Characterization of the Serum from a Patient with Insulin Resistance and Hypoglycemia
Evidence for Multiple Populations of Insulin Receptor Antibodies with Different Receptor Binding and Insulin-mimicking Activities

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SUMMARY
The serum from a patient with lupus nephritis, insulin resistance, and hypoglycemia was studied. This serum both inhibits the binding of $^{125}$I-insulin to its receptor and has insulin-like activity on fat cells (see refs. 1 and 2). The IgG fraction from this patient’s serum one-half maximally inhibited $^{125}$I-insulin binding to IM-9 cells at 1 $\mu$M, but did not markedly inhibit $^{125}$I-monovalent antibody binding even at concentrations as high as 4 $\mu$M. The IgG was then subjected to affinity chromatography on a protein A-Sepharose column. Four protein peaks were eluted from this column by a step pH gradient from 5.5 to 2.3. Three of the four peaks inhibited $^{125}$I-insulin binding to its receptors, but none was more potent than the unfractionated IgG itself. One IgG peak, however, was able to inhibit $^{125}$I-monovalent antibody binding at tenfold lower concentrations than the unfractionated IgG. When the ability of the four IgG fractions to stimulate 2-deoxy[$^3$H]-D-glucose transport in rat adipocytes was studied, two fractions showed stimulatory activity. Compared with unfractionated IgG, one had a weak ability to inhibit $^{125}$I-insulin binding, but tenfold more potency to mimic insulin action. The other had a strong ability to inhibit $^{125}$I-insulin binding but less potency to mimic insulin action. These studies indicate, therefore, that the serum contains multiple populations of antibodies to the insulin receptor, or portions of the plasma membrane adjacent to the receptor, which have different biologic effects.


Patients with immunologic diseases, who have antibodies that have the ability to both inhibit the binding of labeled insulin to its receptor and to mimic the actions of insulin on many biologic functions, have been reported.1-7 Generally, these patients have the clinical symptoms of hyperglycemia, but occasionally, patients may have hypoglycemia, or a combination of hyper- and hypoglycemia.8,9 It is not known, however, whether the ability to inhibit the binding of insulin and the ability to mimic insulin action reside in one population of antibodies in several different classes of antibodies that have different effects. In order to investigate these possibilities, we have studied the serum of a patient with lupus nephritis, insulin resistance, hypoinsulinemia, and hypoglycemia.1 This serum both inhibits $^{125}$I-insulin binding to its receptor and stimulates glucose transport and oxidation in isolated rat adipocytes.2 To test whether multiple populations of antibodies were involved in these activities, the IgG from this serum was fractionated on a protein A-Sepharose affinity column and the separate fractions were then tested for their ability to interact with the insulin receptor and to stimulate glucose transport in isolated rat adipocytes.

MATERIALS AND METHODS
The patient’s serum was precipitated in 33% ammonium sulfate, suspended in potassium phosphate 0.01 M (pH 8.0) buffer, and applied to a DEAE cellulose (DE 52, Whatman, Ltd., Kent, UK) column equilibrated in 0.01 M phosphate buffer (pH 8.0). The eluate was concentrated against dry Sephadex G-200 and subsequently dialyzed three times at 4°C against phosphate (pH 7.4) buffer. Thereafter, aliquots used to perform binding or glucose transport studies were dialyzed twice at 4°C for 24 h against the same buffer used in subsequent experiments. An aliquot (~40 mg/ml) of the patient’s IgG suspended in Tris 0.05 M, NaCl 0.15 M (pH 9.0) buffer was absorbed at 4°C into a column containing ~14 mg protein A (protein A-Sepharose CL 4B, Pharmacia Fine Chemicals, Uppsala, Sweden), gel volume 7 ml, equilibrated in Tris 0.05 M, NaCl 0.15 M (pH 9.0) buffer. The column was then washed with 30 ml Tris buffer and eluted by applying sequentially: (1) citrate 0.05 M, NaCl 0.15 M
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RESULTS

Unlabeled insulin inhibits the binding of labeled insulin to IM-9 lymphocytes with one-half maximal activity occurring at 200 pM, mouse monoclonal antireceptor antibody with one-half maximal activity occurring at 4 nM, and the unfractonated IgG with one-half maximal activity occurring at 1 μM. In contrast, normal human IgG was without effect on 125I-insulin binding (Figure 1A). Next, unlabeled insulin competed with the binding of labeled monoclonal antibody with one-half maximal activity occurring at 400 pM, and with the binding of unfractonated monoclonal antibody with one-half maximal activity occurring at 2 nM. Unfractonated IgG, in contrast, was a very weak inhibitor of labeled monoclonal antibody binding since at a concentration of 4 μM only 35% of the labeled monoclonal antibody was inhibited (Figure 1B); moreover, at this concentration, normal human IgG interfered with labeled monoclonal antibody binding (Figure 1B).

The four antibody fractions isolated from the protein A column were then studied for their ability to inhibit the binding of labeled insulin to IM-9 lymphocytes. Antibodies eluted at pH 3.3 inhibited binding approximately one-half as well as the unfractionated IgG; the fractions at pH 5.5 and 2.3 had even less potency, and the fraction eluted at pH 4.3 was without activity (Figure 2A). Next, the effect of these fractions were studied on labeled monoclonal antibody binding to the insulin receptor (Figure 2B). The fraction at pH 3.3 one-half maximally inhibited labeled antibody binding at concentrations tenfold lower than those of the unfractionated IgG. In contrast, the other fractions had negligible effects on labeled monoclonal antibody binding, and were not different from normal IgG (Figure 2B).

(pH 5.5) buffer; (2) acetate 0.05 M, NaCl 0.15 M (pH 4.3) buffer; (3) glycine/HCl 0.05 M, NaCl 0.15 M (pH 3.3) buffer; (4) glycine/HCl 0.05 M, NaCl 0.15 M (pH 2.3) buffer. Fractions referring to antibodies eluted at pH 5.5, 4.3, 3.3, and 2.3 were collected, dialyzed immediately against Hapes 100 mM, NaCl 120 mM, EDTA 1.5 mM, Na-acetate 15 mM (pH 7.8) buffer at 4°C for 24 h, concentrated against dry Sephadex G-200, and finally dialyzed twice at 4°C for 24 h against the buffer used in the subsequent experiments.

125I-insulin binding and 125l-monoclonal antibody binding to IM-9 human lymphocytes were carried out in buffer containing 100 mM Hapes, 120 mM NaCl, 1.5 mM EDTA, 15 mM Na-acetate, 10 mM glucose (pH 7.8) with bovine serum albumin at 10 mg/ml. One to two × 10^5 cells/ml were incubated with either 125I-insulin (100 pM; 130 Ci/g) or 125I-monoclonal antibody (60 pM; 10 Ci/g), prepared as previously reported in detail, in the presence or absence of different amounts of insulin, monoclonal antibody, normal human IgG (Sigma Chemicals, St. Louis, Missouri), and unfractonated or fractionated patient’s IgG for 70 min at 15°C in a total volume of 0.5 ml. 125I-insulin binding and 2-deoxy-D-glucose uptake in isolated adipocytes were carried out on cells prepared from epididymal fat pads obtained from male Sprague-Dawley rats (140-180 g) according to the method of Rodbell. 12 125I-insulin binding studies were carried out by adding 125I-insulin (100 pM) to 2 × 10^6 cells/ml suspended in 35 mM Tris, 120 mM NaCl, 5 mM MgSO4, 10 mM glucose, 2 mM CaCl2, 24 mM Na-acetate, and 10 mg/ml bovine serum albumin (pH 7.6), in the presence or absence of insulin, unfractonated or fractionated patient’s IgG for 90 min at 24°C. Glucose transport studies were carried out on 2 × 10^6 cells/ml incubated in Krebs-Ringer bicarbonate (pH 7.4) buffer in the presence or absence of insulin, normal human IgG (Sigma Chemicals), and unfractonated or fractionated patient’s IgG for 60 min at 24°C; thereafter, each sample was incubated with 2-deoxy-[1-3H]-D-glucose (0.4 μCi/ml) (New England Nuclear, Boston, Massachusetts) plus 0.1 mM 2-deoxy-D-glucose (Sigma Chemicals) at 24°C for 3 min. 12

FIGURE 1. The effect of insulin (--), monoclonal antibody (—), unfractonated IgG (—), and normal human IgG (control) (—) on the binding of 125I-insulin and 125I-monoclonal antibody binding to IM-9 lymphocytes. (A) Specific 125I-insulin binding. (B) Specific 125I-monoclonal antibody binding. Each value was the mean of the triplicate determinations.

FIGURE 2. The effect of IgG fractions on the binding of 125I-insulin and 125I-monoclonal antibody binding to IM-9 lymphocytes. (A) Specific 125I-insulin binding. (B) Specific 125I-monoclonal antibody binding. Each value was the mean of duplicate determinations.
3H]-D-glucose was added and the incubation continued for 3 min, at
fractions (pH 5.5, 4.3, 3.3, and 2.3) inhibited the binding of insulin to
its receptor, but no fraction was more potent than unfractionated IgG by itself (data not shown).

We have previously reported that the serum from this patient stimulated glucose transport and other activities in iso-
ated rat adipocytes. In the present study, the unfractionated
IgG from this serum was active in stimulating deoxyglucose
transport with one-half maximal effect occurring at 5 µM
(Figure 3). When the IgG fractions were studied the fraction
at pH 5.5 was more potent than unfractionated IgG, with
an activity on transport that was slightly less than that for the
unfractionated IgG. The remaining two fractions, as well as
normal human IgG, were without effect (Figure 3).

DISCUSSION

In the present study, we have analyzed the serum from a
different populations of antireceptor antibodies. In order to
understand the ability of these sera to inhibit the binding of insulin to its receptor and to mimic insulin action, we pre-
viously prepared from mice a monoclonal antibody to the
human insulin receptor. This monoclonal antibody competes
with insulin for the binding to its receptor in human but not
animal cells, and does not mimic the actions of insulin, and
when added with insulin it blocks insulin's effects.

To study the nature of the antibodies present in this pa-
tient's serum, we first investigated the ability of the patient's
IgG to inhibit the binding of both labeled insulin and labeled
monoclonal antibody to the insulin receptor. The IgG from
the patient's serum inhibited the binding of insulin to its re-
ceptor but showed relatively lower potency in inhibiting the
binding of labeled monoclonal antibody to the insulin re-
ceptor. Next, we fractionated the patient's IgG by affinity
chromatography over protein A-Sepharose. Four protein
peaks were obtained with this procedure. Three of the frac-
tions (pH 5.5, 3.3, and 2.3) inhibited the binding of insulin to
its receptor, but no fraction was more potent than unfrac-
tionated IgG by itself. In contrast, however, one fraction (pH
3.3) was able to inhibit the binding of the monoclonal anti-
tibody to its receptor and this fraction was more potent than
unfractionated IgG. These studies suggested, therefore, that
(1) the patient's serum contains several IgG populations that
displayed varying abilities to inhibit the binding of insulin to
its receptor; (2) the insulin binding site is a complex molecule
with several antigenic sites since the monoclonal antibody
and major part of the patient's antibodies were directed to-
ward different determinants.

When deoxyglucose transport was studied, only two frac-
tions (pH 5.3 and 3.3) mimicked the stimulation of insulin.
Of interest was that the fraction eluted at pH 5.5 had a rel-
atively weak ability to inhibit insulin binding but had a marked
ability to stimulate transport. These data further indicate that
the serum from this patient contains multiple populations of
antibodies. Moreover, comparison of data obtained with IgG
eIgG eluted at pH 5.5 and 3.3 indicates that certain antibodies
have major effects on insulin binding, whereas other anti-
bodies have major effects to mimic insulin action.

The present studies suggest, therefore, that there are dif-
erent populations of antibodies that recognize different an-
tigenic sites. Thus, there are antibodies whose predominant
activity is to inhibit insulin binding and antibodies whose
predominant activity is to mimic insulin action. The latter may
not even be directed against the insulin receptor since it has
been shown that antibodies directed toward various mem-
brane proteins of adipocytes mimic the effects of insulin. These findings may explain, therefore, the clinical symptoms
of either hyper- or hypoglycemia seen in patients since the
clinical course may depend on the relative concentrations
of these different types of antibodies.

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The effect of fractionated IgG on labeled insulin binding
to isolated adipocytes was comparable with that obtained
in experiments carried out on IM-9 cells. In fact, the pH 4.3
fraction was unable to inhibit insulin binding to adipocytes,
whereas three fractions (pH 5.5, 3.3, and 2.3) inhibited the
binding of insulin to its receptor; however, no fraction was more potent than unfractionated IgG by itself (data not shown).

Studies with cells in vitro have indicated that the antire-
ceptor sera from patients with both hyper- and hypoglycemia
inhibit the binding of labeled insulin to its receptor in several
cell types, and, in addition, mimic many of the actions of
insulin. It has been observed, however, that sera from some patients have a marked ability to inhibit insulin binding
and a relatively weaker ability to stimulate biologic func-
tions. Conversely, other sera have a relatively weak potency
to inhibit insulin binding and a relatively stronger potency to
stimulate biologic activity. On the basis of these data, it
has been hypothesized that these antireceptor sera contain
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REFERENCES