A NEW TECHNIQUE FOR EVALUATING ANTIGENIC RELATEDNESS AMONG VIRUSES

Frank M. Hetrick

Maryland University

Prepared for:

Office of Naval Research

29 January 1975

050152

AD A004664

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Department of Microbiology University of Maryland	None
	2b. GROUP –

3. REPORT TITLE

A New Technique for Evaluating Antigenic Relatedness Among Viruses.

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final report

5. AUTHOR(S) (First name, middle initial, last name)

Frank M. Hetrick

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
January 29, 1975	7	3

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
N 00014-67-A-0239-0018	–
b. PROJECT NO. NR-136-902	
c. –	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) –
d. –	

10. DISTRIBUTION STATEMENT

–

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
–	Office of Naval Research 800 North Quincy Street Arlington, Va. 22217 Project Officer - Dr. Arthur J. Emery, Jr.

13. ABSTRACT

The major objectives of the work were to evaluate the effectiveness of the macrophage migration inhibition (MMI) test for: (a) differentiating between closely related viruses that cross-react in the standard seriological tests available and (b) rapid identification of a viral agent in a diagnostic context. Essentially, objective (a) was realized but objective (b) was not. The MMI test was found to be an effective means of differentiating (a) two serologically cross-reacting strains of infectious bronchitis virus, an avian coronavirus (b) two nuclear polyhedrosis viruses which are being investigated for possible use in the biological control of insects - these 2 viruses are not separable by any other technique (c) Adenovirus types 1, 4, 5, and 7 - this study also showed that there is an optimal sensitizing virus dose which if exceeded results in the production of humoral antibody which competitively inhibits the delayed hypersensitivity response.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Security Classification

FINAL REPORT

for

OFFICE OF NAVAL RESEARCH

Contract N 00014-67-A-0239-0018

Task No. NR 136-902

A NEW TECHNIQUE FOR EVALUATING ANTIGENIC RELATEDNESS AMONG VIRUSES

by

Frank M. Hetrick
Department of Microbiology
University of Maryland
College Park, Maryland 20742

Contract Period Covered: March 1, 1971 - December 31, 1974

INTRODUCTION

The major objectives of the work were to evaluate the effectiveness of the macrophage migration inhibition (MMI) test for: (a) differentiating between closely related viruses that cross-react in the standard serological tests available and (b) rapid identification of a viral agent in a dical agnostic context. Essentially, objective (a) was realized but objective (b) was not. The methodologies employed and the specific data obtained are detailed in the previously submitted Annual Reports. In addition, the support provided equipment and supplies for the research activities of three individuals who obtained advanced degrees in our department -- Daniel Woodman (Lt., U.S.N.), Ph.D. degree; James Novotny, Ph.D. degree; and Mr. Charles Benton, M.S. degree, and for an undergraduate honors thesis by Mr. David Via.

Major Accomplishments of the Work

1. The first year of the contract work involved establishing the optimal conditions needed for performing macrophage migration inhibition (MMI) tests in our laboratory. The initial studies were performed with the P 22 and P 221 phage of Salmonella typhimurium. Highly purified preparations of P 22 and P 221 phage were used to sensitize Hartley strain guinea pigs. Following sensitization, guinea pig peritoneal exudate cells were placed in capillary tubes and the degree of MMI in the presence of the two antigens was measured. In all tests, the migration of macrophages was inhibited to a greater degree by the sensitizing antigen than by the heterologous antigens. A dose response curve for migration inhibition caused by increasing concentrations of P 22 protein used for sensitization was established. The P 22 and P 221 bacteriophages were clearly distinguished with good statistical significance as measured by Student's paired t test. P 221 proved to be a more effective antigen in eliciting cellular hypersensitivity reactions than P 22.

The MMI test was also able to distinguish between two serologically cross-reacting strains of infectious bronchitis virus (IBV), an avian coronavirus. A dual system for propagating IBV was used for the sensitizing and test antigen preparations. Following guinea pig immunization with chicken tracheal explant grown IBV-1, the MMI test was able to differentiate IBV-1 from either IBV strains 8 or 10 using embryonated egg propagated test antigen. No significant MMI was observed with any of the control peritoneal exudate cells studied. Neither nonsensitized cells in the presence of the test antigens nor sensitized cells in normal media showed any significant reduction in their ability to migrate.

This study indicated that a dual propagation system, i.e., preparation of sensitizing and test antigens in different hosts, may prove useful in future MMI studies and eliminate the need for extensive purification of viral antigens to be used for the sensitization procedure. This work, by Dr. Woodman, was published.

2. Work with insect viruses

One of my graduate students, Mr. Charles Benton, was evaluating the possible use of insect viruses for the biological control of insect pests. When it became apparent that two of the viruses he was studying were indistinguishable with the methodology available, we decided to employ MMI test procedures to ascertain if the two agents were antigenically different or not.

The macrophage migration inhibition test (MMI) was found to be an effective means of differentiating two nuclear polyhedrosis viruses (NPV) which infect the following insects: the cabbage looper (Trichoplusia ni) and the alfalfa looper (Autographa californica). Peritoneal exudate cells from guinea pigs sensitized to T. ni virions demonstrated significant MMI in the presence of T. ni virions but not with the A. californica antigen. Similarly, when A. californica virions were employed as sensitizing antigen, only the homologous antigen gave a significant MMI response. These two insect viruses are not separable by any other technique. This work was published in 1973.

Following the success in determining that the two insect viruses were antigenically distinct, it was of interest to us to determine if the polyhedral protein in which the two viruses were embedded was identical or not since both viruses were propagated in the same host, namely the fall armyworm, Spodoptera frugiperda. Cross MMI test results were confusing as polyhedral protein preparations from either virus inhibited the migration of macrophages from pigs sensitized to the heterologous protein as well as those from pigs sensitized to the homologous polyhedral protein. Subsequently it was shown that peritoneal exudate cells from non-sensitized guinea pigs demonstrated 100% MMI in the presence of 10 ug/ml of either T. ni or A. californica polyhedral protein or intact polyhedral inclusion bodies. Currently, studies are in progress that are designed to characterize this "non-specific" migration inhibition factor. This factor also appears to be a more potent mitogen than phytohemagglutinin. Any publications resulting from these studies will acknowledge ONR support.

3. Work with human Adenoviruses -- by Dr. Novotny and Mr. Via

The MMI test was evaluated for its ability to differentiate between human Adenovirus types 1, 4, 5 and 7. These viruses were selected because they are of importance as etiologic agents of acute respiratory disease in recruits and because they contain a common group-specific complement fixing (CF) antigen. Although type-specific antigens are present in virions, cross-reactions have been reported with neutralization and hemagglutination-inhibition test procedures as well as in the CF test.

The macrophage migration inhibition (MMI) test was found to be a satisfactory procedure for distinguishing between Adenovirus types 1, 4, 5 and 7. Highly purified virus preparations were used for the sensitization of Hartley strain guinea pigs, whereas the MMI test antigen consisted of crude virus preparations grown in KB cells. With all four virus types, a significantly greater MMI response was noted when peritoneal exudate cells

were exposed to the homologous sensitizing antigen as compared to that obtained with the three heterologous antigens. Studies with Adenovirus type 1 indicate that sensitizing doses between 70 and 150 ug of viral protein per guinea pig gave the optimal MMI response. Doses below 70 ug did not stimulate the delayed response, whereas doses above 120 ug produced MMI reactions which were non-specific, as differences between homologous and heterologous antigens were not demonstrable.

As to the latter observation, it is postulated that if the sensitizing antigen is of sufficient concentration to also stimulate the synthesis of humoral antibody, the antibody may competitively inhibit induction of the delayed response. This work was published in 1974.

While in all four of the above cases, the MMI test was shown to be equal or superior to existing test procedures for differentiating viruses, several troubling observations were made: (a) not all guinea pigs that were sensitized with a given antigen responded with a significant MMI response--this animal variability has plaqued other investigators using this system and (b) the macrophages in the peritoneal exudates of some animals did not migrate at all, irrespective of the presence or absence of antigens. Because of these problems, the utility of the MMI test in a diagnostic laboratory would be minimal.

In an attempt to circumvent these difficulties, a second major area of investigation was launched. Reports in the literature indicated that the delayed hypersensitivity response could be conferred to normal peritoneal exudate cells by first exposing them to RNA extracted from the spleens and lymph nodes of sensitized animals. When these cells were subsequently exposed to the sensitizing antigen, the migration of the macrophages was inhibited. This approach was particularly appealing for several reasons--RNA could be extracted from the tissues of guinea pigs giving good MMI responses to specific antigens and stored under ethanol; this RNA could then be used to confer sensitivity to normal peritoneal exudate cells which could then be used to identify specific antigens. The previously mentioned difficulties with "non-responders" would be eliminated because we could use only RNA extracts from pigs giving "good" MMI responses.

All guinea pigs sensitized with either the four Adenovirus types or the two insect viruses were studied in this regard. At the time the animals were sacrificed to obtain peritoneal exudate cells, the spleens were harvested and immediately frozen. After the MMI data on the pigs became available, RNA extracts were prepared from the spleens of those animals giving significant MMI responses to the sensitizing antigen. Despite exhaustive efforts (1 year by Dr. Novotny, 1-1/2 years by Mr. Via, and 1 year by Leslie Bass, another graduate student of mine) which included evaluating various extraction procedures and test conditions, we were not able to transfer the MMI response to normal guinea pig cells with any of the RNA extracts tested.

Publications resulting from the work (ONR support acknowledged).

1. Benton, C. V., C. F. Reichelderfer, and F. M. Hetrick. 1973. Differentiation of Trichoplusia ni MEV and Autographa californica MEV by macrophage migration inhibition tests. J. Invertebrate Pathol. 22, 42-49.

2. Woodman, D. R., R. B. Johnson, and F. M. Hetrick. 1974. Differentiation of two serotypes of infectious bronchitis virus by the macrophage migration inhibition test. Avian Diseases 18, 262-265.

3. Novotny, J. F., F. M. Hetrick, and D. Via. 1974. Differentiation of four Adenovirus types by macrophage migration inhibition tests. Inf. and Imm. 10, 475-480.