genetically divergent from its host (Sarcoma 180) showed the most striking response to diet and radiation. Tumors less divergent from their hosts were markedly less responsive. It is proposed that the varying responses obtained in these studies can be explained on the basis of the protein reserves of the host, and the genetic composition of the tumor with respect to its host and the resulting immunological consequences.

We wish to thank Dr. Kelly H. Clifton for his interest and advice, and H. Maseehuddin for technical assistance.

1. Elson, L. A. and Lamerton, L. F., Brit. J. Cancer 3, 414 (1949).

2. Devik, F., Elson, L. A., Koller, P. C., and Lamerton, L. F., Brit. J. Cancer 4, 298 (1950).

3. Holland, J. F. and Heidelberger, C., Cancer Res. 20, 975 (1960).

4. Day, D., Cancer Res. 21, 581 (1961).

5. Hoffman, N. A., Gollin, F. F., and Clifton, K. H., Radiology 88, 568 (1967).

6. Clifton, K. H. and Draper, N. R., Intern. J. Radiation Biol. 7, 515 (1963).

7. Allison, J. B., Wannemacher, R. W., Jr., Hilf, R., Migliarese, J. F., and Crossley, M. L., J. Nutr. 54, 593 (1954).

8. Allison, J. B., Wannemacher, R. W., Jr., Hilf, R., and Hetzel, C. A., J. Nutr. 59, 27 (1956).

9. Allison, J. B., Wannemacher, R. W., Jr. Prosky, L., and Crossley, M. L., J. Nutr. 60, 297 (1956).

10. Wannemacher, R. W., Jr. and Yatvin, M. B., J. Nutr. 85, 393 (1965).

11. Graham, J. B., Graham, R. M., Neri, L., and Wright, K. A., Immunology 75, 103 (1955).

12. Graham, J. B. and Leskowitz, S., Immunology 75, 110 (1955).

Received Jan. 16, 1968. P.S.E.B.M., 1968, Vol. 128.

## Specific Response of the Immunoglobulins to Rubella Infection* (32979)

JOSEPH V. BAUBLIS AND GORDON C. BROWN

*Department of Pediatrics, School of Medicine, and the Virus Laboratory, Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48104*

The teratogenic consequences of rubella infection early in pregnancy are well known. However, experience with the rubella epidemic in 1964 revealed unexpected virologic and serologic consequences of maternal infection. Despite the fact that the rubella syndrome was caused by maternal infection prior to the immunologic maturity of the fetus, serologic immunity rather than tolerance was observed in older children with the syndrome (1,2). Furthermore, rubella virus was found to persist in the affected infants for many months despite the presence of serum antibodies (3–5). Attempts to equate the presence of rubella antibodies in these infants with an active immune response by means of conventional serology was complicated by the presence of passively acquired maternal antibodies of the IgG variety which persist for several months. Since IgA and IgM do not cross the placental barrier readily, the association of antibody activity within the IgM and /or IgA class of globulins of the infant's serum would indicate active immunity. The following studies were carried out in order to characterize the classes of immunoglobulin which responded specifically to rubella infection and to determine their chronology. Observations were also made regarding the classes of rubella antibodies in sera from "normal" newborns and infants with congenital rubella.

*Materials and Methods.* Rubella antibodies were demonstrated by the indirect fluorescent antibody method as described by Brown *et al.* (6). Whereas conventional methods of serum fractionation and antibody titration were cumbersome, this technique provided a rapid and reliable method for identification of the antigenic class of antibodies (IgG, IgA, or IgM) in whole serum which had combined

* Supported in part by PHS Grant AI-05876 from the National Institute of Allergy and Infectious Diseases, N.I.H., and in part by 5-TO-1 HD00057 from the National Institute of Child Health and Human Development, N.I.H.

TABLE I. Titer of Specific Classes of Rubella Antibody during the First Month of Convalescence.

| Specific class of antibody | | Titer of rubella antibody[a] | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days after onset of rash: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| IgM | L.B. | | | | | | | | 64 | | | | | | | | 16 | | | | | | <8 |
| | E.V. | 16 | | | | | | | 8 | | | | | | | 8 | | | | | | | <8 |
| | J.B. | | 8 | | | 32 | | | | | | | | 16 | | | | | | | | | |
| | M.E. | | | | | 8 | | | | | 16 | | | 8 | | | | | | | <8 | | |
| IgA | L.B. | | | | | | | | 64 | | | | | | | | 16 | | | | | | 32 |
| | E.V. | 8 | | | | | | | 64 | | | | | | | 32 | | | | | | | 16 |
| | J.B. | | 16 | | | 8 | | | | | | | | 8 | | | | | | | | | |
| | M.E. | | | | | 32 | | | | | 16 | | | 16 | | | | | | | 16 | | |
| IgG | L.B. | | | | | | | | 32 | | | | | | | | 64 | | | | | | 64 |
| | E.V. | <8 | | | | | | | 32 | | | | | | | 64 | | | | | | | 64 |
| | J.B. | | <8 | | | 8 | | | | | | | | 16 | | | | | | | | | |
| | M.E. | | | | | 32 | | | | | 32 | | | 64 | | | | | | | 64 | | |

[a] The titer is expressed as the reciprocal of the highest dilution showing specific antibody activity by the fluorescent antibody method for sera from patients L.B., E.V., J.B., and M.E.

with viral antigen. Coverslip cultures of a chronically infected line of monkey kidney cells (7) fixed in acetone were employed as an antigen source. The conjugated antisera used in this study were purchased from Hyland Laboratories as fluorescein conjugated antisera prepared in goats against human IgG, IgA, and IgM globulins, respectively. Before use these sera were fractionated by DEAE chromatography as described by Riggs *et al.* (8). The specificity of each conjugate for a single class of globulin was established by immunodiffusion techniques. Furthermore, direct fluorescent antibody techniques demonstrated that they did not stain rubella infected monkey kidney cells. Dilutions of human sera prepared in phosphate buffered saline (PBS) adjusted to pH 7.2 were applied to pieces of infected coverslip cultures and allowed to react for one hour at 37°C. After a 30-min wash with three changes of PBS, a conjugated antiserum was applied to a section of coverslip and allowed to remain at 37°C for 1 hour. A final 30-min wash in PBS was performed and the pieces of coverslip were mounted in elvanol. The presence of rubella antibody in this system was indicated by a brilliant green fluorescence in the cytoplasm of the virus infected cells. The degree of interaction between the antiserum and the antigen was rated semiquantitatively according to the fluorescence observed on a subjective scale from 0 to 4 for the serum dilution tested. The antibody titer of a serum was expressed as the reciprocal of the greatest dilution which yielded a reaction graded at least as "2."

Serum samples were obtained from nonpregnant adults with clinical rubella at intervals during the first month following onset of rash. Sera from infants were obtained from those hospitalized at the University of Michigan Medical Center who showed the stigmata of the rubella syndrome and who had a history of in utero exposure to maternal rubella. Additional sera were obtained from apparently normal newborn infants whose mothers stated they had clinical rubella or were exposed to rubella during pregnancy. Serum was obtained from the mothers of some infants in both these groups. All sera were stored at 4°C until tested.

*Results. Convalescent adults.* Sera obtained by serial bleeding of four adult patients convalescent from rubella were titrated for rubella antibodies by the fluorescent antibody method utilizing fluorescein conjugated antisera specific to IgG, IgA, and IgM, respectively. The results of these antibody titrations are shown in Table I. In two of the patients,

TABLE II. Specific Class of Rubella Antibodies in the Rubella Syndrome.

| Case | Age of infant | Virus isolation[a] | IgM | IgA | IgG |
|---|---|---|---|---|---|
| 1. Mother[b] | | ND | + | + | + |
| Infant | 2 days | + | + | + | + |
| 2. Mother[b] | | ND | + | + | + |
| Infant | 8 days | + | + | + | + |
| 3. Mother[b] | | ND | — | + | + |
| Infant | 4 months | ND | + | + | + |
| 4. Infant | 1 day | ND | + | + | + |
| 5. Infant | 5 days | + | + | + | + |
| 6. Infant | 10 days | + | + | + | + |
| 7. Infant | 10 days | ND | + | + | + |
| 8. Infant | 2 months | + | — | — | + |
| | 7 months | ND | — | + | + |
| 9. Infant | 3 months | ND | — | + | + |
| 10. Infant | 10 months | + | — | — | — |

[a] Rubella isolation from the infant is indicated by a + in the "Virus isolation" column; ND indicates that no attempt was made.

[b] Rubella infection during the first trimester of pregnancy.

IgM and IgA antibodies were detected within 2 days after the onset of rash and prior to the appearance of IgG antibodies. In sera obtained between the fifth and the seventeenth day, antibodies of all three immunoglobulin classes were present. The IgM antibodies were no longer detected in sera obtained from three of the four patients 20 days or more after rash, although both IgG and IgA rubella antibodies were present. These observations demonstrate that in adults, convalescence from rubella is accompanied by the development of a heterogenous group of antibodies which includes IgG, IgA, and IgM, and that the duration of the latter is distinctly limited in time.

*Infants with rubella syndrome.* The class of antibodies which could be detected by the fluorescent antibody technique in sera from infants with the rubella syndrome is shown in Table II, together with the results of tests on sera from three of their mothers collected within 4 months of delivery. It can be seen that rubella antibodies were present in IgA, as well as IgG fractions of gamma globulin in all three mothers and in the IgM of two at this time, indicating persistence in these globulins for up to six months throughout pregnancy. Rubella antibodies of IgG, IgA, and IgM classes were found in sera collected at various ages up to 4 months from seven of the nine infants with the rubella syndrome. The serum of infant no. 8 was negative for IgA and IgM antibodies at 2 months but evidence of active immunity is suggested by the appearance of IgA rubella antibodies by 7 months of age. No evidence of rubella antibodies was found in infant no. 10 at 10 months, despite the isolation of rubella virus from his cerebrospinal fluid at that time and again at 11 months of age.

*Normal newborn infants.* On the basis of the results described above an attempt was made to diagnose subclinical, in utero infection retrospectively by testing cord sera from apparently normal infants. Although each mother gave a history of exposure, a history of clinical rubella during pregnancy was obtained from the mothers of only 3 of the 11 infants in this study. The mother's serum was available for study in four cases. Two of these adult sera were obtained from women who reported clinical rubella during the second trimester of pregnancy. The results of the immunofluorescent tests for the three classes of rubella antibody are shown in Table III.

TABLE III. Specific Class of Rubella Antibodies in Maternal and Normal Newborn Infant Sera.

| Case | | IgM | IgA | IgG |
|---|---|---|---|---|
| 11. | Mother | + | + | + |
| | Cord | — | — | + |
| 12. | Mother | — | + | + |
| | Cord | — | — | — |
| 13. | Mother[a] | — | + | + |
| | Cord | — | — | + |
| 14. | Mother[a] | + | + | + |
| | Cord | — | — | + |
| 15. | Cord | — | — | — |
| 16. | Cord | — | — | + |
| 17. | Cord | — | — | + |
| 18. | Cord | — | — | + |
| 19. | Cord | — | — | + |
| 20. | Cord | — | — | + |
| 21. | Cord[a] | + | + | + |

[a] A history of rubella during second trimester of pregnancy was obtained.

The IgG and IgA rubella antibodies were demonstrated in all of the maternal sera. The IgM antibodies were present in sera from one of the mothers with a history of rubella during pregnancy and in one other mother who denied clinical rubella.

Rubella antibodies of the IgG variety were found in 10 of the 11 infant sera, reflecting passively acquired immunity. The IgA and IgM antibodies indicating active immune response were found in the serum of a single apparently normal newborn infant whose mother gave a history of rubella early in the second trimester. By 3 months of age, this child was suspected of deafness and that suspicion was subsequently confirmed.

The successful application of the fluorescent antibody method in the detection of congenital rubella in this infant dramatically illustrates the potential value of this technique in the detection of prenatal infection. During an outbreak, the routine screening of cord sera for IgM and IgA antibodies to rubella could identify those infants who have congenital infection even though maternal disease was unrecognized or unreported.

*Discussion.* The specific IgG, IgA, and IgM immunoglobulin response to rubella infection was demonstrated by fluorescent microscopy. In four nonpregnant adults, rubella antibodies of all three classes appeared within 1 week following the onset of rash but IgM antibodies were not detected for longer than 16 days. In pregnant women, however, the IgM response was greatly prolonged since some who had experienced the infection early in pregnancy still retained these antibodies months later at the time of delivery. This unusual persistence of IgM antibodies to rubella probably reflects a response to the continuing antigenic stimulus of a virus-infected conceptus. The presence of IgM and IgA rubella antibodies in infants with the rubella syndrome clearly indicates the active nature of the immunity to rubella in the infants and is in contrast to the passively acquired immunity seen in normal infants who showed only IgG antibodies.

The participation of IgA immunoglobulins in the antibody response to rubella infection was of particular interest in this study. Although antibodies of the IgA class appeared early in the first week following onset of rash, unlike IgM antibodies they are present in sera from women with a history of rubella in childhood. The number of observations and the sensitivity of the method do not permit the conclusion that the IgM and IgA response clearly predate that of the IgG, although the results obtained with sera from two subjects within the first 3 days after infection suggest this possibility.

The unusual amount of IgM and IgA globulins in cord serum from infants with congenital rubella which have been reported by others (9,10) have now been observed in other congenital infections, including syphilis, cytomegalovirus infection and toxoplasmosis (11–13). The presence of specific IgM antibodies which has been shown for rubella has also been demonstrated following congenital toxoplasmosis (14, 15) and in four cases of cytomegalovirus infection studied in our laboratory. Three patterns of antibody response were encountered in infants with congenital rubella. The first pattern was associated with the presence of IgM and IgA antibodies soon after birth and would be compatible with in utero responsiveness. A second pattern consisted of absence of IgM or IgA antibody in the early specimen but with the appearance of IgA antibodies in later bleedings, suggesting a response to rubella antigen after birth. One infant with the rubella syndrome had not produced IgM, IgA, nor IgG antibodies by 10 months of age and may represent an example of tolerance. This variability suggests the need for complementary studies including serology as well as viral isolation.

The immunofluorescent technique described above has already provided invaluable assistance in the prompt serologic diagnosis of congenital rubella infection. Serology based upon the antibody activity of IgM and IgA globulin in infant sera provides the only clear separation of active immunity from the confusing background of IgG antibodies which cross the placenta from the mother. The inherent immunologic specificity of immunofluorescent serology, and the elimination of cumbersome preparatory procedures for

fractionation of serum make the serologic diagnosis of perinatal infection a practical procedure for diagnostic serologic laboratories already employing fluorescent microscopic techniques. Continuing studies will reveal whether the immunologic responsiveness of the fetus to rubella, toxoplasma, and cytomegalovirus is exceptional or is encountered with other infectious agents.

1. Weller, T. H., Alford, C. A., and Neva, F. A., New Engl. J. Med. **266**, 1233 (1962).

2. Plotkin, S. A., Dudgeon, J. A., and Ramsay, A. M., Brit. Med. J. **2**, 1296 (1963).

3. Alford, C. A., Neva, F. A., and Weller, T. H., New Engl. J. Med. **271**, 1275 (1964).

4. Plotkin, S. A., Boné, A., and Boné, J. G., Am. J. Epidemiol. **81**, 71 (1965).

5. Veronelli, J. A., Baublis, J. V., Heyn, R. M., and Hoggatt, J. E., Mich. Med. **64**, 923 (1965).

6. Brown, G. C., Maassab, H. F., Veronelli, J. A., and Francis, T., Science **145**, 943 (1964).

7. Maassab, H. F. and Veronelli, J. A., J. Bacteriol. **91**, 436 (1966).

8. Riggs, J. L., Loh, P. C., and Eveland, W. C., Proc. Soc. Exptl. Biol. Med. **105**, 655 (1960).

9. Alford, C. A., Amer. J. Diseases Children **40**, 455 (1965).

10. Bellanti, J. A., Artenstein, M. S., Olson, L. E., Buescher, E. L., Lulvis, C. E., and Milstead, K. L., Amer. J. Diseases Children **110**, 464 (1965).

11. McCracken, G. and Shinefield, R., Pediatrics **36**, 933 (1965).

12. Stiehm, E. R., Amman, A. J., and Cherry, J. D., New Engl. J. Med. **275**, 971 (1966).

13. Alford, C. A., Schaefer, J., Blankenship, W. J., Straumford, J. V., and Cassady, G., New Engl. J. Med. **277**, 437 (1967).

14. Eichenwald, H. F. and Shinefield, H. R., J. Pediat. **63**, 870 (1963).

15. Remington, J. S. and Miller, M., Proc. Soc. Exptl. Biol. Med. **121**, 357 (1966).

Received Jan. 18, 1968. P.S.E.B.M., 1968, Vol. 128.

## Mammary Gland Growth during Pseudopregnancy and Pregnancy in the Rat* (32980)

R. R. ANDERSON AND C. W. TURNER[1]

*Department of Dairy Husbandry, University of Missouri, Columbia, Missouri 65201*

Pseudopregnancy in the rat results in an extension of the lifespan of the corpora lutea to approximately 12 days. Mammary glands differentiate from a ductal system characteristic of virgin cycling rats to a lobule–alveolar system characteristic of pregnancy (1). The extent of the lobule-alveolar development in normal pseudopregnancy has been estimated by whole-mount observations to be similar to that of 13 days of pregnancy in the rat (2). Since it has been demonstrated that hysterectomy and uterine deciduomata prolong pseudopregnancy in the rat (3), one would expect that mammary gland growth continues during the more prolonged periods of diestrum which result from such manipulations. A quantitative assessment of mammary gland growth and function is available based on the chemical determination of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (4). The present investigation was undertaken to determine by quantitative measurements the extent of mammary gland growth in the intact or normal pseudopregnant rat, the pseudopregnant rat that was previously hysterectomized, the pseudopregnant rat having concomitant deciduomata, and the normal pregnant rat.

*Materials and Methods.* Two hundred and four female albino rats of the Sprague-Dawley-Rolfsmeyer strain were maintained at a constant temperature of 78 ± 2°F on Purina lab pellets under an artificial lighting regimen of 14 hours light and 10 hours darkness. Estrous cycle patterns were obtained by the vaginal lavage technique. At least two normal cycles were followed before pseudopregnancy or pregnancy was initiated.

* Contribution from the Missouri Agr. Expt. Sta. Journal Series No. 5095.

[1] This investigation was aided in part by a grant from the USPHS.