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Cytomegalovirus Infection in Children Undergoing Open-Heart Surgery[1]

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A group of 124 children undergoing open-heart surgery was followed prospectively in order to estimate the risk of cytomegalovirus (CMV) infection due to transfused blood.

Ninety-three patients (75%) had complement fixation (CF) titers of < 1:4 against CMV on admission. Of this seronegative subgroup, nine patients (9.7%) subsequently became infected with CMV. All nine showed seroconversion, and six were viruric 12–14 weeks after surgery. Comparative seroepidemiological studies of the hospital population showed that in the age ranges studied (3–16 yr), the infections seen in the study group represented a significant excess over expectation. This infection rate was consistent with a model of transmission by blood transfusion with a risk of 2.7% per unit but not proven.

Thirty-one patients had CF antibody to CMV on admission. CMV was isolated from 14% of urines of seropositive children both before and after surgery, but only two patients showed CF antibody rises to CMV. Thus the frequency of CMV infection associated with open-heart surgery and transfusion could not be calculated in the seropositive subgroup.

CMV infection was not related to the primary diagnosis or to Down's syndrome.

INTRODUCTION

Since Kääriäinen *et al.* (4) originally implicated blood transfusion in the transmission of cytomegalovirus (CMV), a number of quantitative estimates of the risk per unit of blood have been made. Henle *et al.* (3) estimated that the risk of CMV infection in recipients was proportional to the number of units transfused and could be estimated as 5, 10.5, and 12% per unit, respectively, for three groups of patients in Helsinki and Philadelphia. Prince *et al.* (10) found that of 152 patients who received a total of 1269 units of blood, 31 showed subsequent seroconversion and estimated the overall risk as 2.4 seroconversions per 100 units transfused. For those receiving only 1 unit, the risk was 6.8%/unit and 0.9%/unit for those receiving 15 or more units. Recently, Kane *et al.* (5) showed that 7 of 223 (3%) volunteer blood donors were shedding CMV in the urine and that recipients of blood from three of these donors showed serological evidence of subsequent CMV infection. In 1969, Diosi *et al.* (2) were able to isolate CMV from 2 of 35 asymptomatic blood donors. These studies strongly suggest that CMV can be transmitted by blood. However, subsequent efforts to isolate CMV from blood involving a total of more than 800 donors have been unsuccessful (5, 8, 15). This experience has been repeatedly confirmed by us and by other contributors in this volume. It is possible that attempts to isolate CMV from normal volunteer donor blood are not a useful measure of the risk of blood. The virus may exist in a form that is not readily isolated, or blood may operate in ways other than in direct viral transmission.

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In seropositive individuals, evidence of CMV infection following transfusion could represent activation of latent endogenous infection by the antigenic stimulus of the transfusion, which can be considered an allograft (7). Recently, Olding *et al.* (9) have shown that the mixed leukocyte reaction as well as nonspecific mitogens will activate murine CMV latent in leukocytes. Wu *et al.* (16) have shown enhancement of chronic murine CMV infection by application of skin allografts. Activation of latent infection has long been considered an important cause of the frequent CMV infections seen in renal transplant recipients (1). Therefore, in order to exclude possible factors involved in reactivation and concentrate on blood as a potential vehicle for viral transmission, we chose to study CMV infection in a group of largely seronegative children, most of whom would not be expected to harbor latent CMV. Patients admitted to the Children's Hospital of Pittsburgh (CHP) for open-heart surgery were identified as such a group. These children received a variable number of units of blood and were accessible to follow-up.

MATERIALS AND METHODS

Study Population

One hundred twenty-four children undergoing open-heart surgery at the Children's Hospital of Pittsburgh were followed from admission through their subsequent visits to the surgical clinic (6–8 weeks after surgery) and cardiology clinic (12–14 weeks after surgery). Blood and urine specimens were obtained prior to surgery and at each clinic visit. Patients were unselected in that efforts were made to follow all patients on whom presurgical specimens could be obtained during the period from July 1972 to December 1974. Data obtained on each patient included age, sex, race, previous admissions to the hospital, diagnosis, number of units of blood used during the procedure.

The open-heart procedure in these patients was accomplished with a 1300-ml Sarns heart–lung machine and Bently oxygenator. In general, 2 units (600 ml) of packed red blood cells were used to prime the machine, although this was somewhat variable depending on the size and hematocrit of the patient. The remaining volume was made up with lactated Ringer's solution with 5% dextrose. While on bypass, approximately 20% of the patient's blood was sequestered in the machine, to be returned at the end of the procedure. Additional blood requirements were met by standard transfusion methods.

Virus Isolation

Urine was centrifuged and penicillin (200 units/ml), streptomycin (200 μg/ml) and amphotericin B (5 μg/ml) were added to the supernatant. The pH was adjusted to 7.0 with 4% $NaHCO_3$ and 0.5 ml was inoculated onto duplicate monolayer cultures of human foreskin fibroblasts (MA-184, Microbiological Associates, Inc., Bethesda, Md.) or locally-derived human embryonic fibroblasts. Cell cultures were maintained in Eagle's Minimal Essential Medium (MEM) supplemented with 2% fetal calf serum and observed microscopically for viral cytopathology over an 8-week period. Leukocytes obtained from buffy coats of heparinized blood specimens were also inoculated into cell cultures for the first 18 months of this study. Since no specimens from the study population yielded CMV, the practice was discontinued. Unselected volunteer donor blood specimens were also collected for virus isolation studies. Heparinized or EDTA-treated blood was centrifuged. The buffy coat was aspirated in about 1 ml, and sufficient MEM was added to make 2 ml. Duplicate 0.5-ml samples were inoculated onto human fibroblast cultures as described above. When

toxicity was seen, the cultures were "patched" by the addition of sufficient fibroblasts to restore the monolayer.

Serology

The complement fixation test was performed by the micromethod described by Takatsky (12) as modified by Sever *et al.* (11) using commercial antigen prepared from CMV strain AD-169 (Microbiological Associates, Inc.). Two units of antigen and five units of complement were used in the test, and the end point was taken as the highest dilution of serum giving 1+ (25%) fixation. Titers $\geq$ 1:4 were considered positive, and changes in titer $\geq 4\times$ were considered significant. Titers of 1:4 were uncommon, occurring in about 2% of specimens. Sera were obtained from the Clinical Chemistry and Hematology Laboratories of Children's Hospital and the Central Blood Bank of Pittsburgh for CF antibody prevalence studies.

RESULTS

The Study Group

The study group ranged in age from 3–16 years with an average age of 7.6. Infants less than 3 years of age were excluded owing to the difficulty in obtaining urine specimens. The study group was 56% male and 6% black which may be compared with 52% male and 7% black for 484 unselected admissions in the same age range. Twenty-five percent (31) of the study group had CF titers $\geq$ 1:4 on admission, which may be compared with 28.5% (68/239) for unselected sera obtained on admission to CHP. Most frequent diagnoses included atrial or ventricular septal defects, endocardial cushion defects and infundibular or valvular pulmonic stenoses.

Evidence of CMV Infection

The evidence for CMV infection during the course of the study was an increase of at least fourfold in CF antibody titer and/or isolation of CMV from urine. The data are summarized in Table 1. Viruria was detected in a total of 15 patients. It can be seen that of 31 patients seropositive at admission, five were viruric at that time and four became viruric after surgery. This suggested that viruria was not a useful index of acquisition of infection in seropositive children. In Table 2, the significance of viruria is reexamined, classifying urine specimens on the basis of the serologic status of the patient and the time of collection of urine relative to surgery. It is clear that in the seropositive children, about 14% were excreting CMV in the urine irrespective of surgery or transfusion. CMV was isolated from 6 out of 321 specimens obtained from seronegative patients. Repeated positive isolations from the same patient are excluded from Table 2. All isolations were made at least 12–14 weeks after surgery. All six seronegative patients who developed viruria also showed seroconversion, with peak titers ranging from 1:32 to 1:256. The good correlation between viruria and seroconversion, and the statistically significant ($\chi^2_{(2)} = 10.894$, $P < .005$) clustering in the period 12–14 weeks after surgery suggests that, in contrast to the seropositive group, development of viruria in the seronegative group was a useful indication of newly-acquired infection. The significance of serological rise in the seropositive patients who were not excreting CMV at the time of surgery is not clear. It is to be noted that the rate of rise (2/26 = 7.7%) in this group is somewhat lower than the infection rate of the seronegative group. This may indicate that there is some immunity. However, the data are insufficient. Viruria not accompanied by change in CF antibody titer was detected in 4 of these 26 seropositive children.

There were five patients who had seroconversion alone without viruria. As shown in Table 1, two of these were seropositive on admission and three were previously

TABLE 1
Evidence of CMV Infection in Study Group

Status on admission and evidence of infection		Number of patients
Seronegative on admission		
No change		84
Viruria and Seroconversion following surgery	6	
Seroconversion only	3	
Seropositive and viruric on admission		
No Seroconversion		5
Seropositive on admission		
No change		20
Viruria following surgery, no seroconversion	4	
≥ Fourfold CF rise following surgery	2	
Totals	15	109
	124	

seronegative. Based on the frequency of CMV viruria in seropositive patients, the relative rarity of serological rise, and the uncertainty of the origin of infection, CMV transmission by blood transfusion was estimated only in the seronegative subgroup.

CMV Infection Rates at CHP

Although 9 out of 93 (9.7%) seronegative patients showed evidence of postsurgical CMV infection, usually at a time related to their surgery with concomittant transfusion/perfusion, it seemed necessary to compare this rate with that seen in other patients at CHP to determine whether it was a significant increase. Therefore, 421 sera were collected, without selection, from the clinical chemistry and hematology laboratories. The age-specific prevalence of CMV-CF antibody titers ≥ 1:4 is shown in Fig. 1. Prevalence changed little over the age range of 1–16 years, averaging 29.2%. The incidence compatible with these changes in prevalence must be substantially less than 1% per year which is, in any case, much too low to account for the 9.7% observed in the 3-month period during which the individual members of the study group were followed. This provides additional evidence that the CMV infections observed were related to events occurring during hospitalization. Next, an attempt was made to determine the effect of hospitalization alone. Of the 421 sera included in the prevalence survey, 182 were from patients with previous admissions to CHP which exceeded 7 days in duration. Fifty-five (30.2%) of these patients had CMV-CF antibody, whereas 68 out of 239 (28.5%) children who had had hospital

TABLE 2
CMV Isolation from Urines of Children
Undergoing Open-Heart Surgery

CF status on admission	Time of urine collection			
	Presurgical	6–8 weeks post surgery (surgical clinic)	12–14 weeks post surgery (cardiology clinic)	Totals
Seropositive	6/43[a] (14%)	6/40 (15%)	4/30 (13.3%)	16/113 (14.2%)
Seronegative	0/103 (0%)	0/104 (0%)	6/114 (5.3%)	6/321 (1.9%)

[a]Number of virus isolations/number of urine specimens tested.

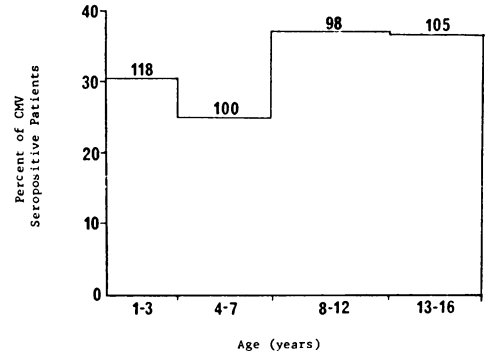

FIG. 1. Age-related prevalence of CMV-CF antibody in patients admitted to Children's Hospital of Pittsburgh. Above the histogram are the number of patients per group. All patients were over 6 months of age.

stays of less than 7 days were seropositive. It is therefore unlikely that exposure to the CHP environment itself caused CMV infection. These survey findings are summarized in Table 3. It should be noted that in Table 3 the majority of specimens from children categorized as having hospital stays of less than 7 days were collected at the time of first admission.

The relationship between CMV infection and diagnosis is shown in Table 4, where the status of the whole study group with respect to CMV-CF antibody and viruria prior to surgery and subsequent evidence of CMV infection is examined. There was no discernible correlation between diagnosis and incidence of infection. Although the rate of infection (38%) for patients with pulmonic stenosis is apparently high, the number is too small for meaningful analysis. Eight patients with Down's syndrome were identified; one of these subsequently developed infection. In spite of the fact that such patients are thought to have poor resistance to infections and are frequently institutionalized, in our series they were not shown to differ from the total study group.

The Relationship of CMV Infection to Number of Units Transfused

As shown in Fig. 2, the number of units received by seronegative patients ranged from 0 to 20 with a mean of 3.9. Packed erythrocytes were used almost exclusively. We failed to isolate CMV from 120 units of blood used in these and other patients or from freshly drawn blood samples from 200 healthy volunteer donors. The distribution of infected patients according to the number of blood units received (Fig. 2) does not appear to indicate any dose–response relationship. However, more detailed analysis showed that the distribution was consistent with a transmission rate of about

TABLE 3
CMV-CF Antibody Prevalence in Unselected Sera from CHP

Length of stay at CHP	CMV-CF Antibody		Totals
	Positive	Negative	
<7 days	68 (28.5%)	171	239
≥7 days	55 (30.2%)	127	182
Totals	123 (29.2%)	298	421

TABLE 4
CMV Infections According to Diagnosis

Diagnosis	Number of patients	Preoperative status: Viruric CF ≥ 1:4	Preoperative status: CF ≥ 1:4 (no viruria)	Postoperative CMV infection: Number	Postoperative CMV infection: Percentage
Atrial septal defect	32	0	5	3	9.3
Ventricular septal defect	23	2	4	2	8.7
Tetralogy of Fallot	18	0	5	2	11.1
Aortic stenosis	17	2	5	3	17.6
Transposition of the great vessels	8	0	4	0	0.0
Endocardial cushion defect	8	1	2	1	12.5
Pulmonic stenosis	8	0	0	3	37.5
Other	10	1	2	1	10.0
Total	124	5	27	15	12.1
Down's syndrome[a]	8	1	1	1	12.5

[a]Down's Syndrome was not a diagnosis indicating open-heart surgery and these patients are included in the categories above.

2.7% per unit of blood. The calculations were made as follows: If p equals the fraction of all blood units that contains CMV, and m equals the number of units received by a recipient, then P, the probability of receiving blood containing CMV, is given as:

$$P = 1 - (1 - p)^m$$

For example, if 5% of the units contain CMV and a patient received 20 units, $P = 1 - (1 - .05)^{20} = .6415$.

This method of analysis was applied to the data of Fig. 2 using the observed numbers of patients in the study group receiving the number of blood units shown. It was found that for $p = .027$, the total expected number of infections was 9.2 for the study

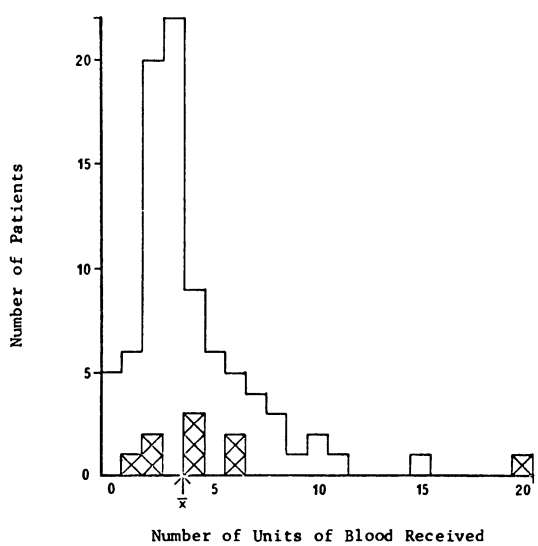

FIG. 2. Blood units received by open-heart surgery patients who were seronegative at admission. Those patients who became infected are shown by cross-hatched bars. The plain histogram represents the total group. $\bar{x}$ = mean.

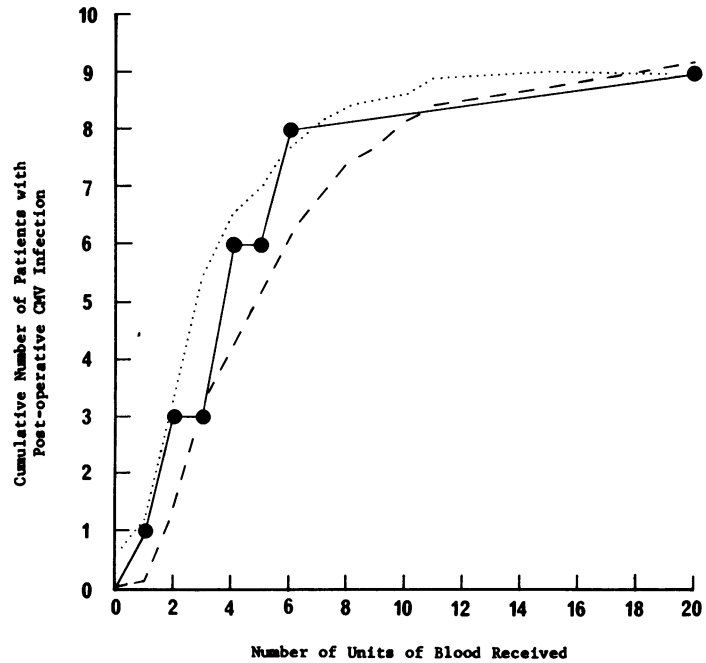

FIG. 3. Relationship between blood transfusion and CMV infection in patients seronegative on admission. The number of infections are plotted as a cumulative function of the number of units received. Circles and solid line: Observed data. Broken line: Theoretical model assuming a risk of transmission of 2.7%/unit (see text). Dotted line: Theoretical model assuming independence between number of units transfused and risk of infections in which all patients have a risk of 9.68% (see text).

population. That is, $\Sigma\, n_i(1 - [1 - 0.027]^{m_i}) = 9.2$ where n_i is the number of patients receiving m_i units of blood. The theoretical cumulative distribution curve derived in this manner is shown, together with the observed values, in Fig. 3. The third curve in this figure is based on an independent model in which each patient has the same probability (9/93 = .0968) of acquiring CMV infection. The three curves are not statistically distinguishable (Kolmogorov–Smirnov test) and hence we can only say that our results are consistent with a model in which 2.7% of the blood units are capable of transmission of CMV. We cannot, from this analysis, eliminate the possibility of involvement of other factors in the CMV infections observed.

DISCUSSION

Evaluation of the role of blood transfusion in the transmission of CMV has been complicated by the recognized ability of this virus to remain latent in man and to be reactivated by modification of the host–parasite relationship (6, 14). We have therefore depended only upon data derived from the seronegative subpopulation to estimate CMV transmission by blood. In actuality, the data from our seropositive patients were intrinsically unreliable since viruria was shown to occur with equal frequency before and after transfusion. Rises in antibody titer must also be viewed with suspicion in seropositive patients in view of the temporal variation in CMV-CF titers reported by Waner and Weller (13) in patients undergoing plasmapheresis.

We believe that the nine infections seen in seronegative children are probably primary, although it is possible that those detected by seroconversion only may represent reactivations or normal fluctuations in titer not associated with new infection. It

is likely that viruria could have been detected in association with seroconversion if urine samples had been obtainable more frequently.

Seroepidemiological studies of the general hospital population showed that our study group was very similar to the total hospital population in terms of age, race, and prevalence of CMV-CF antibody. The low annual incidence rate of CMV (< 1% year) in the age group studied, estimated from the prevalence data, enabled us to conclude that the observed incidence (9.7% over a 3-month period or 40% per year) in the seronegative study group represented a real excess of infection occurring after transfusion.

The observed infection rate of 2.7% per unit of blood administered to seronegative recipients is surprisingly similar to the estimate made by Kane *et al.* (5) that 3% of healthy volunteer donors are viruric and possibly capable of transmitting CMV, although we, like many others (5, 8, 15) were unable to detect CMV in blood. The findings of Diosi *et al.* (2) that a proportion of asymptomatic donors yield CMV from their blood remains to be confirmed with American volunteer donor populations. Nonetheless, our results are consistent with transmission by blood although dose-responsiveness could not be statistically established.

Our estimate of the risk of acquiring CMV by blood transfusion cannot be directly compared with estimates made by Henle *et al.* (3) and Prince *et al.* (10). These estimates considered both seropositive and seronegative patients and the mathematical model used, although correct for single-unit transfusion, was not well adapted for estimation of risk in multiple-unit transfusions.

Examining physicians reported no clinically important morbidity attributable to CMV infection in our study group.

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