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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

AD-A203 862

1b RESTRICTIVE MARKINGS NA		3 DISTRIBUTION AVAILABILITY OF REPORT Distribution Unlimited	
MODULE		5 MONITORING ORGANIZATION REPORT NUMBER NA	
NUMBER(S)		7a NAME OF MONITORING ORGANIZATION Office of Naval Research	
Carnegie Mellon University		7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	
6a NAME OF PERFORMING ORGANIZATION Carnegie Mellon University	6b OFFICE SYMBOL (if applicable) NA	7c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	
8a NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research	8b OFFICE SYMBOL (if applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0063	
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO 61153N PROJECT NO RR04108 TASK NO WORK UNIT ACCESSION NO	
11 TITLE (Include Security Classification) Behavior, Immunologic Response, and Upper Respiratory Infection			
12 PERSONAL AUTHOR(S) Sheldon Cohen, Carnegie Mellon University & David Tyrrell, Common Cold Unit			
13a TYPE OF REPORT Annual		13b TIME COVERED FROM 1/1/88 TO 12/31/88	
		14 DATE OF REPORT (Year, Month, Day) 11 January 1989	
15 PAGE COUNT			
16 SUPPLEMENTARY NOTATION			
17 COSATI CODES FIELD GROUP SUB-GROUP 08		18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Stress, social support, lymphocytes, antibody, (AW) HSV-1, viral challenge, RV	
19 ABSTRACT (Continue on reverse if necessary and identify by block number) Our study involves predicting infection and symptomatology in humans from psychological and immunologic measures taken prior to a viral challenge (Rhinoviruses 2, or 9, or 14, or Corrona virus 221E). Subjects are approximately 1,000 volunteers kept in isolation and monitored closely for viral shedding, and symptomatology over nine-day trails. ONR funds are primarily used to examine the usefulness of antibody titers to herpes simplex virus 1 (HSV-1) as an indirect measure of cellular immune function in this context. At study completion, HSV-1 antibody titers will be correlated with psychological measures, other humoral and cellular measures of immunity, and will be tested as a possible predictor of susceptibility to infection and clinical disease among infected subjects. The first year of funding has been spent hiring and training technicians and developing HSV-1 assays. An ELISA assay for IgG has been developed for the whole viral antigen and an IgA assay is also in development with the current			
20 DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS		21 ABSTRACT SECURITY CLASSIFICATION (u)	
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DD Form 1473, JUN 86

Previous editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

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19. ABSTRACT

proposed protocol involving an indirect ELISA and test-sera pre-absorbed with protein A. Measurement of specific IgM was considered but a preliminary trial revealed negligible amounts in sera with high IgG and IgA titers. Necessary antigens are being obtained for development of an anti-rubella antibody assay to provide a control for humoral (as opposed to cellular) implications of HSV-1 activation. *Keywords:*

See 19.A

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NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
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Availability Codes	
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ANNUAL REPORT

Office of Naval Research Contract
N00014-88-K-0063

January 1, 1988 - December 31, 1988

The purpose of the research is to determine the roles of stress and natural social support systems in individual susceptibility to upper respiratory infection, and clinical colds. We also focus on the role of immunologic function in predicting susceptibility to colds and in linking stress and support to susceptibility. The general design involves predicting infection and symptomatology in humans from psychological and immunologic measures taken prior to inoculation with a cold virus (Rhinoviruses 2, or 9, or 14, or Corona virus 221E). Subjects are approximately 1,000 volunteers kept in isolation and monitored closely for viral shedding, and symptomatology over nine-day trials.

ONR funds are used to examine the usefulness of antibody titers to herpes simplex virus 1 (HSV-1) as an indirect measure of cellular immune function in this context. This work was stimulated by Glaser's argument (e.g., Glaser & Gottlieb-Stematsky, 1982) that latent herpes viruses are held in check by cellular immune function and hence virus activation among HSV-1 infected persons suggests a suppression of cellular immune competence. In our study, HSV-1 activation is (a) correlated with various psychological measures; (b) compared with viral specific and nonspecific antibody titers, and with white blood cell counts including lymphocyte subset; and (c) evaluated as a predictor of susceptibility to infection and clinical disease. During the first year of the project we hired and trained technicians and developed appropriate assays for detection of HSV-1 antibodies. Data collection will be completed by June 1989 and all of the HSV-1 assays will be conducted at that time.

A review of the literature concerning human antibody response to HSV-1 revealed that, while some antibody rises were found during recurrence, the majority of investigations had failed to detect rises corresponding to virus reactivation. Although an assay of immunoglobulin G (IgG) against whole virus antigen is necessary to compare with the work of Glaser and his colleagues, it was considered that assay of IgA levels and IgG against more specific viral antigens would possibly be better indicators of viral reactivation. Enzyme labelled immunosorbent assays (ELISAs) were selected as being the most suitable and sensitive for the task.

To assay immunoglobulin against whole virus antigen an accredited HSV-1 strain has been obtained (strain 10798 from the Centre for Applied Microbiological Research) and a pool of viral antigen has been prepared after growing the virus in human diploid fibroblasts.

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A control antigen pool has been prepared from uninfected cell cultures. A pool of IgG-positive sera has also been selected, for use as a positive standard in the IgG assays. The format for the IgG ELISAs is similar to the protocol we employ for antirhinovirus antibody assays. One half of the microtitre plates are coated with viral antigen and the other half with control antigen. A titration of the positive serum standard and duplicate samples of each test serum (at a single dilution) are added to each half of the plate. After reading the final optical densities from the assays, the control antigen values are subtracted from the viral antigen values. The positive serum standard is assigned an arbitrary value of 1×10^5 units so the corrected optical densities from the test samples can be equated with a point on the standard dilution curve and allocated a value. We will attempt to calibrate the positive serum standards against commercial sera of known immunoglobulin content, thus enabling the specific antibody content of the test-sera to be expressed in ng ml^{-1} . Test-sera are not given a value if their optical densities fall outside the calibration curve and the sera are retested at a different dilution. Sera are also retested if there is a large variation between the duplicate samples.

In pursuit of a more specific assay, monoclonal antibodies were obtained against viral glycoproteins D and B. In preliminary trials, viral antigen, which had been detergent solubilised, was captured by the monoclonal antibodies and this was recognised by human IgG. It could not be proven, however, that the monoclonal antibodies captured purified glycoprotein and discussion with other virologists indicated that glycoproteins tend to form heterologous aggregates and that it might be impossible to capture pure glycoprotein in its native form.

As an alternative means of measuring antibody against a specific glycoprotein, samples of glycoprotein D expressed in a baculovirus (grown in an insect cell line) were obtained. This proved successful as an antigen for ELISAs but assaying a selection of human sera revealed that the antibody activities detected were very similar to those obtained with the assay using whole viral antigen.

It is possible that antibody raised against viral early antigens/control proteins are less persistent than those against glycoprotein and are, therefore, better indicators of viral reactivation. Some of these early antigens have been successfully expressed in cell culture systems and contact has been made with someone working in this field with a view to eventually obtaining some antigen for use in assays.

It was hoped to measure anti HSV-1 IgA using a simple "Indirect" assay (as for IgG) but pre-absorption of the test-sera with protein-A (to remove IgG) resulted in higher IgA measurements. This indicated that the presence of IgG in unabsorbed sera was artificially depressing the perceived IgA titers presumably due to competition for antigen binding sites. The most convenient form of ELISA to overcome this problem would be an IgA capture assay but despite trials with different reagents and protocols the background optical densities

with capture assays could not be reduced to an acceptable level. This appeared to be due to non-specific absorption of the viral antigen. In view of this impasse, the protocol now adopted for IgA measurement utilizes an indirect ELISA and test-sera pre-absorbed with protein-A. A suitable pool of IgA-positive sera has been selected for use as a standard, so assay of the test-sera can now be begun.

Measurement of specific IgM was considered but a preliminary trial revealed negligible amounts in sera with high IgG and IgA titres. Other workers have reported measurable quantities of specific IgM only in response to primary or severe recurrent infections.

Before deciding upon the desirability of assaying the convalescent sera (returned by volunteers after departure from the Common Cold Unit) it was decided to test both pre infection and convalescent sera from the first 100 volunteers and review the results. This has now been completed for IgG and the IgA assays are about to be started. The results should also indicate whether it is necessary to look for IgA in sera which contain no anti HSV-1 IgG.

Because increased antibody production to HSV-1 could reflect a general activation of humoral immunity as opposed to cellular immune suppression, we decided to use anti-rubella antibody in test sera to control for this alternative interpretation. An approach has been made to the Central Public Health Laboratory regarding their protocol for such assays and the purchase of necessary antigens: a reply is being awaited.

References

Glaser, R., & Gottlieb-Stematsky, T. (Eds.) (1982). Human herpesvirus infections. Clinical aspects. New York: Marcel Dekker.

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