CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1996, p. 216–218 Vol. 3, No. 2
1071-412X/96/$04.00+0

Detection of Rubella Virus-Specific Immunoglobulin M Antibodies with a Baculovirus-Expressed E1 Protein

MICHEL SCHMIDT,[1,2] CHRISTER LINDQVIST,[3] AIMO SALMI,[4] AND CHRISTIAN OKER-BLOM[2]*

Department of Biochemistry and Pharmacy[1] and Department of Biology,[3] Åbo Akademi University, and Department of Virology, University of Turku,[4] FIN-20520 Turku, and VTT Biotechnology and Food Research, FIN-02044 VTT,[2] Finland

Received 7 July 1995/Returned for modification 18 October 1995/Accepted 14 December 1995

The structural proteins of rubella virus (RV) were expressed in insect cells by using the baculovirus expression vector system. The recombinant E1 envelope glycoprotein was purified by immunoaffinity chromatography and used to detect RV-specific immunoglobulin M antibodies in a time-resolved fluoroimmunoassay. Correlation analysis between the reactivities of antibodies against this recombinant E1 and the reactivities against authentic RV antigen shows that purified E1 can detect RV antibodies of the immunoglobulin M type.

Rubella virus (RV) is a positive-stranded RNA virus and the only member of genus *Rubivirus* within the family *Togaviridae* (16). The virion consists of three structural proteins, capsid protein C and two envelope glycoproteins, E1 and E2, with molecular masses of 33, 58, and 42 to 47 kDa, respectively (22, 35, 39, 42). Most of the neutralizing antibody binding epitopes have been localized to the E1 glycoprotein (5, 7, 15, 37). The structural proteins are proteolytically processed from a p110 polyprotein precursor, which is translated from a subgenomic 24S mRNA consisting of the 3′ one-third (3,189 nucleotides) of the genome (4, 6, 24). The order of translation is NH_2-C-E2-E1-COOH (20).

Normally, RV infections are not harmful, and the disease German measles occurs only in humans. However, if RV infection is acquired during the first trimester of pregnancy, the fetus has a high probability of developing serious malformations or becoming mentally retarded; this is known as congenital rubella syndrome (43). Today, authentic RV is generally used as an antigen in screening for RV-specific antibodies (17, 32, 38, 40, 41). However, the production and purification steps of authentic RV antigen in mammalian cell cultures are laborious, and the product is infectious. The entire genome of RV has been cloned and sequenced, which has made it possible to produce recombinant antigens in various biological systems as well as synthetic peptides (1, 3, 9, 19, 23, 27, 29, 31, 34).

We have expressed the structural proteins of RV in lepidopteran insect cells by using baculovirus technology (18, 25, 33) and purified the E1 glycoprotein in order to study the reactivity of the corresponding recombinant antigen with human antibodies of the immunoglobulin M (IgM) type.

MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* (Sf9) cells were propagated as monolayer or suspension cultures in TNM-FH medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, N.Y.) containing 100 U of penicillin per ml, 40 μg of streptomycin per ml, and 2.5 μg of amphotericin B (Fungizone; Gibco BRL) at 27°C. Recombinant baculovirus VL1392-RV24S (21), containing the entire coding region of the structural proteins of RV, was amplified by standard procedures (33), gaining a stock virus with a titer of 10^8 PFU/ml.

The Therien strain of RV was grown in B-Vero cells, and the virus was purified as previously described (22, 35).

Preparation of cytoplasmic extracts. Suspension cultures (2×10^6 cells per ml) in spinner flasks (Bellco Glass, Inc., Vineland, N.J.) were infected with recombinant baculovirus VL1392-RV24S at a multiplicity of infection of 5 to 10 PFU per cell. At 48 to 72 h postinfection, cells were collected by low-speed centrifugation ($1,000 \times g$ for 5 min) and washed with ice-cold phosphate-buffered saline (PBS; pH 7.4). The cell pellet was resuspended in TNE buffer (10 mM Tris-HCl, 0.15 M NaCl, 1 mM EDTA [pH 7.8]) containing 1% (vol/vol) Triton X-100 and 0.2 mM phenylmethylsulfonyl fluoride to a concentration of 10^7 cells per ml. Samples were incubated on ice for 30 min, sonicated, and thereafter clarified by centrifugation ($5,000 \times g$ for 10 min). The supernatant fraction containing soluble E1 protein was further processed as described below.

Purification of recombinant E1. The supernatant fraction was applied to an immunoaffinity column with the monoclonal antibody, 4E10, coupled to a CNBr-activated Sepharose 4B gel (Pharmacia, Uppsala, Sweden), as recently described (12). The cytoplasmic extract containing recombinant RV-E1 protein was mixed (end over end) with the affinity matrix at 4°C overnight. The unbound protein fraction was removed by washing the matrix four times with aliquots of 7 mM Na- or K-phosphate buffer (pH 7.3). Recombinant RV-E1 was eluted with 0.01 M Na_2CO_3 buffer (pH 11.0) containing 0.005% (wt/vol) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (Sigma Chemical Co.) and neutralized before storage with 1 M Tris-HCl (pH 7.4).

SDS-PAGE and immunoblot analysis. Eluted protein samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (11) with MINI-PROTEAN II dual slab gels (Bio-Rad Laboratories, Richmond, Calif.). After separation, protein samples were transferred to Zeta-Probe blotting membranes by using a MINI TRANS-BLOT electrophoretic transfer cell (all from Bio-Rad Laboratories). The recombinant E1 protein was identified by immunoblot analysis (36) with rabbit RV antiserum (22). RV-specific antibodies were detected with horseradish peroxidase-conjugated goat anti-rabbit antibodies (Caltac Laboratories, San Francisco, Calif.). The fractions containing recombinant RV-E1 were pooled and used in a time-resolved fluoroimmunoassay (TR-FIA), as described below.

Determination of protein contents. The protein level in each sample was determined by the method of Bradford (2), with bovine serum albumin (BSA) as the reference. The protein concentration of the final reagent was found to be 35 to 50 μg/ml. The purity of the E1 protein was analyzed by SDS-PAGE and Coomassie brilliant blue staining (11).

Delayed fluorescence immunoassay. All diagnostic tests were performed by TR-FIA with the DELFIA system (Wallac Oy, Turku, Finland). Microtiter plates (Nunc, Roskilde, Denmark) were coated at 4°C for 12 h with purified recombinant RV-E1 (2 μg/ml) or authentic RV antigen (2 μg/ml) in PBS. Plates were then blocked with 1% (wt/vol) BSA in PBS for 1 h. Human sera diluted in PBS containing 1% (wt/vol) BSA were added to wells at a dilution of 1/100, and the levels of IgM antibodies were determined with europium (Eu^{3+})-labelled mouse anti-human IgM antibodies (Wallac Oy). The labelling of corresponding antibodies, murine monoclonal antibodies directed against human IgM (anti-human IgM clone 7408; Medix Biochemica Oy, Kauniainen, Finland), was carried out as previously described (8, 30). Fluorescence was monitored with a DELFIA research fluorometer (Wallac Oy) (13, 14).

Human serum samples. A total of 64 human serum samples were used. Of these, 20 (Department of Virology, University of Helsinki) were from clinical rubella patients with positive IgM enzyme immunoassay tests, in which purified RV had been used as the antigen. Corresponding samples were not tested for the rheumatoid factor (RF). The other 44 serum samples (Department of Virology,

* Corresponding author. Mailing address: VTT Biotechnology and Food Research, P.O. Box 1500, FIN-02044 VTT, Finland. Phone: 358-0-4565831 or 358-0-4565108. Fax: 358-0-4552103. Electronic mail address: Christian.Oker-Blom@vtt.fi.

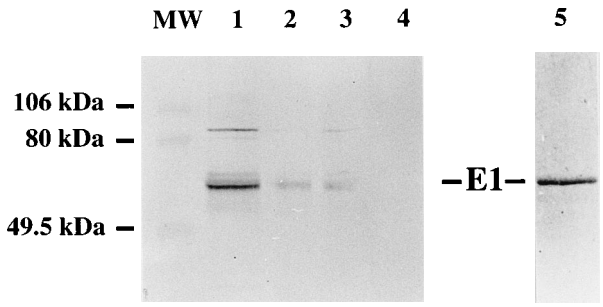

FIG. 1. SDS-PAGE and immunoblot analysis of purified recombinant RV E1 protein expressed in VL1392-RV24S-infected *S. frugiperda* insect cells. Western blot (immunoblot) analysis of samples from four consecutive fractions of this protein eluted at pH 11.0 (lanes 1 through 4). Proteins were identified with rabbit RV antibodies. Coomassie brilliant blue staining of the pooled E1-containing fractions used in TR-FIA (lane 5). Lane MW, molecular mass markers.

University of Turku) were tested for RF by using a human gamma globulin containing latex reagent for the detection of rheumatoid arthritis (RapiTex RF; Behringwerke AG, Marburg, Germany). Corresponding samples were found to be RF negative. All of these serum samples had not been confirmed to be IgM positive.

RESULTS

Preparation and purification of the recombinant antigen. Sf9 insect cells infected with recombinant baculovirus VL1392-RV24S were collected at 48 h postinfection. Cytoplasmic extracts were prepared, and the recombinant E1 antigen was immobilized by using murine anti-E1 monoclonal antibodies coupled to a Sepharose matrix, as described above. The corresponding protein was eluted at a high pH, and the protein content of each fraction was analyzed by SDS-PAGE, followed by immunoblot analysis. The protein contents of four consecutive fractions were analyzed with rabbit polyclonal antibodies directed against whole RV antigen (Fig. 1, lanes 1 through 4). As shown, the major portion of the E1 protein was found in the first fraction, indicating efficient elution of this protein. The protein content of these pooled E1-containing fractions was analyzed by SDS-PAGE and Coomassie brilliant blue staining, showing the purity of the antigen (Fig. 1, lane 5). This final preparation was found to contain between 35 and 50 μg of purified protein per ml.

Correlation analysis. Purified recombinant antigen and whole authentic RV antigen were used for coating microtiter plates, as described above. After incubation with human serum samples, IgM-specific antibodies were detected with murine anti-human IgM monoclonal antibodies labelled with Eu^{3+}. The binding of corresponding antibodies was monitored by TR-FIA. A total of 64 human serum samples were used, and the TR-FIA results obtained from both assays were compared. We were able to show a positive correlation ($r = 0.843$) between the binding of RV IgM antibodies to recombinant RV-E1 and the binding to authentic RV antigen in the TR-FIA (Fig. 2). The mean fluorescence values were slightly higher (9.5%) in assays in which authentic RV antigen was used.

DISCUSSION

It has previously been shown that the baculovirus expression vector system is capable of producing significant amounts of RV-specific proteins (21, 23, 29) and that recombinant proteins have potential in screening for RV-specific antibodies (12, 28). In addition, procaryotic and other eucaryotic systems, as well as synthetic peptide technology, have been used to

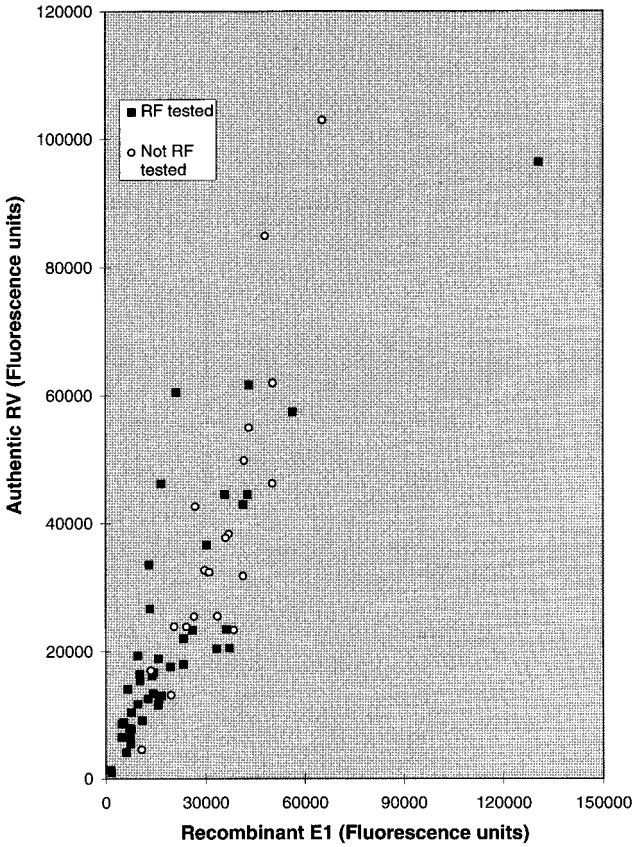

FIG. 2. Correlation analysis of human RV IgM antibodies bound to immunoaffinity-purified recombinant E1 or partially purified authentic RV antigen. A total of 64 serum samples from patients with recent or past rubella infections were analyzed. The binding of IgM antibodies to the corresponding antigen was detected by TR-FIA. The correlation coefficient was calculated to be 0.843.

produce RV-specific antigens in hopes of replacing the expensive and infectious authentic RV antigen (1, 9, 19, 31, 34). Recently, nonreplicative RV-like particles have been produced in mammalian cell systems by introducing 24S cDNA sequences of RV encoding the components needed for virus assembly into CHO and BHK cells (10, 26). The corresponding antigen, which closely resembles that of the authentic RV particle, could be useful in screening for RV-specific antibodies.

In this study, we used the strong polyhedrin gene promoter of *Autographa californica* nuclear polyhedrosis virus to control expression of the 24S cDNA of RV in *S. frugiperda* insect cells (33). During infection with this recombinant baculovirus, lepidopteran cell cultures were found to synthesize structural RV-specific protein products that very much resembled those of the authentic RV particle (21). The availability of monoclonal antibodies directed against the E1 protein (12) made it possible to purify the corresponding protein from lysates of recombinant-baculovirus-infected insect cells. By utilizing this approach, we were able to prepare highly purified antigen at concentrations of about 50 μg/ml. We have shown that this baculovirus-expressed recombinant E1 protein is capable of detecting RV antibodies of the IgM type by using 64 human serum samples from two different sources. Since TR-FIA has previously been shown to be highly sensitive (8, 30), it was selected as the detection method for our assays. Interestingly, we were able to see a clear correlation between the reactivity of the recombinant E1 antigen and that of authentic RV antigen. In many cases, however, the reactivity was somewhat

stronger with authentic RV antigen. Although most of the neutralizing binding epitopes have been localized to the E1 protein, this may partially be explained by the fact that many patients with recent rubella infections also develop antibodies against the two other viral components, i.e., the capsid and E2 proteins.

In conclusion, the baculovirus expression vector system clearly seems to have potential for the production of a recombinant RV-specific antigen that is capable of detecting human antibodies of the IgM type. The possibility of upscaling (43) also makes it an attractive alternative for large-scale production of foreign proteins of this type. The results presented here should provide a framework for further development of this assay with the recombinant E1 antigen either alone, as described here, or in combination with recombinant E2 and capsid proteins.

ACKNOWLEDGMENTS

We thank Kim Pettersson and Pirjo Laaksonen (Wallac Oy) for materials and help with TR-FIA, Helena Seppänen (Labsystems Oy, Helsinki, Finland) for rabbit anti-RV antibodies, and Klaus Hedman (Department of Virology, University of Helsinki, Helsinki, Finland) for human serum samples and valuable discussions. We also thank Robert Kronqvist, Mia Käldman, and Monica Österblad for technical assistance and advice, as well as David Mottershead for proofreading the manuscript.

This study was financed by The Academy of Finland and The Ella and Georg Ehrnrooth, The Magnus Ehrnrooth, and The Åbo Akademi Foundations.

REFERENCES

1. **Al-Mumin, S., J. Newcombe, W. Starkey, J. M. Best, R. E. Spier, and P. G. Sanders.** 1992. Production of recombinant rubella antigens, p. 596–598. *In* R. E. Spier, C. Macdonald, and B. Griffiths (ed.), Animal cell technology: development, processes and products. Pergamon Press, Oxford.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
3. **Clarke, D. M., T. W. Loo, H. McDonald, and S. Gillam.** 1988. Expression of rubella virus cDNA coding for the structural proteins. Gene **65:**23–30.
4. **Dominguez, G., C. Y. Wang, and T. K. Frey.** 1990. Sequence of the genome RNA of rubella virus: evidence for genetic rearrangement during togavirus evolution. Virology **177:**225–238.
5. **Dorsett, P. H., D. C. Miller, K. Y. Green, and F. I. Byrd.** 1985. Structure and function of the rubella virus proteins. Rev. Infect. Dis. **7:**150–156.
6. **Frey, T. K.** 1994. Molecular biology of rubella virus. Adv. Virus Res. **44:**69–160.
7. **Green, K. Y., and P. H. Dorsett.** 1986. Rubella virus antigens: localization of epitopes involved in hemagglutination and neutralization by using monoclonal antibodies. J. Virol. **57:**893–898.
8. **Hemmilä, I., S. Dahubu, V.-M. Mukkala, H. Siitari, and T. Lövgren.** 1984. Europium as a label in time-resolved immunofluorometric assays. Anal. Biochem. **137:**335–343.
9. **Hobman, T. C., M. L. Lundström, and S. Gillam.** 1990. Processing and intracellular transport of rubella virus structural proteins in COS cells. Virology **178:**122–133.
10. **Hobman, T. C., M. L. Lundström, C. A. Mauracher, L. Woodward, S. Gillam, and M. G. Farquhar.** 1994. Assembly of rubella virus structural proteins into virus like particles in transfected cells. Virology **202:**574–585.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
12. **Lindqvist, C., M. Schmidt, J. Heinola, R. Jaatinen, M. Österblad, A. Salmi, S. Keränen, K. Åkerman, and C. Oker-Blom.** 1994. Immunoaffinity purification of baculovirus-expressed rubella virus E1 for diagnostic purposes. J. Clin. Microbiol. **32:**2192–2196.
13. **Lövgren, T., I. Hemmilä, K. Pettersson, and P. Halonen.** 1985. Time-resolved fluorometry in immunoassay, p. 203–217. *In* W. P. Collins (ed.), Alternative immunoassays. John Wiley & Sons, Ltd., Chichester, United Kingdom.
14. **Lövgren, T., and K. Pettersson.** 1990. Time-resolved fluoroimmunoassay, advantages and limitations, p. 233–253. *In* K. Van Dyke and R. Van Dyke (ed.), Luminescence immunoassay and molecular applications. CRC Press, Inc., Boca Raton, Fla.
15. **Lozzi, L., M. Rustici, M. Corti, M. G. Cusi, P. E. Valensin, L. Bracci, A. Santucci, P. Soldani, and P. Neri.** 1990. Structure of rubella E1 glycoprotein epitopes established by multiple peptide synthesis. Arch. Virol. **110:**271–276.
16. **Matthews, R. E. F.** 1982. Classification and nomenclature of viruses. Third report of the International Committee on the Taxonomy of Viruses. Intervirology **17:**1–199.
17. **Meurman, O. H., I. A. Hemmilä, T. N.-E. Lövgren, and P. E. Halonen.** 1982. Time-resolved fluoroimmunoassay: a new test for rubella antibodies. J. Clin. Microbiol. **16:**920–925.
18. **Miller, L. K.** 1989. Insect baculoviruses—powerful gene expression vectors. Bioessays **11:**91–95.
19. **Mitchell, L. A., T. Zhang, M. Ho, D. Décarie, A. J. Tingle, M. Zrein, and M. Lacroix.** 1992. Characterization of rubella virus-specific antibody responses by using a new synthetic peptide-based enzyme-linked immunosorbent assay. J. Clin. Microbiol. **30:**1841–1847.
20. **Oker-Blom, C.** 1984. The gene order for rubella virus structural proteins is NH_2-C-E2-E1-COOH. J. Virol. **51:**354–358.
21. **Oker-Blom, C., M. Blomster, M. Österblad, M. Schmidt, K. Åkerman, and C. Lindqvist.** 1995. Synthesis and processing of the rubella virus p110 polyprotein precursor in baculovirus infected *Spodoptera frugiperda* cells. Virus Res. **35:**71–79.
22. **Oker-Blom, C., N. Kalkkinen, L. Kääriäinen, and R. F. Pettersson.** 1983. Rubella virus contains one capsid protein and three envelope glycoproteins, E1, E2a, and E2b. J. Virol. **46:**964–973.
23. **Oker-Blom, C., R. F. Pettersson, and M. D. Summers.** 1989. Baculovirus polyhedrin promoter-directed expression of rubella virus envelope glycoproteins, E1 and E2, in *Spodoptera frugiperda* cells. Virology **172:**82–91.
24. **Oker-Blom, C., I. Ulmanen, L. Kääriäinen, and R. F. Petterson.** 1984. Rubella virus 40S genome RNA specifies a 24S subgenomic mRNA that codes for a precursor to structural proteins. J. Virol. **49:**403–408.
25. **O'Reilly, D. O., V. A. Luckow, and L. K. Miller.** 1992. Baculovirus expression vectors—a laboratory manual. W. H. Freeman & Co., New York.
26. **Qiu, Z., D. Ou, H. Wu, T. Hobman, and S. Gillam.** 1994. Expression and characterization of virus-like particles containing rubella virus structural proteins. J. Virol. **68:**4086–4091.
27. **Sanchez, A., and T. K. Frey.** 1991. Vaccinia-vectored expression of rubella virus structural proteins and characterization of the E1 and E2 glycosidic linkages. Virology **183:**636–646.
28. **Seppänen, H., M.-L. Huhtala, A. Vaheri, M. D. Summers, and C. Oker-Blom.** 1991. Diagnostic potential of baculovirus-expressed rubella virus envelope proteins. J. Clin. Microbiol. **29:**1877–1882.
29. **Seto, N. O. L., and S. Gillam.** 1994. Expression and characterization of a rubella virus E1 envelope protein. J. Med. Virol. **44:**192–199.
30. **Siitari, H., P. Turunen, J. Schrimsher, and M. Nunn.** 1990. New sensitive and specific assay for human immunodeficiency virus antibodies using labeled recombinant fusion protein and time-resolved fluoroimmunoassay. J. Clin. Microbiol. **28:**2022–2029.
31. **Starkey, W. G., J. Newcombe, K. M. Corbett, K. M. Liu, P. G. Sanders, and J. M. Best.** 1995. Use of rubella virus E1 fusion proteins for detection of rubella virus antibodies. J. Clin. Microbiol. **33:**270–274.
32. **Stewart, G. L., P. D. Parkman, H. E. Hopps, R. D. Douglas, J. P. Hamilton, and H. M. Meyer, Jr.** 1967. Rubella virus hemagglutination-inhibition test. N. Engl. J. Med. **276:**554–557.
33. **Summers, M. D., and G. E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. Texas Agricultural Experiment Station Bulletin no. 1555. Texas Agricultural Experiment Station, College Station, Tex.
34. **Terry, G. M., L. Ho-Terry, P. Londesborough, and K. R. Rees.** 1989. A bioengineered rubella E1 antigen. Arch. Virol. **104:**63–75.
35. **Toivonen, V., R. Vainionpää, A. Salmi, and T. Hyypiä.** 1983. Glycopolypeptides of rubella virus. Arch. Virol. **77:**91–95.
36. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
37. **Umino, Y., T. A. Sato, S. Katow, T. Matsuno, and A. Sugiuro.** 1985. Monoclonal antibodies directed to E1 glycoprotein of rubella virus. Arch. Virol. **83:**33–42.
38. **Väänänen, P., V.-M. Häivä, P. Koskela, and O. Meurman.** 1985. Comparison of a simple latex agglutination test with hemolysis-in-gel, hemagglutination inhibition, and radioimmunoassay for detection of rubella virus antibodies. J. Clin. Microbiol. **21:**793–795.
39. **Vaheri, A., and T. Hovi.** 1972. Structural proteins and subunits of rubella virus. J. Virol. **9:**10–16.
40. **Vejtorp, M., E. Fanoe, and J. Leerhoy.** 1979. Diagnosis of postnatal rubella by the enzyme-linked immunosorbent assay for IgM and IgG antibodies. Acta Pathol. Microbiol. Scand. Sect. B **87:**155–160.
41. **Voller, A., and D. E. Bidwell.** 1975. A simple method for detecting antibodies to rubella. Br. J. Exp. Pathol. **56:**338–339.
42. **Waxham, M. N., and J. S. Wolinsky.** 1983. Immunochemical identification of rubella virus hemagglutinin. Virology **126:**194–203.
43. **Weiss, S. A., G. P. Godwin, S. F. Gorfien, and W. G. Whitford.** 1995. Insect cell culture techniques in serum-free media, p. 79–86. *In* C. D. Richardsson (ed.), Methods in molecular biology, baculovirus expression protocols. Humana Press, Totowa, N.J.
44. **Wolinsky, J. S.** 1990. Rubella, p. 815–837. *In* B. N. Fields and D. M. Knipe (ed.), Virology. Raven Press, Ltd., New York.