JOURNAL OF VIROLOGY, Oct. 1990, p. 5183–5187
0022-538X/90/105183-05$02.00/0

Vol. 64, No. 10

Effect of Antiviral Antibody on Maintenance of Long-Term Rubella Virus Persistent Infection in Vero Cells

EMILY S. ABERNATHY, CHIN-YEN WANG, AND TERYL K. FREY*

Department of Biology and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30302-4010

Received 26 March 1990/Accepted 22 June 1990

A Vero cell line with a long-term rubella virus persistent infection was maintained for 45 weeks in the presence of anti-rubella virus antibody of sufficient titer to completely neutralize the virus in the culture fluid to determine the effect of the presence of antibody on the maintenance of the persistent infection. Prior to antibody treatment, virus was continuously detected as plaque-forming units in the persistently infected culture fluid. Virus clones that were plaque purified from the persistently infected culture fluid were temperature sensitive and exhibited a reduced efficiency of replication and ability to induce cytopathic effects in Vero cells at the persistently infected culture temperature compared with the standard virus used to initiate the persistently infected culture. Defective interfering RNAs were the major intracellular virus-specific RNA species present in the persistently infected cells. Treatment with antibody failed to cure the persistently infected culture of virus, and the cells retained the ability to release virus after antibody treatment was discontinued. Interestingly, the presence of antibody led to the selection of a population of virus which was markedly less cytopathic for Vero cells than the virus population which was selected during persistent infection in the absence of antibody.

Rubella virus (RUB) has been shown to be capable of persistent infections in both congenitally infected individuals and individuals infected after birth. Chronic diseases associated with RUB persistence in congenital rubella syndrome patients include thyroiditis (31), acute-onset diabetes (10, 18), and progressive rubella panencephalitis (28). In patients infected after birth, RUB persistence has been associated with several forms of chronic arthritis (3, 7, 24, 29).

Not surprisingly, RUB establishes a persistent infection in every cell culture line which has been tested. The striking feature of RUB persistence in cell cultures is the fact that the cultures cannot be cured by inclusion of antiviral antibody in the medium. In five independent studies in which different cell lines were used, workers have reported maintenance of RUB persistence in the continuous presence of antibody for as long as 3 months (17, 19, 20, 22, 27). In cell cultures persistently infected with other nonretro-RNA viruses, curing has been effected by antibody treatment (25). However, in the case of cells persistently infected with paramyxoviruses (measles virus and Newcastle disease virus), 3 months or more of antibody treatment was required for curing of the persistent infection, and in some sublines curing did not occur despite treatment with antibody for 1 year or more (12, 23). Therefore, we decided to treat a long-term RUB persistently infected Vero cell line which we had been studying with antiviral antibody for a more prolonged period to determine whether the culture could be cured.

The establishment of RUB persistence in Vero cells with plaque-purified RUB (Therien strain) free of detectable defective interfering (DI) particles has been described previously (8). After establishment of persistence, the culture was maintained by weekly subculturing at a 1:2 dilution. The persistently infected cells appeared to be similar to Vero cells except that rounded, detached cells adhering to the monolayer were always observed. A previous study showed that Vero cells lack a functional interferon system (4). The persistently infected culture was resistant to superinfection with RUB but not to superinfection with Sindbis virus or vesicular stomatitis virus, verifying that interferon was not a factor in maintenance of persistence. As determined by immunofluorescence (11), between 10 and 31% of the cells expressed virus antigen. As determined by an infectious center assay (11), only between 0.3% and 1.5% of the cells produced plaque-forming virus.

Virus was continuously detected as plaque-forming units in the persistently infected culture fluid. The titer generally ranged from 10^3 to 10^5 PFU/ml (during productive infections titers between 1×10^7 and 7×10^7 PFU/ml were produced). After 23 passages, we observed reductions in the plaque size of the persistent virus from the standard virus plaque size of 3 to 4 mm in diameter to a size of less than 1 mm in diameter. As Table 1 shows, when seven plaques isolated from the persistently infected cell culture at passages 165 and 175 were tested for the ability to replicate, we found that all of them exhibited a reduced ability to replicate compared with the standard virus at 35°C, the persistently infected culture temperature. All seven plaques were temperature sensitive (*ts*) at 39°C.

During RUB productive infections in Vero cells, two positive-polarity virus-specific RNA species are produced, the genomic RNA (9,757 nucleotides long) and a subgenomic RNA containing the 3′-terminal 3,327 nucleotides of the genomic RNA, which serves as an mRNA for the translation of the virus structural proteins (5, 9, 11, 21). A Northern hybridization experiment (Fig. 1) showed that the predominant species of RUB-specific intracellular RNAs in the cell line with the long-term persistent infection were DI RNAs. In this experiment, two probes were used, an oligonucleotide complementary to the 3′ 30-nucleotide end of the genome and negative-polarity RNA transcribed from a plasmid containing the 3′-terminal 3,300 nucleotides of the RUB genome. The oligonucleotide probe (Fig. 1A) was more sensitive than the RNA probe (Fig. 1C) for detection of DI

* Corresponding author.

TABLE 1. Replication in Vero cells of virus clones isolated from persistent infections

Clone[a]	Titer (PFU/ml) at:		
	30°C	35°C	39°C
Standard	ND[b]	2.0×10^7	1.3×10^6
E1	9.0×10^4	1.0×10^6	$<10^2$
E3	ND	1.8×10^4	$<10^1$
E4	1.9×10^5	3.1×10^5	$<10^2$
E5	ND	4.0×10^4	$<10^1$
E6	ND	3.2×10^4	$<10^1$
E7	1.7×10^5	1.1×10^5	$<10^1$
E8	1.0×10^4	$<10^2$	$<10^1$

[a] Plaques were picked from plaque assay plates containing standard virus or persistently infected culture fluids from passages 165 (clones E1 through E5) and 175 (clones E6 through E8) and eluted in 1 ml of minimal essential medium containing 1% fetal bovine serum; 0.1-ml portions of each fluid were used to infect 1×10^6 Vero cells in wells of 24-well culture trays. After 4 h of adsorption at 35°C, cells infected with each clone were incubated at 30, 35, or 39°C. For the standard clone and clones E1 and E4, culture fluids were harvested when CPE were observed at 35°C (92 h for the standard clone, 146 h for clone E1, 171 h for clone E4). No CPE were observed in cells infected with clones E3 and E5 through E8, and culture fluids were harvested at 192 h.
[b] ND, Not done.

RNAs, while the RNA probe was more sensitive for the detection of the genomic and subgenomic RNAs. This was presumably due to the fact that since the DI RNAs (all but one of which were smaller than the subgenomic RNA) contained significant internal deletions of genomic sequences, the relative specific activity of the RNA probe was much higher for the genomic and subgenomic RNAs than it was for the DI RNAs, while the specific activity of the oligonucleotide probe was the same for all of these RNAs. The number of detectable DI RNAs in the four passages tested ranged from three to seven. Some DI RNAs which were of similar lengths were conserved in these passages. The subgenomic RNA was detected in three of the four passages with the oligonucleotide probe and in all four passages with the RNA probe, while the genomic RNA was detected only in passage 183 with both probes.

Culture fluid from two passages (passages 183 and 184) in which seven intracellular DI RNAs were observed was concentrated 10-fold and used to infect Vero cells either in the absence or in the presence of helper standard virus (multiplicity of infection [MOI], 5 PFU/cell) (a Northern gel of intracellular RNA from passage 184 was similar to the gel of passage 183 RNA shown in Fig. 1A through C). When RNA extracted from the infected Vero cells was analyzed by Northern hybridization, only one DI RNA species was detected (Fig. 1D). This DI RNA species was similar in length (roughly 1,000 nucleotides) to one of the DI RNAs present in the persistently infected cells. The other six DI RNAs present in the persistently infected cells may not have been transmissible, a phenomenon which has been reported in BHK-21 cells that are persistently infected with Sindbis virus (26). Interestingly, although the subgenomic RNA was clearly present in all lanes containing RNA from cells infected with persistently infected culture fluid, the genomic RNA was not detected in any of these lanes. Thus, the 1,000-nucleotide DI RNA was capable of interfering with genomic RNA synthesis, and the inhibition of genomic RNA synthesis observed in the persistently infected cells was due at least in part to DI RNA-mediated interference.

When the persistently infected cell line was subcultured at passage 173, a sample of cells was plated into medium containing 1% goat anti-RUB serum (Fig. 2). This subline (designated the GS line) was subsequently subcultured like the persistently infected cells except that the culture medium contained 1% goat antiserum and the subculture dilution was increased from 1:2 to 1:5 since it has been shown that heavy subculturing can adversely affect efforts to cure a persistent infection with antibody (14). Plaque-forming units were never detected in the culture fluid of the GS line, and therefore neutralization of extracellular virus by addition of the antiserum was effective. To detect the presence of RUB in the GS line, a polymerase chain reaction (PCR) assay was developed to detect the presence of RUB RNA in total cell RNA. When the assay was performed on the GS line after 40 passages in the presence of antiserum, a positive result was obtained (Fig. 3), demonstrating that persistence had been maintained. After 45 passages in the presence of antiserum, the GS line was assayed by immunofluorescence, and we found that 50% of the cells were positive. This finding indicated that the persistent infection was being maintained in a large percentage of the cells and not just in a small residual fraction.

To determine whether the persistently infected cells treated with antiserum had maintained the ability to release virus, at various subculturings of the GS line samples of cells were plated into medium without antiserum (GSR sublines), and the culture fluid was assayed for the presence of virus by using the plaque assay (Fig. 2). In cells released from antiserum after eight passages (subline GSR-8), plaque-forming units were detected within one passage in the absence of antiserum. When GS line cells were released from antiserum after both 22 passages (subline GSR-22) and 40 passages (subline GSR-40), there was no evidence of plaque-forming units in the culture fluids until three or four passages in the absence of antiserum after release. At this point we observed very tiny, opaque plaques which were not consistently present from plaque assay to plaque assay with the same culture fluid. To determine whether virus was present in these culture fluids, Vero cells were infected with the culture fluids and assayed for the presence of virus by using the PCR assay (after 3 days of incubation) or immunofluorescence (after 7 days of incubation). Vero cells infected with these culture fluids did not exhibit cytopathic effects (CPE). Both assays were positive for all culture fluids tested. Thus, virus was present in these culture fluids, and the cells had maintained the ability to release virus during the treatment with antibody. However, the virus released did not induce CPE when total culture fluid was used to infect Vero cells and variably produced small, opaque plaques. In the culture fluids from persistently infected cells maintained in the absence of antiserum, plaque-forming units were always present, and these culture fluids induced CPE in Vero cells within 2 days after infection. Thus, antiserum treatment led to selection of a virus population which was less cytopathic for Vero cells than the virus population that was present before antiserum treatment. In concurrence with this conclusion, we observed that the rounded and detached cells that were routinely observed in the persistently infected culture disappeared within a few passages in the presence of antibody and the culture became morphologically indistinguishable from Vero cells.

In summary, two important observations about RUB persistent infection were made in this study. First, RUB persistence was maintained in the presence of antibody for a much longer period of time than previously reported. In the case of other steady-state virus persistent infections, when cell-to-cell spread of virus is blocked by the presence of

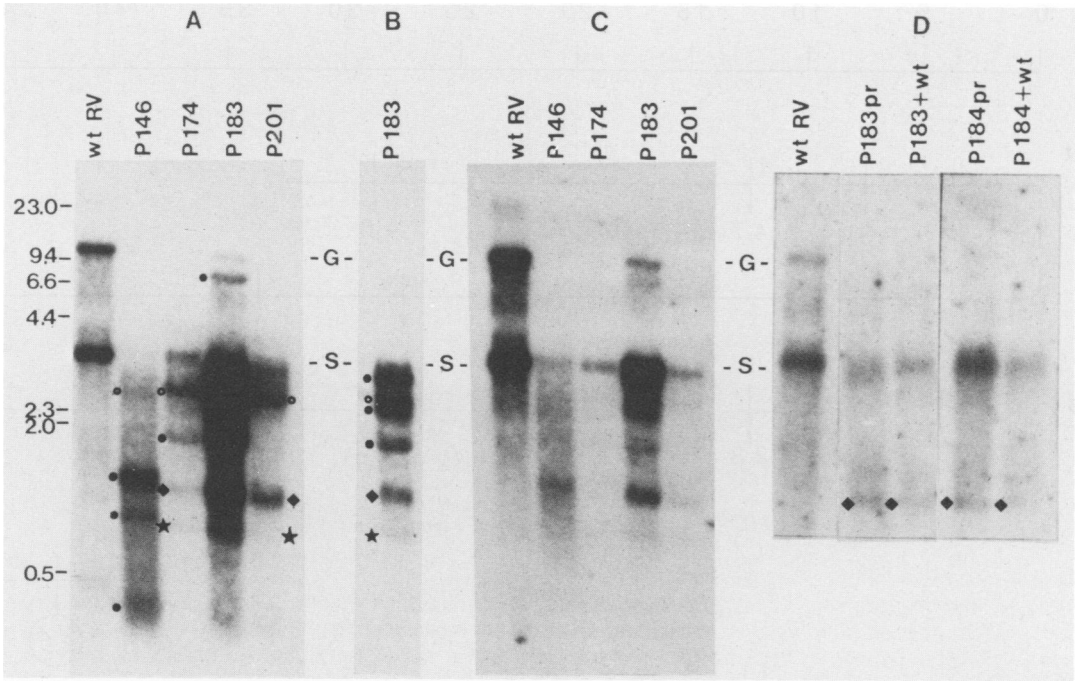

FIG. 1. Northern analysis of intracellular RNA from persistently infected cells. (A through C) Total cell RNA was extracted from Vero cells that were productively infected with standard RUB (MOI, 10 PFU/cell, 50 h postinfection) or from the persistently infected cell line at different passages as previously described (11). Denaturation with glyoxal, electrophoresis in 1% agarose gels, transfer to nitrocellulose, and hybridization with labeled probes were done as previously described (9, 11). Each lane of the gel contained 4.5 μg of total cell RNA. (A) Blot hybridized against an oligonucleotide (oligonucleotide 9) with the sequence TATACAGCAACAGGTGCGGGAATCTAGTGG, which was synthesized with a model 381A DNA synthesizer (Applied Biosystems, Foster City, Calif.) and which is complementary to the 3′-terminal 30 nucleotides of the RUB genome (5). The oligonucleotide was labeled with [γ-^{32}P]ATP (6,000 Ci/mmol; Dupont-New England Nuclear, Wilmington, Del.) and polynucleotide kinase (Promega Biotec, Madison, Wis.) (9). The results of a 24-h exposure in the presence of an intensifying screen are shown. (B) A 2.5-h exposure of the passage 183 (P183) lane. (C) The same Northern blot as in panels A and B was stripped of hybridized oligonucleotide probe by incubation in water at 95°C and rehybridized with ^{32}P-labeled negative-polarity RNA synthesized from an RNA polymerase promoter plasmid (pGEM 3; Promega Biotec) containing the 3′-terminal 3,300 nucleotides of the RUB genome (pLEE-1) by using SP6 RNA polymerase (Promega Biotec) in the presence of [α-^{32}P]UTP (3,000 Ci/mmol; Dupont-New England Nuclear) (11). The positions of ^{32}P-labeled lambda *Hin*dIII fragments which were electrophoresed in the same gel are indicated on the left (sizes given in kilobase pairs). The positions of the RUB genomic (G) and subgenomic (S) RNAs are also indicated. The positions of DI RNAs are indicated to the left of the P146, P174, and P183 lanes and to the right of the P201 lane as follows: ●, DI RNAs present in a single passage; ○, ◆, and ★, DI RNAs of similar sizes present in three or more passages. (D) Culture fluids from passages 183 and 184 were concentrated 10-fold by polyethylene glycol 8000 precipitation and used to infect Vero cells in the presence (+wt) or absence (pr) of standard RUB (MOI, 5 PFU/cell). The MOI of the persistent virus was 0.2 PFU/cell. Total cell RNA was extracted at 50 h postinfection. Equal samples of RNA were applied to each lane of the gel, and the blot of the gel was probed with the oligonucleotide probe. The position of a DI RNA having migration similar to that of one of the DI RNAs (◆) in the gel shown in panels A through C is indicated. wt RV, Vero cells infected with standard RUB.

antibody, curing eventually occurs, indicating that occasional reinfection is important in maintenance of persistence (14). Thus, in RUB persistently infected cell cultures some mechanism must exist to ensure maintenance of the virus in each cell and passage of the virus to both daughter cells during cell division. RUB buds both at the cytoplasmic membrane and into intracellular vacuoles (1), providing an intracellular repository for virus which may play a role in cellular maintenance of the virus. In the case of paramyxovirus persistence, virus can be spread from cell to cell in the presence of antibody by cell fusion (16). However, RUB is capable of fusing cells only at low pH values (15), and thus it does not seem likely that this mechanism plays any role in the maintenance of RUB persistence.

The second important observation made in this study was that antibody treatment had an effect on the evolution of the persistent virus population in that a less cytopathic virus population was selected. Presumably, the less cytopathic virus had evolved prior to antiserum treatment. In the presence of antiserum, the only repository for virus was the infected cells. Eventually, the cells infected with cytopathic virus were killed, thus eliminating the cytopathic virus from the persistent virus population. Cells infected with less cytopathic viruses survived indefinitely, and thus this type of virus was maintained in the persistent virus population.

Much work has been done on the evolution of virus variants which exhibit attenuated cytopathogenicity, such as DI particles and *ts* mutants, during persistent infection with other viruses (13, 30). Evolution of both DI particles and *ts* mutants was observed in our Vero cell line that was persistently infected with RUB in the absence of antibody treatment (in RUB persistent infections in other cell lines, evolution of *ts* mutants [27] and DI particles [20] has been reported). In this report we document that in the case of RUB, antibody treatment leads to further selection of less cytopathic virus variants.

The ability of the cells that were persistently infected with RUB to release virus was maintained throughout the antise-

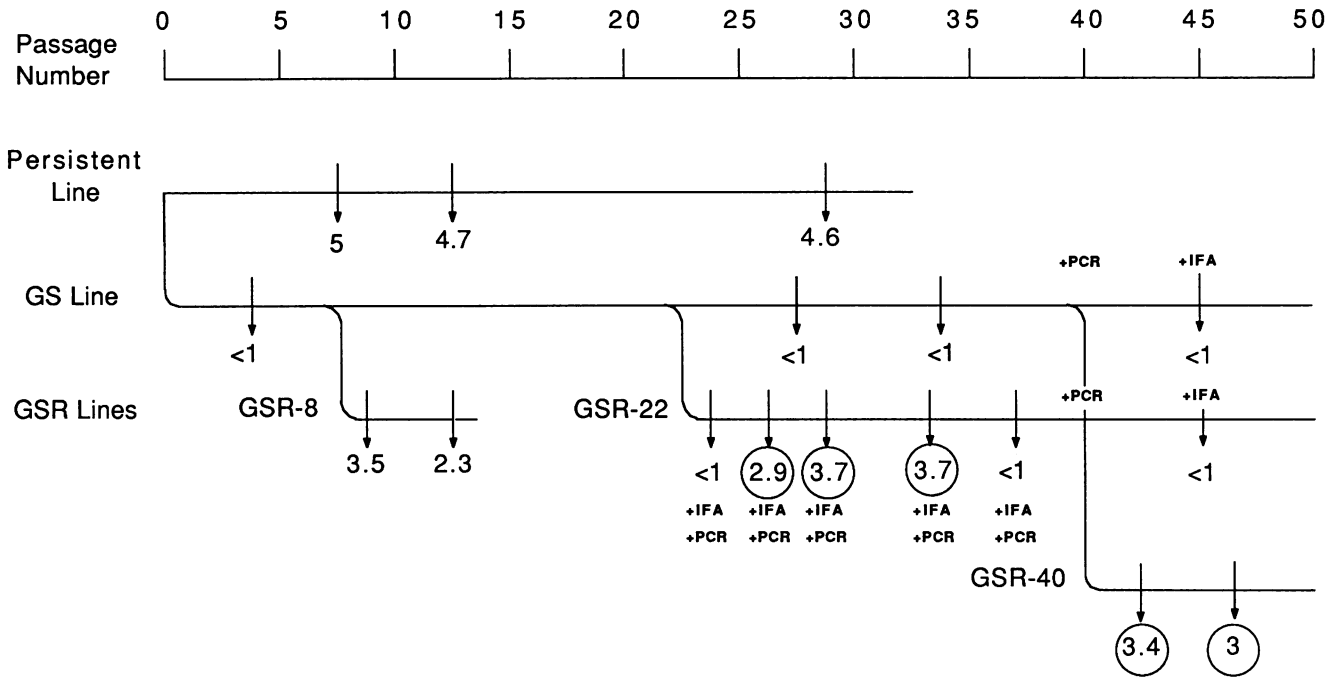

FIG. 2. Passage history of the persistently infected cell line in the presence of goat anti-RUB antiserum. Beginning at passage 172 of the persistently infected Vero cell line (denoted as passage 0 in the passage number line), a subline was established and maintained in medium containing 1% anti-RUB antiserum (the GS line). The goat anti-RUB antiserum was obtained from Preston Dorsett (University of Tennessee College of Medicine, Memphis) and had a neutralization titer of 1,600, as determined by a plaque reduction assay in which our standard RUB was used. At different passages of the GS line, sublines were established and maintained in medium without antiserum (the GSR lines). Passages at which the presence of virus was confirmed by an immunofluorescence assay (IFA) and a PCR assay of total cell RNA extracted from the line (11) are indicated by +IFA and +PCR, respectively. Passages at which the titers of the culture fluids were determined by using a plaque assay are indicated by arrows; titers are given in $\log_{10}$ plaque-forming units per milliliter. In plaque assays done on culture fluids from sublines GSR-22 and GSR-40, small opaque plaques were present which were not consistently detected from plaque assay to plaque assay with the same culture fluid (titers given are from assays in which plaques were detected; these titers are circled). The presence of virus in these culture fluids was confirmed by immunofluorescence (+IFA) and PCR (+PCR) analyses of Vero cells infected with these culture fluids where indicated.

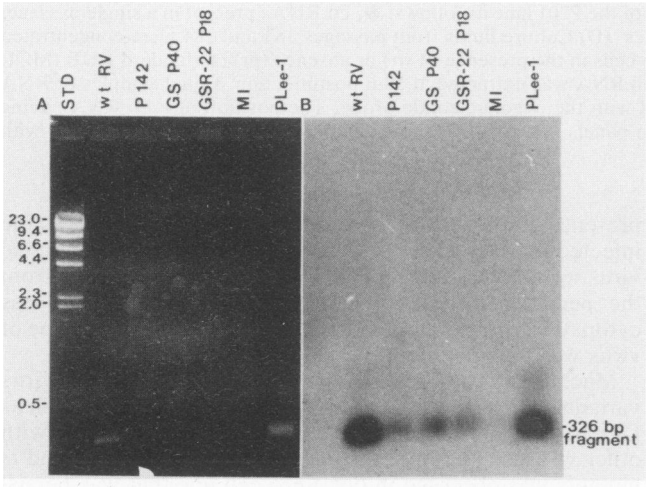

FIG. 3. PCR detection of RUB RNA. Total cell RNA extracted as described previously (11) was reverse transcribed by using an oligo(dT) primer and then subjected to PCR amplification by using as primers oligonucleotide 9, which is complementary to the 3′ end of the RUB genome (see the legend to Fig. 1), and an oligonucleotide with the sequence CCCCCGCCCTACCAAGTCAGCTGCGGGGG, which is identical to the sequence of nucleotides 298 through 326 from the 3′ end of the RUB genome (5). This pair of primers was expected to yield a 326-base-pair (bp) product. Buffers and enzymes were supplied in a Gene Amp kit (Perkin-Elmer-Cetus, Norwalk, Conn.). Amplification (95°C for 1 min, 37°C for 2 min, and 72°C for 2 min for 30 cycles) was done by using a thermal cycler (Perkin-Elmer-Cetus). The products of PCR amplification were electrophoresed in a 1% agarose gel, stained with ethidium bromide (A) (the sizes of the *Hin*dIII lambda fragment standards [STD] [in kilobase pairs] are indicated on the left), and then transferred to nitrocellulose and hybridized against ^{32}P-labeled negative-polarity RNA transcribed from pLEE-1 (B) (see the legend to Fig. 1). The following RNA samples were used: wt RV, Vero cells infected with standard RUB (MOI, 5 PFU per cell) for 48 h; P142, passage 142 of the persistently infected cell line; GS P40, GS line after 40 passages in the presence of goat anti-RUB antiserum; GSR-22 P18, GS line released from antiserum treatment after 22 passages and maintained for 18 passages in medium without anti-RUB antiserum. MI, Mock infected; pLEE-1, plasmid control.

rum treatment. Interestingly, in a study of HeLa cells that were persistently infected with measles virus, a different observation was made (23). Prolonged antibody treatment of the cells that were persistently infected with measles virus led to selection of a population of cells in which the virus was completely cell associated and extracellular virus was not produced after antibody treatment was discontinued. This resembled the state of measles virus in subacute sclerosing panencephalitis patients.

Both of the observations made in this study are applicable

to congenital rubella syndrome patients from whom virus is recoverable for 6 months to 1 year after birth despite a vigorous humoral response (6). In these patients, the cell-mediated response is defective (2), and, as shown in this study, RUB can persist for prolonged periods of time in the presence of antibody. The reason for virus disappearance in congenital rubella syndrome patients after 6 months to 1 year is not clear. On the basis of the results of this study, perhaps a population of attenuated virus that is not demonstrable by routine methods evolves in these patients in the presence of antibody.

We thank Lee D. Marr for expert technical assistance and critical reading of the manuscript, Mark Hemphill and Cynthia Derdeyn for help with some of the experiments, Bill Walthall for assistance with microscopy, Robert B. Simmons for photographic work, and Margo Brinton for critical reading of the manuscript.

This work was supported by Public Health Service grant AI-21389 from the National Institutes of Health, by Public Health Service Basic Research Support Grant RR-07171 from the National Institutes of Health, by grant 211-2142A from the Central New York Arthritis Foundation, and by grants from the Georgia State University Research Office. T.K.F. was the recipient of Public Health Service Research Career Development Award AI-00923 from the National Institutes of Health.

LITERATURE CITED

1. **Bardeletti, G., J. Tektoff, and D. Gautheron.** 1979. Rubella virus maturation and production in two host cell systems. Intervirology **11**:97–103.
2. **Buimovici-Klein, E., P. B. Lang, P. R. Ziring, and L. Z. Cooper.** 1979. Impaired cell mediated immune response in patients with congenital rubella: correlation with gestational age at time of infection. Pediatrics **64**:620–626.
3. **Chantler, J. K., A. J. Tingle, and R. E. Petty.** 1985. Persistent rubella virus infection associated with chronic arthritis in children. N. Engl. J. Med. **313**:1117–1123.
4. **Desmyter, J., J. Melnick, and W. Rawls.** 1968. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). J. Virol. **2**:955–961.
5. **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.
6. **Dudgeon, J.** 1969. Congenital rubella. Am. J. Dis. Child. **118**: 35–44.
7. **Fraser, J. R. E., A. L. Cunningham, K. Hayes, R. Leach, and R. Lunt.** 1983. Rubella arthritis in adults: isolation of virus, cytology and other aspects of the synovial reaction. Clin. Exp. Rheumatol. **1**:287–293.
8. **Frey, T. K., and M. L. Hemphill.** 1988. Generation of defective interfering particles by rubella virus in Vero cells. Virology **164**:22–29.
9. **Frey, T. K., L. D. Marr, A. Sanchez, and R. B. Simmons.** 1989. Identification of the 5′ end of the rubella virus subgenomic RNA. Virology **168**:191–194.
10. **Ginsberg-Felner, F., M. E. Witt, B. Fedun, F. Taub, M. J. Doberson, R. C. McEvoy, L. Z. Cooper, A. L. Notkins, and P. Rubenstein.** 1985. Diabetes mellitus and auto-immunity in patients with congenital rubella syndrome. Rev. Infect. Dis. **7**:S170–S176.
11. **Hemphill, M. L., R. Y. Forng, E. S. Abernathy, and T. K. Frey.** 1988. Time-course of virus specific macromolecular synthesis in rubella virus infected Vero cells. Virology **162**:65–75.
12. **Henle, W.** 1963. Interference and interferon in persistent viral infections of cell cultures. J. Immunol. **91**:145–150.
13. **Holland, J., S. Kennedy, B. Semler, C. Jones, L. Roux, and E. Grabaw.** 1980. Defective interfering RNA viruses and host cell response, p. 137–192. *In* H. Fraenkel-Conrat and R. Wagner (ed.), Viral invasion, persistence, and disease. Comprehensive virology, vol. 16. Plenum Publishing Corp., New York.
14. **Holland, J. J., L. P. Villarreal, R. M. Welsh, M. B. Oldstone, D. Kohne, R. Lazzarini, and E. Scolnick.** 1976. Long-term persistent vesicular stomatitis virus and rabies virus infection of cells *in vitro*. J. Gen. Virol. **33**:193–211.
15. **Katow, S., and A. Sugiura.** 1988. Low pH-induced conformational change of rubella virus envelope proteins. J. Gen. Virol. **69**:2797–2807.
16. **Kingsbury, D. W.** 1986. Orthomyxo- and paramyxoviruses and their replication, p. 491–512. *In* B. Fields and D. Knipe (ed.), Fundamental virology. Raven Press, New York.
17. **Maassab, H., and J. Veronelli.** 1966. Characteristics of serially propagated monkey kidney cell cultures with persistent rubella infection. J. Bacteriol. **91**:436–441.
18. **Menser, M., J. Forrest, and R. Bransby.** 1978. Rubella infection and diabetes mellitus. Lancet **i**:56–60.
19. **Mifune, K., J. Desmyter, and W. Rawls.** 1970. Effect of exogenous interferon on rubella virus production in carrier cultures of cells defective in interferon production. Infect. Immun. **2**:132–138.
20. **Norval, M.** 1979. Mechanisms of persistence of rubella virus in LLC-MK2 cells. J. Gen. Virol. **43**:289–298.
21. **Oker-Blom, C., I. Ulmanen, L. Kääriäinen, and R. Pettersson.** 1984. Rubella virus 40S genomic RNA specifies a 24S subgenomic mRNA that codes for a precursor to structural proteins. J. Virol. **49**:403–408.
22. **Rawls, W., and J. Melnick.** 1966. Rubella virus carrier cultures derived from congenitally infected infants. J. Exp. Med. **123**: 795–816.
23. **Rustigian, R.** 1966. Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected HeLa sublines and recovery of a HeLa clonal line persistently infected with incomplete virus. J. Bacteriol. **92**: 1805–1811.
24. **Tingle, A. J., J. K. Chantler, K. H. Pot, D. W. Paty, and D. K. Ford.** 1984. Postpartum rubella immunization: association with development of prolonged arthritis, neurological sequelae, and chronic rubella viremia. J. Infect. Dis. **152**:606–612.
25. **Walker, D. L.** 1964. The viral carrier state in animal cell cultures. Prog. Med. Virol. **6**:111–148.
26. **Weiss, B., R. Levis, and S. Schlesinger.** 1983. Evolution of virus and defective-interfering RNAs in BHK cells persistently infected with Sindbis virus. J. Virol. **48**:676–684.
27. **Williams, M., T. Brawner, H. Riggs, and J. Roehrig.** 1979. Characteristics of a persistent rubella infection in a human cell line. J. Gen. Virol. **52**:321–328.
28. **Wolinsky, J. S., M. N. Waxham, J. Hess, J. Townsend, and J. Baringer.** 1982. Immunochemical features of a case of progressive rubella panencephalitis. Clin. Exp. Immunol. **48**:359–366.
29. **Wouters, J. M., J. van der Veen, L. B. van der Putte, and D. J. de Rooij.** 1988. Adult onset Still's disease and viral infections. Ann. Rheum. Dis. **47**:764–767.
30. **Youngner, J. S., and O. T. Preble.** 1980. Virus persistence: evolution of viral populations, p. 73–135. *In* H. Fraenkel-Conrat and R. Wagner (ed.), Viral invasion, persistence, and disease. Comprehensive virology, vol. 16. Plenum Publishing Corp., New York.
31. **Ziring, P. R., G. Gallo, M. Finegold, E. Buimovici-Klein, and F. Ogra.** 1977. Chronic lymphocytic thyroiditis: identification of rubella virus antigen in the thyroid of a child with congenital rubella. J. Pediatr. **90**:419–420.