

Radio-immuno Assays for the Rapid Detection of Dengue Antigens

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OBJECTIVE : To develop a system to detect dengue antigen.

BACKGROUND : It has been hypothesized that antigen-antibody complexes form in the blood of Dengue Hemorrhagic Fever (DHF) patients. These are thought to trigger an immunologic mechanism which leads ultimately to the development of shock. This hypothesis is supported by a great deal of indirect evidence. Until recently, however, the technology has not been available to investigate this point directly.

Studies at the Walter Reed Army Institute of Research have indicated that antibody to structural and nonstructural dengue antigens can be measured independently by radio-immune assays (RIA). Using these techniques it may be possible to detect dengue antigens in patient's sera as well as in mosquitoes inoculated with patient's sera and in wild-caught mosquitoes.

DESCRIPTION : Immunoglobulin G was prepared from pooled human sera with high titered dengue antibody and without hepatitis B antigen or antibody. The globulin in pooled serum was precipitated with 50% ammonium sulfate. Adjustment of the pH of an aliquot of saturated $(\text{NH}_4)_2\text{SO}_4$ to about pH 7.8 was done by addition of 2N NaOH just prior to precipitation of the gamma globulin. The precipitate was dissolved in 0.01 M phosphate buffer pH 7.5, and dialyzed overnight (approximately 18 hours) at 4°C against 0.01 M phosphate buffer, pH 7.5. The dialyzed protein was chromatographed on a diethylaminoethylcellulose column (0.9 x 15 cm) equilibrated with 0.01 M phosphate buffer pH 7.5. The first peak (IgG) was concentrated to original volume in an amicon filtration unit over a XM 50 membrane. The IgG preparations were stored at 4°C.

IgG was labeled with ^{125}I by a modification of the method of Hunter and Greenwood (1). The following reagents were added in order to a small beaker: 20 microliters of 0.25 M phosphate buffer, pH 7.3; 200 μCi of high-specific activity ^{125}I (in 1 to 2 microliters of a solution of chloramine T (3.5 $\mu\text{g}/\text{microliter}$): 20 microliters of a solution of sodium metabisulfate (4.8 $\mu\text{g}/\text{microliter}$); and 20 microliters of a solution of sucrose (22.5%), potassium iodide (2 mg/ml), and aqueous phenol red (0.025%). After the addition of chloramine T, the reaction was allowed to proceed for 15 seconds before being terminated by the addition of sodium metabisulfate. The mixture was applied to the top of a column (made from a 5 ml syringe) packed with sephadex G-50 and equilibrated with phosphate buffered saline, pH 7.4. The protein was eluted with the same buffer; fractions containing the first peak of radioactivity were pooled and diluted with an equal volume of calf serum. This stock mixture was stored at 4°C and diluted 1:2 with calf serum just before use.

Polyvinyl U-bottom microtiter plates (Cooke Engineering) served as the solid phase for the radio-immuno assay. The wells were coated with a 1:10 dilution of dengue antibody containing material (convalescent serum or purified IgG) in normal saline solution with 0.1% sodium azide. After four hours incubation at 4°C, the wells were washed and secondarily coated overnight with 1% bovine

serum albumin in saline. Following a second wash with normal saline solution 25 microliters of the sample to be tested for the presence of antigen was put into the wells and the plates were incubated at 4°C for approximately 40 hours. After incubation the plates were washed and 50 microliters of ¹²⁵I labeled IgG were added to the wells. Plates were again incubated at 37°C for 4 to 6 hours, washed and cut apart with scissors. The wells were transferred to individual gamma counting tubes for quantification of residual radioactivity in a gamma spectrometer.

PROGRESS : Preliminary tests of this technique of radioimmune assay with antigen containing samples of 20% dengue 2 infected suckling mouse brain or dengue 2 infected tissue culture fluids failed to demonstrate specific antigens. Two areas of possible failure were identified. Questions of whether the IgG, used to identify the presence of antigen, was actually labeled by the ¹²⁵I and /or whether the initial high titer dengue antibody was binding to the plastic plate, are being investigated further.

Searches for optimal conditions for the test systems are underway.

REFERENCE :

1. Hunter, W.M., and Greenwood, F.C.: Preparation of Iodine-131 Labeled Human Growth Hormone of High Specific Activity. *Nature (London)*, **194** : 495, 1962.