1) This procedure clearly differentiates fluorescent patterns on three specific chromosome regions; this allows new research on the specialized structural and functional properties of these regions, and identification of the similar regions that may exist in other species as well.

2) The orange-red fluorescent bands produced by daunomycin and adriamycin do not appear as bright as those produced by the Q-banding technique. However, the fading of fluorescence and the disruptive effect of ultraviolet light on chromosomes is less pronounced as compared with the Q-banding technique. D-bands can still be observed several days after staining. The use of this antibiotic as fluorochromes for chromosome binding is not as sensitive to variations in ionic strength as has been observed in the use of quinacrine (*5*). Thus, it may be more convenient to use the D-band technique for routine chromosome analysis.

3) Anthracycline antibiotics have been shown to have some base specificity in their interaction with DNA (*11*). This class of compounds could provide information on the mechanism of chromosome bands produced by the fluorochrome. Preliminary data suggest that the daunomycin binding patterns may be due to differential fluorescent quenching by DNA regions with specific base sequences (*12*).

The anthracycline antibiotics used in this study are anticanceral agents and are shown to fluoresce brightly in nuclei and meiotic chromosomes of hamster cells shortly after in vivo injection (*13*). Further investigations can be initiated to observe both the biological and the pharmacokinetic effect of the neoplastic cells at a fluorometric level.

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Antibodies That Impair Insulin Receptor Binding in an Unusual Diabetic Syndrome with Severe Insulin Resistance

Abstract. *Six patients with a unique form of diabetes associated with extreme insulin resistance have markedly reduced insulin binding to specific receptors on their circulating monocytes. When normal insulin receptors were exposed to serum or immunoglobulin fractions from three of these patients in vitro the specific binding defect was reproduced.*

We have studied six patients, all nonobese females, with a unique diabetic syndrome associated with severe insulin resistance and the skin condition acanthosis nigricans (*1*). These patients are unique in that their plasma insulin concentrations, both basal and stimulated by glucose, are increased 10- to 100-fold, and their responses to injected insulin are markedly blunted, with some patients receiving 1000 times the usual dose of insulin to control blood glucose. Furthermore, the specific binding of [^{125}I]insulin to insulin receptors on their circulating monocytes is only 5 to 30 percent of normal. In this report, we demonstrate that a serum factor (most probably an antibody) from three of these patients alters the insulin receptor and im-

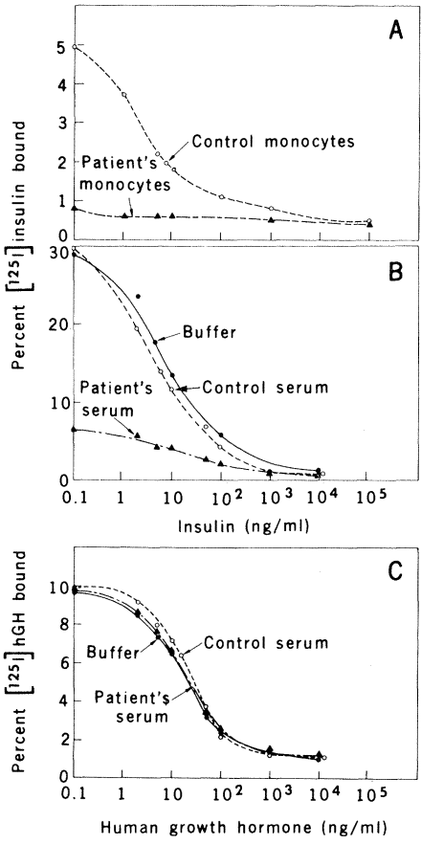

Fig. 1. (A) Binding of [^{125}I]insulin to circulating monocytes from a normal control and patient B with the syndrome severe insulin resistance. Mononuclear leukocyte preparations were obtained from buffy coats of each patient by passage through a Ficoll-Hypaque gradient (*9*). Cells were washed and resuspended in HEPES buffer (*p*H 8.0) at a concentration of 50×10^6 cells per milliliter; 20×10^6 cells were incubated in 0.5 ml of buffer containing 100 pg of [^{125}I]insulin (specific activity, 100 to 200 μc/μg) and increasing amounts of unlabeled insulin to give the final concentrations indicated in the figure. After 3 hours at 22°C, duplicate 200-μl samples were sedimented through 100 μl of cold buffer in a Beckman Microfuge. Supernatant was aspirated and the [^{125}I]insulin bound to the pellet was counted. The number of monocytes, which constitute the major insulin binding cell in this preparation, was determined by ingestion of latex beads (*3*). Binding was expressed as the percentage of [^{125}I]insulin bound per 10^7 monocytes. (B) Effect of serum preincubation on insulin binding to cultured lymphocytes. Human lymphoblastoid cells (IM-9) maintained in continuous culture (*2*) were grown to stationary phase in Eagle's minimum essential medium with 10 percent fetal calf serum. The cells were sedimented and resuspended at a concentration of 15×10^6 cells per milliliter in HEPES buffer (*p*H 7.6). Samples of cells (0.2 ml) were incubated with 0.2-ml samples of normal serum (control), serum from patient B, or buffer for 60 minutes at 4°C. Cells were then washed three times, once through 1.0 ml of cold fetal calf serum and twice in 1.0 ml of cold buffer. The cells were then resuspended in 0.5 ml of buffer at final concentration of 4.8×10^6 cells per milliliter and incubated with [^{125}I]insulin (100 pg/ml) in the presence of increasing amounts of unlabeled insulin for 90 minutes at 15°C. The percent of [^{125}I]insulin bound to duplicate 200-μl samples was determined as above. (C) Effect of serum preincubation on growth hormone binding to cultured lymphocytes. The IM-9 lymphocytes were preincubated and washed as above; 6×10^6 cells in 0.5 ml of HEPES buffer (*p*H 7.6) were then incubated with 100 pg/ml of human growth hormone (hGH) labeled with ^{125}I in the presence of increasing amounts of unlabeled hormone. After 90 minutes at 30°C binding was determined as above.

pairs subsequent binding of insulin. This factor recreates in vitro the defect in insulin binding observed with these patients' own cells and accounts reasonably for their extreme resistance to the effects of insulin.

In an attempt to demonstrate a serum factor that might alter the insulin receptor interaction, we studied the binding of [^{125}I]insulin to the insulin receptor in four well-characterized systems—circulating human monocytes, cultured human lymphocytes (IM-9 line), highly purified plasma membranes of rat liver (Sprague-Dawley rats from Dr. David Neville, National Institutes of Health), and fresh avian (*Meleagus gallopava* from NIH) erythrocytes. These insulin receptors have remarkably similar characteristics of insulin binding by a variety of precise and sensitive criteria (*2, 3*). For these studies, cells or membranes were exposed to serum or buffer for 1 hour at 4°C, washed extensively, and then exposed to [^{125}I]insulin for measurements of specific insulin binding.

When the cultured lymphocytes were exposed to the serum of patient B at a 1 : 2 dilution, the binding of insulin was reduced to 20 percent of normal (Fig. 1B). This decrease in insulin binding was comparable to that observed in direct binding studies in which the patient's own monocytes were used (Fig. 1A). In contrast, binding of [^{125}I]hGH (human growth hormone labeled with ^{125}I) to its receptors on the cultured lymphocytes (*4*) exposed to serum was unaffected over the entire range of growth hormone concentrations (Fig. 1C). Neither insulin nor growth hormone binding was altered by preincubation with normal control serum. In similar experiments, preincubation with serums from patients A and C (referred to as serums A, C, and so forth) reduced insulin binding to cultured human lymphocytes to 6 and 28 percent of control, respectively (Fig. 2). Serum from patient A, which had the greatest inhibitory effect, reduced binding to 50 percent of control even at a dilution of 1 : 400.

This inhibitory effect was observed in other tissues containing insulin receptors. Thus, normal human monocytes preincubated with serums A, B, and C exhibited a marked decrease in subsequent insulin binding (5 to 55 percent of control) (Fig. 2). With rat liver plasma membranes, serum A markedly reduced insulin binding, while serums C and F produced a small effect (Fig. 2). With avian erythrocytes, only serum A reduced insulin binding (Fig. 2).

With the exception of a small effect of serum from patient F on insulin binding to rat liver membranes, serums D, E, and F did not affect insulin binding to receptors on any of these four tissues. In addition, preincubation with normal serums and serums from 55 other patients, including a wide spectrum of diabetics, did not reduce insulin binding below 70 percent of buffer control. Similarly, serums from patients with lupus erythematosus containing high titers of autoantibodies, serums with high titers of anti-insulin antibodies, and normal serums to which high concentrations of insulin had been added were without significant effect (Fig. 2).

It is highly likely that the serum factor that inhibits [^{125}I]insulin binding to its receptor is an immunoglobulin (*5*). The inhibitory activity was precipitated by 33 percent ammonium sulfate and recovered almost quantitatively in the redissolved precipitate. On gel filtration on Sephadex G-200, nearly all the inhibitory activity coeluted with the immunoglobulin peaks. Finally, when rabbit antiserums (Behring Diagnostics) to human immunoglobulins were added to serum from the patients, the inhibitory activity precipitated in parallel with the immunoglobulins.

The inhibitory effect is not due to anti-insulin antibodies or insulin bound to such antibodies, since the effect was seen with serum from patient C who had no insulin antibodies, and this effect could not be reproduced with serums containing high titers of anti-insulin antibodies alone or in combination with insulin (Fig. 2). Furthermore, the variation in effect seen with a single serum specimen on different receptor preparations excludes the possibility that the decreased binding is accounted for by the presence of insulin, bound or free, in these serums, since insulin (as well as proinsulin and other well-characterized insulin-like peptides) would inhibit binding to all of these receptors equally (*3*).

Antibodies might affect the hormone-receptor interaction by several mechanisms.

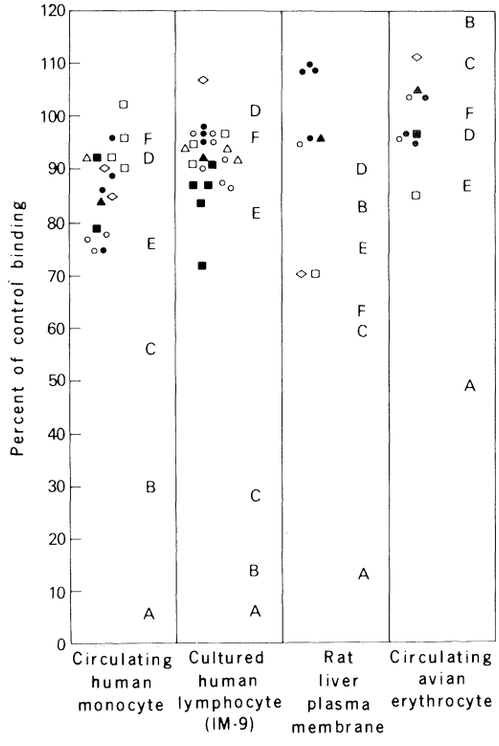

Fig. 2. Effect of serum preincubation on subsequent binding of [^{125}I]insulin to insulin receptors. Mononuclear leukocytes were obtained from normal human buffy coats by Ficoll-Hypaque separation (*9*), IM-9 human lymphocytes were obtained from continuous culture as noted in Fig. 1, and rat liver plasma membranes were prepared by the method of Neville (*10*). Avian erythrocytes were obtained from turkey blood by centrifugation and washed three times to remove any white cell contamination (*3*). Samples of cells or cell membranes were incubated for 1 hour at 4°C with an equal volume of serum from patients (A through F) with the syndrome of severe insulin resistance, from patients with other diseases as indicated, or with buffer. The receptor preparations were then washed three times as described in the legend of Fig. 1, with the exception of the rat liver membranes for which the fetal calf serum wash was omitted. These serum preincubated and washed receptors were tested for specific insulin binding as follows. (i) Human mononuclear cells (25×10^6) were incubated in 0.5 ml of HEPES buffer (*p*H 8.0) with [^{125}I]insulin (100 pg/ml) for 90 minutes at 15°C (*3, 11*). (ii) Cultured human lymphocytes (2.4×10^6) were incubated in 0.5 ml of HEPES buffer (*p*H 8.0) with [^{125}I]insulin (100 pg/ml) for 90 minutes at 15°C (*12, 13*). (iii) Rat liver plasma membranes (100 μg of membrane protein) were incubated in 0.5 ml of Krebs-Ringer phosphate buffer (*p*H 7.5) with [^{125}I]insulin (100 pg/ml) for 60 minutes at 30°C (*3, 11, 13*). (iv) Avian erythrocytes (3×10^8 cells) were incubated in 0.5 ml of tris buffer (*p*H 7.8) with [^{125}I]insulin (100 pg/ml) for 180 minutes at 15°C (*3*). In all cases duplicate sets of incubation tubes were prepared, one set of which also contained unlabeled insulin to give a final concentration of 10 μg/ml. At the end of incubation duplicate 200-μl samples were taken from each sample and centrifuged in a Beckman Microfuge for 1 minute. 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 the unlabeled insulin (*3*). Nonspecific insulin binding varied between 2 and 10 percent of the total insulin bound, depending on the receptor preparation, but was not significantly affected by the serum preincubation. Results, expressed as percent of control binding, were calculated as [(percent specifically bound to serum preincubated cells)/(percent specifically bound to buffer preincubated cells)] × 100. Patients A to F with syndrome: ●, normal control; ▲, control plus insulin (50 ng/ml); ○, obese diabetic; □, juvenile diabetic; ■, systemic lupus; Δ, thin hyperinsulinemic diabetic; and ◇, serum with insulin antibodies.

The antibody might occupy the receptor site directly or bind to the cell on or near the insulin receptor producing steric hindrance of insulin binding. Alternatively, the antibody might interact with a membrane component distant from the receptor, inducing a change in the membrane which subsequently alters the insulin-receptor interaction. Our data suggest that in these patients the antibodies have a wide spectrum of specificities. Although the antibodies are clearly much more active in inhibiting insulin binding to human tissues, serum A was reactive with cells as distant from man as those of the avian erythrocyte.

Although the decrease in receptors could be accounted for by "blocking antibodies" in only three of the six patients, it is possible that the insulin resistance in the others is due to similar antibodies with concentrations or affinities too low to be detected. An antibody in the other three patients might be directed at some membrane site which does not immediately alter insulin binding, but which decreases insulin binding on more prolonged exposure by altering kinetics of receptor turnover. Alternatively, the receptor defect in these patients might have an entirely different pathophysiology.

There are precedents for the interaction of antibody with functional receptors on the cell surface. Lindstrom and others have shown that patients with myasthenia gravis have high titers of antibodies that bind to preparations of human and rat muscle that contain receptors for the neurotransmitter acetylcholine (*6*). Active immunization of laboratory animals with preparations of the acetylcholine receptor produces a syndrome with many features of myasthenia gravis and circulating antibodies that bind to skeletal muscle preparations containing acetylcholine receptors (*6*). Other recent studies indicate that immunoglobulins from patients with Graves disease may compete with thyrotropin for binding to thyrotropin receptors on human thyroid. These antibodies also stimulate the adenylate cyclase activity in this tissue (*7, 8*), producing a clinical state of hormone excess.

In three cases reported here, we demonstrate that autoantibodies may alter the insulin receptor, cause impaired insulin binding, and result in a clinical syndrome of extreme insulin resistance. Similar autoantibodies may be responsible for other idiopathic disorders of hormone resistance.

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Life Cycle Variants of *Physarum polycephalum* That Lack the Amoeba Stage

Abstract. *The myxomycete life cycle ordinarily proceeds in the sequence plasmodium-spore-amoeba-plasmodium. Extraordinary variants are described in which the sequence is plasmodium-spore-plasmodium.*

The life cycle of the acellular slime mold *Physarum polycephalum* normally includes two vegetative stages: small uninucleate amoebae and large multinucleate plasmodia (*1*). The conversion from the amoebal form to the plasmodial form is under genetic control, and has been the subject of a number of recent studies (*2–4*). Sporulation, which occurs only in plasmodia, and spore germination, which gives rise to the amoebal form, complete the life cycle. We now describe the isolation of variants in which the amoebal phase is missing, that is, in which germinating spores release plasmodia rather than amoebae.

The amoeba-less life cycle (ALC) variants were isolated from amoebae carrying heterothallic alleles of the mating type locus. Ordinarily, such amoebae are haploid and produce plasmodia only by crossing between two amoebae carrying different alleles (for example, *mt*1 and *mt*3) of the locus. Plasmodia formed in this way are diploid, and produce spores carrying the two mating types in equal numbers. An asexual mode of plasmodium formation is also known. In this mode, plasmodia form in clones of amoebae and the plasmodia have the same ploidy as the amoebae from which they were formed (*3, 4*). The asexual mode is displayed by amoebae carrying the allele *mt*h (*5*), by amoebae heterozygous for mating type (*4*), and, rarely, by amoebae carrying heterothallic alleles (*4*).

The procedures for growing amoebae, forming plasmodia from amoebae, growing and sporulating plasmodia, and germinating spores have been reported (*4, 6*). All of the strains used in our study were derived from heterothallic strains (*mt*1, *mt*3, or *mt*4) in a Colonia genetic background (*6, 7*). For microscopic observation of germination, a sterile suspension of spores and formalinized *Escherichia coli* was inoculated onto a block of LIA (liver infusion agar) in a glass chamber slide. A cover slip was placed on top of the inoculated agar block. The cover slip also came into contact with the chamber walls and this connection was sealed with paraffin. For observations of spherules, the formalinized *E. coli* was omitted and plasmodial rich medium agar was used (*6*) instead of LIA. The chambers were incubated at 26°C.

When large numbers of heterothallic amoebae are incubated for a sufficient period of time, occasional plasmodia appear.