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Longitudinal, Serological Study of Cytomegalovirus Infections in Nurses and in Personnel Without Patient Contact

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Sera were obtained at intervals from 172 hospital employees for measurement of cytomegalovirus (CMV) complement fixation (CF) and indirect hemagglutination antibody. No fourfold rises or falls in titer were seen over a 19- to 27-month period among 71 employees with initially positive CMV CF titers. The concurrence rate between the CMV CF and the indirect hemagglutination antibody tests in identifying seronegative personnel was 96%. Five seroconversions were identified during an average follow-up period of 15 to 17 months per person among 65 pediatric nurses whose CMV CF titers had initially been <1:8. No seroconversions were seen during an average follow-up period of 29 months per person among 27 hospital employees with CMV CF titers of <1:8 who had little patient contact. The rate of acquisition of CMV infections in seronegative pediatric nurses was 4.1 to 7.7% per year. Sera from 9 of the 172 employees studied (5.2%) gave inconsistent results at the lower limits of the CF test.

The risks of transmission of cytomegalovirus (CMV) from a pregnant woman to the fetus, from the maternal cervix to the newborn, and by transfusion of blood to various types of patients have been investigated, but little is known of other modes of transmission. CMV is considered to be of low communicability. However, it has been isolated from saliva, tears, breast milk, semen, feces, and urine, as well as from the cervix and upper respiratory tract. Virus excretion in newborn infants ranges from less than 0.1 to 2.5% in different populations (2, 3, 13). Excretion of CMV in nasopharyngeal or urine specimens in healthy children between 1 and 12 months of age has been reported to reach 13% in Seattle, Wash., 15% in Bangkok, Thailand, and 56% in Sendai, Japan (8, 10, 11). A study of hospital patients 6 months to 4 years of age has shown excretion of CMV in 4.0 to 5.8% in Manchester, England, 10% in London, England, and 20 to 40% in Helsinki, Finland (3, 7, 13).

Asymptomatic infants who are not suspected of having disease outnumber those with clinically obvious disease (12, 15). Many infants admitted to general pediatric wards are first known to be excreting CMV only when laboratory results return, i.e., days to weeks later. Pediatric nurses are probably continually exposed to the virus, but little specific information exists as to their risk of acquiring infection.

Haldane et al. (4) estimated, from data collected by questionnaires, that pregnant nurses who cared for infants had a higher risk of delivering an infant with a congenital anomaly than those who cared for older age groups or who did not work during pregnancy. This risk appeared to be the highest for nurses who were in contact with premature infants or those with congenital anomalies. Therefore, it appears important to assess the incidence of CMV infections in nurses caring for young children.

Few studies have been published in which antibody titers to CMV have been measured periodically in normal individuals. Currently, there is concern that the complement fixation (CF) test may be insufficiently sensitive as a measure of immune status in previously, but not recently, infected persons. In addition, reports of unstable CMV CF antibody titers and of fluctuations from positive to negative to positive in sequential sera from the same individual have raised doubts as to whether recent infection can be accurately assessed by the conversion of the CMV CF titer from negative to positive.

In this study, sera drawn periodically from nurses and from personnel with little patient contact were evaluated for the incidence of fourfold rises and falls in CMV CF titer in seropositive personnel and for the concurrence of the CF test and the indirect hemagglutination

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(IHA) test in selecting individuals who had no measurable antibody to CMV and who, therefore, would be considered susceptible. In addition, the incidence of new infections, as assessed by a change in CMV CF and IHA titer from negative to positive, was evaluated in two groups of pediatric nurses and in a group of non-nurses to determine whether there were large differences in the incidence of new infections that might be accounted for by the exposure of nurses to infants and young children who were excreting virus.

MATERIALS AND METHODS

Population. Between July 1969 and March 1974, sera were obtained periodically from 46 hospital employees with little patient contact (non-nurses), 62 nurses who worked in a neonatal intensive care unit (group A nurses), and 55 nurses who worked on a general pediatric ward and cared for children 3 months to 3 years in age (group B nurses). All of the personnel were female.

IHA test. Antigen was made by inoculating monolayers of human embryonic lung cells in 32-oz (0.946-liter) bottles with the AD 169 strain of cytomegalovirus. Seven to eight days later, each washed monolayer was covered with 5 ml of buffered physiological saline (pH 7.2) and frozen. After thawing, the saline was removed and clarified by centrifugation at 800 rpm for 10 min. The supernatant fluid was then frozen at −70 C for use as an IHA antigen. The residual monolayer was treated with trypsin and processed to make antigen for the CF test.

The IHA test was performed by the method of Bernstein and Stewart (1).

CF test. The cells were harvested by trypsin treatment, suspended in 2.5 ml of citrated water, and disrupted by sonic treatment. This material was then centrifuged at 800 rpm for 10 min. The supernatant fluid containing the antigen was removed and frozen at −70 C until use. The antigen was used at the concentration that gave the highest antibody titer with a known positive serum sample in a block titration and that was not anti-complementary. The lowest dilution of serum tested was 1:8. All sera from each individual were run simultaneously in the same test at the end of the study. All sera had been stored at −20 C and most had been thawed and refrozen at least once. All tests included known positive and negative controls. The tests were performed with heat-inactivated serum, magnesium-calcium saline, and two complete units of complement and overnight fixation. Sera showing complete fixation and those showing almost complete fixation (3+, slight hemolysis, full cell button) were read as positive.

RESULTS

The average age on entry to the study was 26 years for the non-nurses and 24 years in both group A (neonatal) and group B (pediatric) nurses. Other factors that might influence acquisition of CMV infections, including socioeconomic status and parity, were not rigidly controlled. However, >90% of the people in all groups were Caucasian and most of them held a degree beyond high school.

Of the 172 persons studied, 41.2% had positive CMV CF titers and 53.4% had negative CMV CF titers upon entry to the study. The remaining 5.2% had indeterminate CMV CF titers (fluctuating between <1:8 and 1:8), as discussed below. There were no significant differences among the three groups at the onset of the study regarding the incidence of seropositive and seronegative individuals. An increasing incidence of seropositive individuals was seen with increasing age in all three groups, but it was most evident in the group B nurses who cared for infants and young children (Table 1).

During the study, 763 serum specimens were tested. Of these, 306 were from personnel with positive CMV CF titers upon entry to the study, 392 were from personnel with CMV CF titers of <1:8, and 65 were from personnel with indeterminate CMV CF titers.

As shown in Table 2, 71 personnel with positive CMV CF titers upon entry to the study were followed. Twenty-one were followed for more than 24 months each, and the remainder were followed for shorter periods of time. The average interval between sera was 6.1 months in the group of non-nurses and 4.0 and 4.3 months in the group A and group B nurses, respectively. Although apparent fourfold rises and falls in CMV CF titer were seen in tests conducted over the years against different antigen lots, no fourfold rises or falls were confirmed when all sera from the same individual were run in the same test. Four technicians with laboratory exposure to CMV, who had positive CMV CF titers upon entry to the study, were included in the group of non-nurses. No change in titer was seen over periods of 14, 19, 37, and 41 months. A comparison of IHA and CF titers in 75 seropositive personnel is shown in Fig. 1. Sixty-two percent of the sera had an IHA titer that was at least fourfold higher than the CF titer.

At the time of entry to the study, 27 of the

TABLE 1. *Incidence of ≥1:8 complement fixation titers by age*

Age (yr)	Incidence of titers (%)			
	Non-nurses	Group A: nurses (neonatal)	Group B: nurses (ward)	All personnel
<24	30	28	26	28
25–29	30	30	40	33
≥30	60	57	100	72

TABLE 2. *Hospital personnel with positive CMV CF titers*

Personnel	No. of persons	Avg follow-up/person (mo)	No. followed > 24 mo (% of group)	Avg interval between serum specimens (mo)	No. of persons with fourfold rises or falls in titer
Non-nurses	19	26.9	9 (47)	6.1	0
Group A: nurses (neonatal)	28	18.9	7 (25)	4.0	0
Group B: nurses (ward)	24	18.5	5 (21)	4.3	0

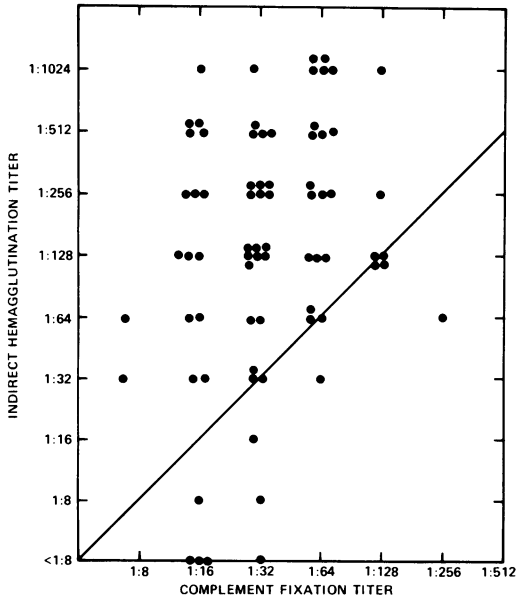

FIG. 1. *Comparison of IHA titers to cytomegalovirus strain AD 169 with CF titers in 75 hospital personnel with positive CF tests.*

non-nurses, 34 group A nurses, and 31 group B nurses had CMV CF titers of <1:8. Of these, 95.7% also had IHA titers of <1:8. Three had IHA titers of 1:8, and one had an IHA titer of 1:64. The average length of follow-up (Table 3) was 29.1 months for the non-nurses, 16.9 months for group A nurses, and 15.2 months for group B nurses. The differences in length of follow-up are accounted for by the higher turnover rate for nurses as compared with other hospital personnel. Seventeen of the 27 personnel (63%) in the group of non-nurses, 10 of the 34 (29%) group A nurses, and 7 of the 31 (23%) group B nurses were followed for more than 24 months.

No seroconversions occurred among the 27 seronegative personnel in the group of non-nurses. Two seroconversions occurred among 34 group A nurses, and three occurred among 31 group B nurses. The rate of seroconversion per year of exposure was, therefore, 0% in the non-nurses, 4.1% in group A nurses, and 7.7% in group B nurses. The five seroconversions occurred 1, 4, 12, 25, and 29 months after entry to the study. These five nurses sustained titer changes from <1:8 to 1:32–1:256. All five nurses initially had titers of <1:8, as measured both by CF and IHA tests, and all five developed positive titers, as measured by both tests. One nurse had a mononucleosis syndrome in association with her seroconversion; the remaining nurses were not aware of symptoms. If the other hospital personnel are compared with the nurses (group A and group B nurses combined) by the Fisher exact test, the difference in the number of seroconversions per number of persons at risk achieves significance at the $P = 0.15$ level. If the same test is done with time of exposure being the unit of comparison between the two groups, the difference in seroconversions achieves significance at the $P = 0.11$

TABLE 3. *Hospital personnel with negative CMV CF titers*

Personnel	No. of persons	Avg age (yr)	Avg follow-up/person (mo)	No. followed >24 mo (% of group)	No. of seroconversions	No. of seroconversions/yr	Seroconversions/yr (%)
Non-nurses	27	26	29.1	17 (63)	0	0	0
Group A: nurses (neonatal)	34	24	16.9	10 (29)	2	1.4	4.1
Group B: nurses (ward)	31	24	15.2	7 (23)	3	2.4	7.7

level. Therefore, the differences between non-nurses and nurses regarding seroconversions to CMV are not highly significant with the numbers available, but a trend toward a greater number of seroconversions among seronegative nurses than among seronegative non-nurses is seen.

Four seronegative technicians with laboratory exposure to CMV were included in the group of non-nurses. Two worked in a diagnostic virology laboratory and were followed 49 and 34 months without serological evidence of infection. Two assisted with laboratory research on CMV and were followed 20 and 28 months, at which time both CMV CF and IHA titers were <1:8.

Sequential sera from two group A nurses, two group B nurses, and five individuals in the group of non-nurses fluctuated between <1:8 and 1:8 in the CF test with most CF antigens; however, six of the nine had CMV CF titers of 1:8 to 1:16 with one of the antigens against which they were tested. When IHA titers were measured in sera from these nine individuals, titers of <1:8 were obtained in six and 1:64 to 1:128 were obtained in three.

DISCUSSION

The prevalence of CMV CF antibodies in adults varies between and within countries, between sexes, and probably within socioeconomic groups living in the same geographic area (6, 17). In 1965, Stern and Elek demonstrated an increase in the percentage of the population with measurable CMV CF titers from 15% in the 5- to 10-year-old age group to 54% in the 25- to 35-year-old age group (14). Age-related increases in the prevalence of CMV CF antibody have also been shown in Seattle and Dallas (9, 17). Thus, it appears likely that in urban areas in the United States, CMV infections are frequently acquired by young adults. A rise with age in the incidence of seropositive individuals was seen in this study in all three study groups, but it was most pronounced among the group B nurses who cared for infants and young children. Our data show that about two-thirds of the nurses entering the profession in their early twenties were susceptible to CMV infection, as judged by the absence of CF or IHA antibody to the stock strain AD 169.

It is increasingly appreciated that apparently normal children may excrete CMV for prolonged periods between birth and 4 years of age, after which time excretion appears to be less common. Undoubtedly, other patient populations with high rates of excretion of CMV exist, such as transplant patients, those with leukemia and lymphomas, and other chronically hospitalized patients who require multiple transfusions. The spread of CMV infections to nurses in hospitals is a risk that is not limited to those who care for newborn infants.

Although the virus is easy to isolate from the secretions of infected patients, little is known about the nature of contact required to transmit CMV from person to person. Attempts to isolate CMV from the diapers of infected infants may fail, even when the virus is present in the urine in titers as higher as 10^6/ml (3).

It has not been established that the presence of CMV CF antibody, as measured by available antigens, confers protection against new infections with CMV stains. Information regarding the longevity and consistency of CMV CF antibody during the years after infection is scant. Waner et al. have reported that 6 of the 20 individuals followed by them over a 16-month period sustained fourfold fluctuations in CMV CF titer when tested with an antigen made from AD 169 (16). Similar changes in titer were looked for among the 71 people in this study who had positive CMV CF titers. Although differences between the studies are many, including differences in preparation and titration of antigen, in reading the end point of the CF tests, in the intervals between sera (1.2 months compared with 4.0 to 6.1 months), and in the population studied (plasmapheresis versus no plasmapheresis), no fluctuations of this degree could be confirmed in our study when all sera from the 71 individuals who had clearly positive CF titers were run in the same test. CMV CF titers of <1:8 are not considered sufficiently reproducible or specific to be reportable. However, our failure to include fluctuations of titers in the 1:4 to 1:8 range would not entirely explain the differences in the two studies, since the population studied by Waner et al. showed titer fluctuation of fourfold or greater above the 1:8 level. Standardization of antigens so that known positive sera give identical titers with different lots has not resulted in reproducible results in our laboratory, and fluctuations of titer are seen when all sera are not run against the same antigen on the same day.

Sera from about 5% of the people in this study had antibody titers that fell at the lower limits of the CF test. Problems with reproducibility and interpretation of specificity of antibody were experienced in this small group. However, we did not note falls in CMV CF titer from >1:8 to <1:8 during this study. Ninety-six percent of the individuals with negative CMV CF titers also had negative CMV IHA titers. Therefore,

it appears unlikely that the seronegative group contained many individuals who had previously experienced a CMV infection.

Five seroconversions were identified among 65 pediatric nurses compared with none among 27 non-nurses who were followed concurrently. Although records were not kept of the exposure of individual nurses to infected children, at least 10 to 13% of the young children in urban areas in the United States can be presumed to be excreting CMV (8, 18). Group A nurses were known to have been exposed to at least 31 different infants who excreted CMV during their hospital stay. Group B nurses were similarly exposed to at least 30 such patients, many of whom were hospitalized for months. Few other studies of CMV infections in nurses exist. Stern (13) attempted to isolate CMV from throat swabs and urine specimens of 99 healthy nurses and laboratory personnel with negative results. Serological evidence of a recent CMV infection was found in 1 of 48 midwives during a 6-month training course in another study (3).

Since the numbers in our study are small and the turnover of nurses is higher than that of the other hospital personnel, it is not certain whether the number of seroconversions among the nurses is greater than that which would be expected in individuals of similar age in the community at large. However, the rate of seroconversion among the nurses of 4.1 to 7.7% per year, if maintained, would be higher than the approximately 2% increase in prevalence of antibody per year of age seen in other populations (5, 14, 17) and is higher than that seen in the non-nurses.

Until the relationship of positive CMV CF titers to susceptibility to CMV infection is better defined and more is known of the nature of the contact required for the transmission of infection, the risk of working on pediatric units while pregnant or attempting to become so can only be defined in general terms. Although the risk of a pediatric nurse acquiring a CMV infection during any given 9-month period appears to be small, it may be greater than the risk sustained by personnel who are not in intimate contact with large numbers of small children. Both the CF and IHA tests appear to be useful in following populations for changes in immune status.

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