Rubella Precipitin Response in Natural Infection and in Vaccination

By

N. R. PAUL, A. J. RHODES, J. B. CAMPBELL, and N. A. LABZOFFSKY

Virus Laboratory, Laboratory Services Branch, Ontario Ministry of Health, and Department of Microbiology, School of Hygiene, University of Toronto, Toronto, Ontario, Canada

With 3 Figures

Received May 4, 1974

Summary

The present investigation was directed towards determination of the significance of the theta and iota precipitins in humoral immunity to rubella. Acutely infected cell cultures with rubella virus strains were found to be inadequate for production of theta and iota antigens, requiring at least 800× concentration of infected tissue culture fluids to obtain weakly reacting theta and iota antigens in gel diffusion. To improve the antigen yield the rubella virus strains were adapted to a porcine stable cell line as a ‘chronically’ infected culture. Three vaccine and three wild rubella virus strains, once established as chronically infected cultures, yielded equally well both the theta and the iota antigens. Examination of sera from cases of natural infection by gel diffusion revealed that the theta precipitin was present in all the sera with HAI titres of 1:64 and higher. This precipitin was first detectable about seven days after the appearance of the HAI antibody. The concentration of theta precipitin increased in parallel with the increase of the HAI titre. The iota precipitin was not detectable in any sera with HAI titres of less than 1:256 and only in 32 per cent of sera with titres of 1:256 or higher. No iota precipitins were found in sera collected earlier than five weeks after the exposure. Examination of post-vaccination sera from adults and children revealed that all sera with HAI titres of 1:64 or higher had detectable levels of theta precipitin but none had iota precipitin, even those that had HAI titres of 1:256 or higher. Even 80× concentration of these sera by immunoglobulin precipitation failed to reveal detectable levels of iota precipitin. The suggestion by others that the iota precipitin plays a major role in humoral immunity is questioned. A test for iota precipitin may serve a useful purpose as a yardstick in evaluation of the efficacy of live rubella vaccine.

1 Taken from the Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science, University of Toronto.
1. Introduction

With the development of live rubella vaccine, the virus diagnostic laboratory is faced with a new problem of determining the immune status of women of childbearing age. Although it is a general consensus that any level of rubella specific hemagglutination inhibiting (HAI) antibodies is indicative of immunity, cases of reinfection in individuals with a low level of antibodies have been reported (1). In a virus laboratory which is engaged in testing large numbers of serum specimens to determine the immune status to rubella, titration of individual serum specimens becomes impractical and, therefore, determination is based on testing an arbitrarily chosen single serum dilution. This approach suffers from a degree of uncertainty. Recently, however, a new promising means for the assessment of immunity to rubella infection was suggested by the work of Le Bouvier (2), who detected two hitherto unrecognized precipitating antibodies in the convalescent sera of patients with natural rubella infection. Le Bouvier named these two antibodies 'theta' and 'iota' and, in the course of his work, observed that although both the theta and the iota are developed in cases of natural infection, only the theta antibodies appear in cases of vaccination with live rubella vaccines currently used. Since vaccine-induced immunity is of a relatively short duration in contrast to naturally acquired immunity, it was suggested that the iota antibody may be mainly responsible for the lasting immunity acquired through natural infection.

Since only a limited amount of work has been done on this subject, it was felt justifiable to undertake further studies in order to determine the applicability of the tests for detection of the theta and the iota antibodies to routine diagnostic virology and particularly to monitoring of the immune status of the population as well as assessing the efficacy of live rubella vaccine.

2. Materials and Methods

2.1. Cell Cultures and Media

One primary and three continuous cell cultures were used in this study.

2.1.1. Primary Cell Culture

African green monkey (Cercocebus aethiops) kidney cells (AGMK) were supplied on a weekly basis as trypsinized cell suspension by the Connaught Laboratories Limited. Growth medium used was Hanks's balanced salt solution with 3 per cent calf serum and as a maintenance medium CMRL 1969 with 2 per cent fetal calf serum (FCS).

2.1.2. Continuous Cell Lines

All continuous cell lines were maintained in the Tissue Culture Production Section of this Laboratory and were provided already planted and ready for use.

a) Porcine kidney stable cell line (PS) was obtained at the fifth passage level through the courtesy of Dr. G. Le Bouvier, School of Epidemiology and Public Health, Yale University. Minimum essential medium of Eagle with 10 per cent FCS was used as a growth medium and the same medium with 5 per cent FCS as a maintenance medium.

b) BHK-21 cell line

The baby hamster kidney cell line used was that established by Stoker and MacPherson (3). As a growth medium CMRL 1969 with 10 per cent FCS was used and for maintenance the concentration of FCS was reduced to 2 per cent.