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Progress Report  
Grant No. DPE-5544-G-SS-6034-00  
Microtubule Associated Proteins (MAPS)-  
A Possible Chemotherapeutic Target Against Trypanosomes (CS-139)

In the last progress report we described vaccination of mice and rats with proteins which copurified with Trypanosome microtubules. Various schemes and times of immunization were used and we reached 100% protection against a challenge with *T. brucei* after 3 vaccinations. These experiments were done in April - June 1988 in Kenya while N. Balaban was working at KETRI. We have tried to repeat these experiments both in KETRI and in the Weizmann Institute but with no success. We therefore decided to try and identify the specific proteins used for vaccination. We electroeluted each protein out of a gel, and were able to show through sequencing that one of the 3 proteins used for vaccination is the enzyme glyceraldehyde 3 phosphate dehydrogenase (GPDH) which is a glycosomal enzyme and which probably copurified with the microtubules because of its positive charge. We were unable to sequence the other 2 proteins because they were N-terminally blocked, but because GPDH was found, we had a suspicion that the second protein was aldolase. We checked the protein fraction used for vaccination for aldolase activity and indeed we saw a high degree of it. We are now checking the sequence of the 3rd protein existing in the injected mixture which we have been meanwhile able to isolate as a separate entity on a mono S FPLC column. We tried to vaccinate animals with commercially available aldolase + GPDH

FEB 27 1989

(purified from rabbit) but with no success, suggesting that under these conditions they were not the protective entity.

In a final attempt to assess the protective entity we will try to vaccinate with the 3rd protein once we can have enough material isolated. We will try to prepare or receive from other labs clean trypanosome aldolase and GPDH.

We were also exploring these months the feasibility that an intracellular antigen can be used as a target site for a vaccine. we showed that sera collected from the vaccinated and protected animals caused aggregation of live trypanosomes. After incubating trypanosomes for 15 min with the sera we fixed them, cryosectioned them and labeled the sections with a gold conjugated antibody. Indeed, we saw intracellular labeling of antibodies and no extracellular binding.

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