in scaphognathite motoneurons which could be due to the slow nonimpulsive component of the oval organ input (*17*).

In fibers Y and Z, the spikes superimposed upon the receptor potentials also reflect the stimulus parameters. For example, the number of spikes evoked by a step pull of constant duration is proportional to the pull amplitude over at least a major part of the response range of both Y and Z (Fig. 2b). Their frequency increases during the dynamic phase (ramp) of a constant velocity pull and varies with its velocity. The threshold amplitude of fiber Y is lower than that of fiber Z, both with rapid pulls of graded amplitude (Fig. 2b) and in the responses to large pulls at low velocities. At suprathreshold amplitudes, fiber Z fires initially at higher frequencies than Y, but adapts more rapidly, probably because of the apparently faster decline of its underlying receptor potential (Fig. 2b).

Thus, at least fibers Y and Z are capable of conveying sensory information normally, by means of trains of impulses, in addition to that coded in the graded potentials. The brief, highly phasic discharge of one or at most a few spikes in fiber X is not likely to have much significance for encoding amplitude, but it probably has other functions. For example, it may provide timing cues about the onset or phase of, or disturbances to, rhythmic movements of the gill bailer (*10, 15*).

We believe this is the first report of a sensory receptor whose afferent fibers carry both spikes and graded depolarizations, over a distance of 1 cm or more, into the central nervous system. Similar dual signaling is known in molluscan photoreceptors (*18*), amacrine cells of the vertebrate retina (*19*), and in motoneurons of the lobster stomatogastric ganglion (*20*); it may be present in a range of cell types in vertebrate and invertebrate nervous systems. The lobster oval organ may thus prove to be a useful model for studying the cellular properties of such neurons.

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13. Average fiber diameters were: X, 41 μm; Y, 32 μm; Z, 22 μm; motor axons MN1 and MN2, 25 μm. Mean nerve length, 12.5 mm (range 10.8 to 15 mm).
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8 July 1981; revised 21 September 1981

Inhibition of Idiotype–Anti-Idiotype Interaction for Detection of a Parasite Antigen: A New Immunoassay

Abstract. *Described in this report is an immunoradiometric assay of general applicability that is based on a new principle: the inhibition of the interaction between monoclonal antibodies by an antigen. The advantages of this assay are that it measures concentrations of single epitopes, purified antigen is not required, and the reagents can be obtained in unlimited amounts and are homogeneous. Its features are particularly attractive when the antigen has not been purified and is a minor component of a complex mixture of molecules.*

A monoclonal antibody (3D11) was recently produced in BALB/c mice against a 44,000-dalton membrane protein (Pb44) of sporozoites of *Plasmodium berghei*, a rodent malaria parasite (*1, 2*). This antibody (of the immunoglobulin IgGl, κ chain isotype), injected intravenously into mice protects the animals against infection with sporozoites.

Here we report the isolation of a second monoclonal antibody against the idiotype of 3D11, and describe an immunoassay based on the specific inhibition of the interaction between the two monoclonal antibodies by Pb44. This assay has been named 4i, which stands for inhibition of idiotype–anti-idiotype interaction. It does not require purified antigen and should have general applicability.

The monoclonal antibody to 3D11 was obtained by injecting BALB/c mice at various skin sites with 3D11 cross-linked with rabbit IgG by treatment with glutaraldehyde (*3*). A total of 1 to 2 mg of protein was given per mouse over a period of 10 weeks. The spleen cells of two animals were used for fusion with the plasmacytoma cell line SP2 (*4*) as described in (*5*). We screened the supernatants of the cultured cells for an activity that would inhibit the reaction between ^{125}I-labeled 3D11 and a conventional rabbit antiserum to the 3D11 idiotype (*6*). From a total of 700 wells originating from two fusions we found one supernatant that contained antibodies with the desired properties. The cells from the positive well were expanded and cloned by limiting dilution. The resulting hybridoma (2D12) was injected intraperitoneally into pristane-treated mice to obtain ascites fluid. A monoclonal antibody (IgG2a, κ chain) was present in this fluid at concentrations of 2 to 4 mg/ml.

We next studied the effect of the Pb44 antigen on the 3D11/2D12 interaction.

0036-8075/82/0326-1637$01.00/0

When Pb44 is associated with the membrane of sporozoites the binding of 3D11 can be detected by the circumsporozoite reaction (*7*) or by indirect immunofluorescence (*8*). Preincubation of 3D11 with 2D12 at equimolar concentrations inhibited both reactions, suggesting that 2D12 bound to an epitope of 3D11 close to or in its antigen-combining site.

The effect of sporozoite extracts on the 3D11/2D12 reaction was evaluated by a solid-phase immunoassay (4i-assay) performed as described in the legend of Fig. 1. As shown, the extracts of *P. berghei* inhibited the 3D11/2D12 reaction in a dose-dependent manner. Significant inhibition was obtained with extracts from as few as 100 sporozoites (*9*). Extracts of *P. cynomolgi* (a simian malaria parasite), or of salivary glands of normal mosquitoes, had no effect. To demonstrate that the inhibition was due to Pb44, we treated the extracts with Sepharose beads coupled with 3D11, or as a control with beads coupled with 2D12. The removal of Pb44 by the specific immunoabsorbent reversed the inhibitory effect of the extracts.

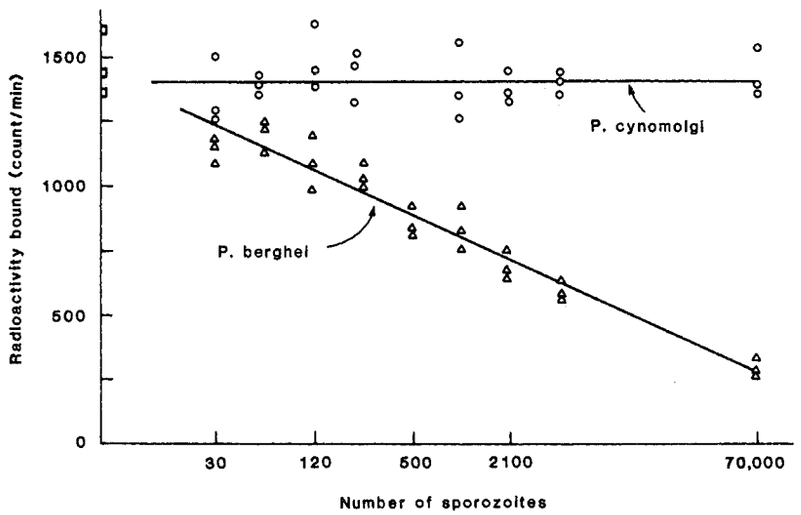

Fig. 1. Inhibition of the 3D11/2D12 interaction by extracts of *P. berghei* sporozoites. Fifty microliters of 3D11 (50 μg/ml in PBS) were placed into wells of disposable flexible plastic plates. After 2 hours of incubation at room temperature, the wells were washed several times with PBS-BSA. Then 25 μl of dilutions of an extract of *P. berghei* in PBS were added to the wells. The *P. berghei* extract was prepared by disrupting the partially purified parasites in a French pressure cell (*1*). After two additional hours of incubation, 10 μl (1.5×10^4 count/min) of a solution in PBS-BSA of ^{125}I-labeled 2D12 monoclonal antibody (specific activity, 2×10^7 count/min-μg) were added. The incubation proceeded overnight at 4°C. The plates were washed several times with PBS-BSA and the individual wells counted in a gamma counter.

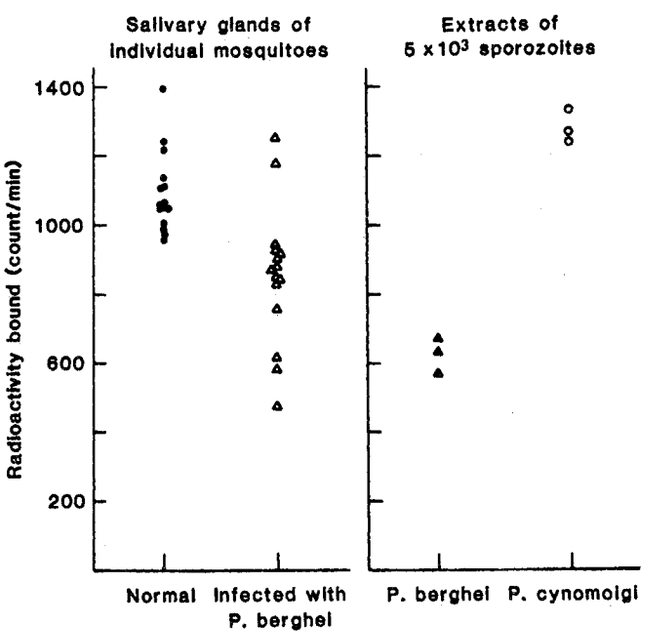

Fig. 2. Inhibition of the 3D11/2D12 interaction by extracts of individual mosquitoes. Whole salivary glands from normal or *P. berghei*–infected mosquitoes were put into propylene plastic tubes containing 40 μl of PBS-BSA. Each sample was frozen and thawed five times and then incubated in boiling water vapors for 5 minutes. After centrifugation at 8000*g* for 2 minutes, the supernatants were assayed for inhibitory activity as described in the legend of Fig. 1. Extracts of partially purified *P. berghei* and *P. cynomolgi* obtained by centrifugation of parasites disrupted in a French pressure cell were used as positive and negative controls.

We also examined the possibility that the 4i-assay was sensitive enough to detect infection in single mosquitoes (Fig. 2). Salivary glands were dissected from a group of *Anopheles stephensi* mosquitoes fed 21 days previously on *P. berghei*–infected hamsters. A sample of 30 glands was examined under phase microscopy, and 90 percent of them were found to be infected with sporozoites. Other salivary glands were extracted individually in 40 μl of phosphate-buffered saline containing 1 percent bovine serum albumin (PBS-BSA) by freezing and thawing followed by boiling for 5 minutes in water vapors. (Preliminary experiments had shown that the Pb44 epitope recognized by 3D11 was heat-stable.) As a control, salivary glands of normal mosquitoes were subjected to the same treatment. A significant difference between the results of the 4i-assay in the two groups ($P < .001$, one-tailed *t*-test) was found.

The 4i-assay differs in one essential aspect from all other radioimmunoassays (RIA) or immunoradiometric assays (IRMA). Whereas the sensitivity and specificity of RIA and IRMA depend primarily on the affinity of an antibody for the corresponding antigen, the 4i-assay is based on two specific reactions which take place at or close to the antigen-combining site of one antibody, and on the inhibitory effects that they have on each other (*10*). In the 4i-assay, antibody 1 binds antigen, and antibody 2 binds antibody 1. The outcome will of course depend on the dissociation constants of both reactions. In the present case we estimated that the apparent dissociation constant for the reaction between 3D11 and 2D12 was 2×10^{-9} mole/liter and between 3D11 and the antigen it was 4.6×10^{-11} mole/liter.

In principle the 4i-assay can be used to detect any antigen present in a complex mixture of molecules, provided that monoclonal antibodies 1 and 2 are available. Different strategies have been successfully developed to obtain monoclonal antibodies against minor antigenic components of biological fluids or cell membranes. The special problem in the development of a 4i-assay can be that of generating monoclonal antibody 2 with the desired properties. However, a growing number of observations indicate

that these may not be difficult to obtain, if a sensitive assay for detection of antibody in hybridoma supernatants is available. For example, Legrain *et al.* (*11*) obtained in BALB/c mice 17 monoclonal antibodies against a single BALB/c idiotype, and in every case the idiotype–anti-idiotype reaction was specifically inhibited by antigen. Similar observations have been made by others (*12, 13*). It should be pointed out that the two monoclonal antibodies do not have to be raised in a single strain of mice. Instead, monoclonal antibody 2 could originate in mice of a different allotype, or even in another animal species, for example, in rats.

Some of the attractive features of the 4i-assay are shared with those of IRMA performed with monoclonal antibodies. For example, the antigen does not have to be purified or labeled, and most labeled antibodies have a long shelf life. Also, because the antibodies are products of hybridomas, they are homogeneous and can be obtained in unlimited amounts. However, most variants of commonly used immunoradiometric methods, such as the sandwich or two-site IRMA (*14, 15*), require a minimum of two separate epitopes, whereas the 4i-assay requires only a single epitope.

This unique characteristic may be particularly attractive when the antigen is a small polypeptide, or weakly immunogenic, or when one is attempting to identify a portion of an antigen associated with an epitope. For example, the epitope of Pb44 recognized by 3D11 seems to be involved in the interaction of sporozoites with their target cell (*16*), and in protective immunity against malaria (*1, 2*). The isolation of a fragment of Pb44 bearing this epitope could be important for the development of a malaria vaccine. This and similar undertakings may be greatly facilitated by the availability of a sensitive and epitope-oriented assay, such as the one described here.

From the point of view of those interested in malaria and other vector-transmitted diseases, the present results indicate that the 4i-assay is sensitive enough to detect a relatively small number of sporozoites in crude extracts of mosquitoes, and might therefore be used in epidemiological surveys.

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11 November 1981; revised 15 January 1982

Prairie Dogs Avoid Extreme Inbreeding

Abstract. *Black-tailed prairie dogs (Rodentia: Sciuridae:* Cynomys ludovicianus*) live in colonies composed of contiguous but separate family groups called coteries. During the 6 years that individuals in a colony were observed, they almost never mated with close genetic relatives. Inbreeding is avoided in four ways: (i) a young male usually leaves his natal coterie before breeding, but his female relatives remain; (ii) an adult male usually leaves his breeding coterie before his daughters mature; (iii) a young female is less likely to come into estrus if her father is in her coterie; and (iv) an estrous female behaviorally avoids mating with a father, son, or brother in her coterie.*

Although inbreeding may sometimes be advantageous (*1, 2*), biologists generally assume that most organisms outbreed (*3*). The reasons, extent, and mechanisms of outbreeding, however, are rarely understood (*4*). Particularly in social species, the details of how and to what extent inbreeding is avoided may provide insights into significant biological and social questions (*5*). To acquire extensive knowledge of exact genetic relationships among social individuals requires long-term field studies. From a 6-year study of 200 marked individuals under natural conditions, I report four mechanisms of outbreeding in a highly social species, the black-tailed prairie dog (Sciuridae: *Cynomys ludovicianus*).

Black-tails are large, diurnal rodents. At Wind Cave National Park, Hot Springs, South Dakota, where I study them, they mate in February and March, and weaned juveniles first emerge from their natal burrows in May and June. The area of my study colony is about 500 m by 130 m (6.6 hectares) and in late spring of each year contains 142.9 ± 33.6 (mean ± standard deviation) yearlings and adults (≥ 2 years) and 72.4 ± 36.9 juveniles arranged into 24.2 ± 1.47 family groups called coteries. A coterie typically contains one adult male, three to four adult females, and several yearlings and juveniles of both sexes. Males and females first breed as 2 year olds, although females occasionally breed as yearlings. Individuals remain within well-defined contiguous territories and are usually amicable toward members of their own coterie and hostile toward members of other coteries (*6*).

From 1975 through 1980, all young were marked with ear tags and fur dye before they mixed with young from other litters (*7*), so that exact genetic relationships through common female ancestors and probable genetic relationships through common male ancestors are now known for more than 90 percent of all colony residents (*8*). With three to six field assistants in February and March 1978, 1979, and 1980, I observed the colony with binoculars from three towers (5 m) for most (≥ 99 percent) of the daylight hours when prairie dogs were aboveground; we observed 94 periods of estrus, involving 74 different females.

Black-tail copulations usually occur underground during daylight hours, and only estrous females enter a burrow with adult males. Because there are specific behaviors associated with what we term an underground consortship (*9*), we are often able to pinpoint when a copulation

0036-8075/82/0326-1639$01.00/0