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.

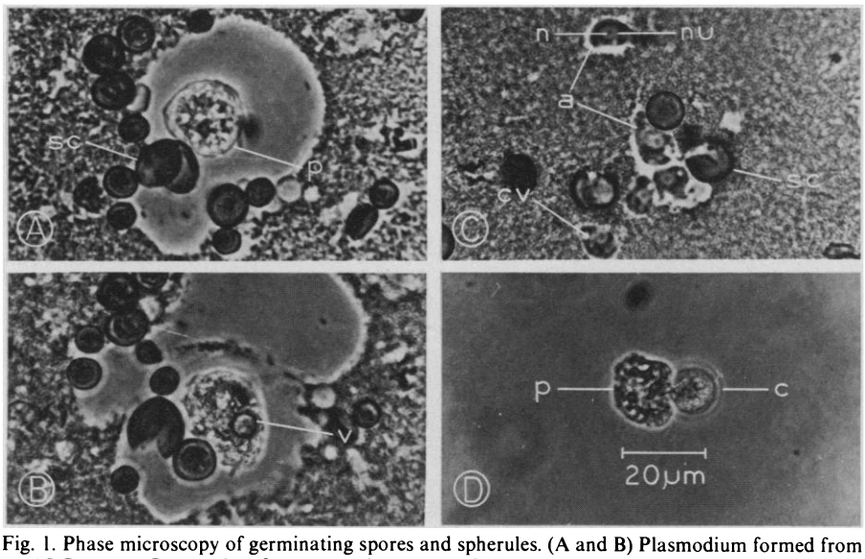

Fig. 1. Phase microscopy of germinating spores and spherules. (A and B) Plasmodium formed from an ALC spore; (C) amoebae from normal spores; and (D) plasmodium from a spherule. Symbols: *a*, amoeba; *c*, spherule casing; *cv*, contractile vacuole; *n*, nucleolus; *nu*, nucleus; *p*, plasmodium; *sc*, spore case; and *v*, food vacuole containing a spore.

As already shown, most of the plasmodia appear not to be the result of mutation, since spores of the plasmodia release amoebae that are indistinguishable from the original heterothallic parent (*4*, *8*). Examination of a large number of plasmodia has revealed that some are certainly variants. One class of variant is characterized by amoebae that tend to form plasmodia asexually. The other class, which is our subject, is characterized by an absence of the amoebal phase.

Wild-type spores plated on a lawn of bacteria yield amoebal plaques after 5 days of incubation. When spores produced by an ALC strain are plated, 5 to 10 days later small plasmodia become visible. The area around such a plasmodium shows no evidence of amoebae. Spores obtained from plasmodia derived from platings of ALC spores germinate to yield plasmodia; thus, the ALC property is transmitted through the life cycle and appears to be the result of mutation. Seven ALC strains have been isolated in an examination of about 100 plasmodia produced asexually by heterothallic amoebae. Two of the ALC's were derived from *mt*1 amoebae, two from *mt*3 amoebae and three from *mt*4 amoebae. The spores of ALC plasmodia germinate with typical frequencies of 10^{-3} to 10^{-4}, although frequencies in the range 10^{-2} to 10^{-5} have been observed. This compares to germination frequencies of 10^{-1} to 10^{-2} for spores of a sexually formed plasmodium and 10^{-3} to 10^{-4} for spores of a clonally formed plasmodium. Depending on the variant, ALC spores may yield plasmodia only, mainly plasmodia with occasional amoebae, or roughly equal numbers of plasmodia and amoebae.

To determine whether plasmodia do indeed germinate from ALC spores we have observed the germination microscopically. As a control, the germination of wild-type spherules and spores was also studied. Culture chambers inoculated with ALC spores were scanned twice a day. No germination was seen until 5 days after plating. At this time, plasmodia were seen emerging from a number of spores. Once a spore was seen germinating, it was observed at intervals ranging from once an hour to twice a day in various experiments. Figure 1A shows a typical plasmodium 2 days after germination. The cytoplasm of the plasmodium is granular and refractile. There is a large clear zone surrounding the plasmodium, which is presumably due to the secretion of slime. The empty spore case from which the plasmodium germinated is seen below it. Clamshell-like spore cases are routinely found after the germination of ALC spores. Newly germinated ALC plasmodia grow very slowly and remain almost stationary for several days. Figure 1B shows the same plasmodium 2 days later at which time it had just started to move and grow more vigorously. A spore is inside a food vacuole in the plasmodium at this time. Ultimately, the entire chamber became covered by one plasmodium as a result of growth and fusion of individual plasmodia. The spore from which the plasmodium shown in Fig. 1, A and B, germinated was larger than a normal spore. This was common for plasmodia-yielding spores, although on two occasions plasmodia were seen to germinate from normal-sized spores. The ploidy of the nuclei in the plasmodia at the time of germination is not yet known. Determination of the size and number of nuclei in vivo was prevented by the granular nature of the plasmodia. The viability and unity of newly germinated ALC plasmodia may not be perfect. On one occasion a plasmodium seemed to split in two and later fuse again. We cannot rule out the possibility that there was a thin strand of cytoplasm connecting the two plasmodia, however. A number of ALC plasmodia appeared to die 2 to 6 days after germination. This may be due to poor conditions in the chamber, as a similar observation was made on plasmodia that germinated from spherules.

For comparison, the germination of spores produced by a plasmodium formed by crossing *mt*3 and *mt*4 amoebal strains that had been inbred to Colonia (*6*) was observed. Amoebae began to germinate 12 to 24 hours after plating. Amoebae divided shortly after germinating from spores, indeed in some cases before the amoebae were completely out of the spore cases, as has been reported by Mohberg *et al.* (*9*). Figure 1C shows a colony of amoebae derived from two spores 2 days after inoculating the chamber. The amoebae are less refractile and granular than plasmodia. A nucleus and nucleolus as well as a contractile vacuole are clearly visible. Two empty spore cases, which resemble the empty spore cases of ALC spores, are visible. The amoebae are not surrounded by a large clear zone. Amoebae grow rapidly and in 4 days the chamber was covered with amoebae.

We have also examined the germination of spherules (*1*) obtained from an old shaker culture of plasmodia (Fig. 1D). A spherule is a plasmodial storage form that hatches to yield a plasmodium that is genetically unchanged. About 80 percent of the spherules germinated in 12 to 24 hours. The plasmodia that germinated from spherules resembled the ALC plasmodia with regard to granulation and the difficulty in visualizing nuclei.

In another type of experiment, we formed a heterokaryon between an ALC plasmodium and a wild-type plasmodium. When the spores of the heterokaryon were germinated, both amoebal and plasmodial clones appeared. Thus, it seems unlikely that the defect in this ALC strain involves a diffusible substance needed during sporulation.

The genotype at the *mt* locus has a marked influence on the rate at which amoebae form plasmodia; *mt* does not, however, influence the properties of the amoebal and plasmodial states, once they are established (*4*). Thus, the system must include the equivalent of a bistable circuit that maintains the differentiated state. The putative ALC mutations could affect either

the establishment or the maintenance of the amoebal state, or both. Since the ALC plasmodia were obtained by screening plasmodia formed from heterothallic amoebae, it seems likely that the mutation causes at least a defect in the maintenance of the amoebal state. The results of the heterokaryon experiment are also compatible with a maintenance defect. The occasional amoebae that arise in platings of ALC spores may be due to reversion, suppression or leakiness of an ALC mutation. If they are due to suppression or leakiness, it will be possible to use them in further genetic analysis of the ALC defect. Preliminary results indicate that three ALC strains yield only phenotypically true revertants, one ALC strain yields only amoebae that are temperature sensitive for growth and form plasmodia in clones, and one ALC strain yields amoebae of more than one phenotype. Thus, we will be able to analyze further at least two of the ALC mutants.

Henny (*10*), studying progeny of a cross between two natural isolates in *Physarum flavicomum*, found spores that seemed to give rise to plasmodia without any evidence of amoebae. In *P. polycephalum*, spores of sexually produced plasmodia occasionally yield amoebae that are heterozygous for *mt* (*4*), and these amoebae form plasmodia at a very high rate. Since the *P. flavicomum* spore germination was not observed microscopically, the apparent plasmodia-producing spores may actually have been mating type heterozygotes.

The ALC variants may prove useful for obtaining plasmodial mutants. Since we have seen plasmodia arise from normal-sized spores, it is likely that such plasmodia are "cloned" from a single nucleus. Large ALC plasmodia could be treated with a mutagen and made to undergo sporulation as the first step in obtaining mutants. Then the spores could be germinated and selection or screening performed on the resulting small plasmodia. This technique would obviate the current need (*7*) of mutagenizing amoebae and picking plasmodia formed from amoebal clones for later screening.

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Vasopressin: Induced Structural Change in Toad Bladder Luminal Membrane

Abstract. *Freeze-fracture electron microscopy demonstrates that vasopressin stimulation of isolated toad bladder alters the structure of the luminal membrane of granular cells. This alteration consists of an ordered aggression of intramembranous particles, and appears to be of functional significance, since the frequency of aggregation sites per area of membrane is closely correlated with vasopressin-induced osmotic water flow.*

The enhanced water permeability of the isolated toad urinary bladder in response to vasopressin is well established (*1*). The mechanism of this response appears to involve both vasopressin stimulation of cyclic adenosine monophosphate formation within epithelial cells of the toad bladder and subsequent alteration of the luminal membrane of these cells (*2*). While many details of this model have not yet been re-

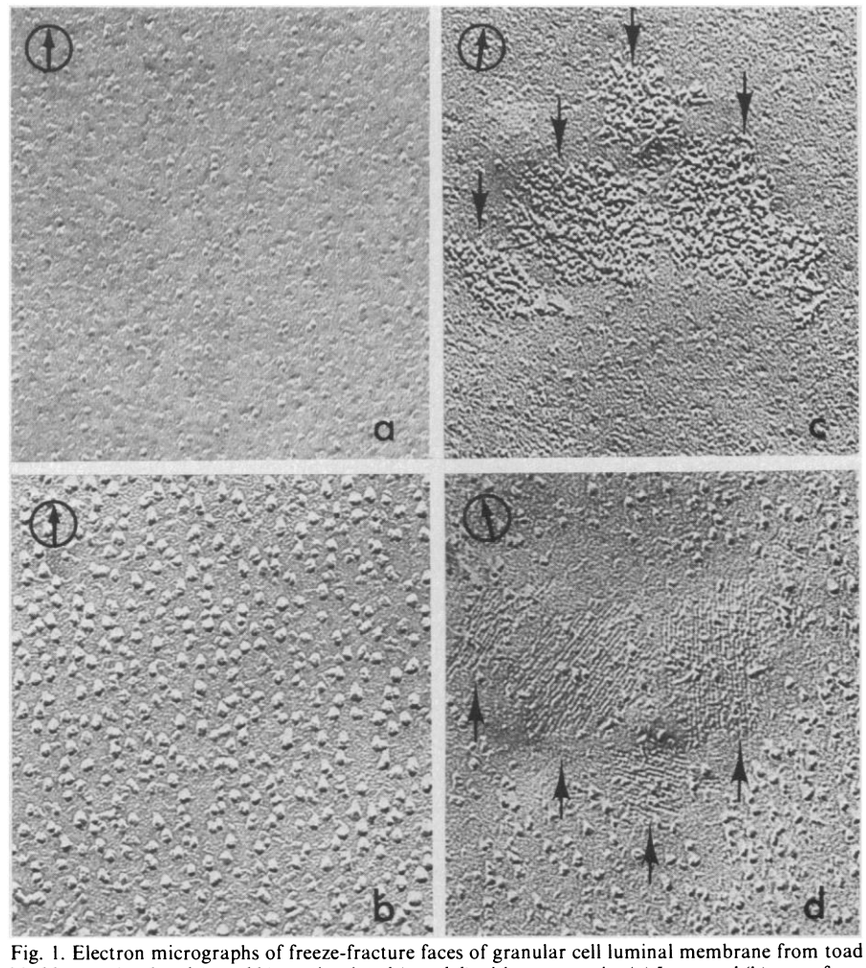

Fig. 1. Electron micrographs of freeze-fracture faces of granular cell luminal membrane from toad bladder unstimulated (a and b) or stimulated (c and d) with vasopressin. (a) Inner and (b) outer fracture faces; without vasopressin stimulation intramembranous particles are not aggregated. (c) Inner fracture face after vasopressin stimulation; separate sites of aggregated intramembranous particles are emphasized (single arrows). (d) Outer fracture face which is complementary to that shown in (c) after vasopressin stimulation; organized linear arrays of depressions corresponding to the aggregated intramembranous particles shown in (c) are emphasized (single arrows). Circled arrows indicate shadowing direction (× 83,000).