay. Serum specimens diluted over 100-fold and immunoglobulin preparations used in these experiments had no detectable insulin by radioimmunoassay.

[4] Maratos-Flier, E. Unpublished data.

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