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Factors Contributing to the Risk of Cytomegalovirus Infection in Patients Receiving Renal Transplants[1]

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INTRODUCTION

Following renal transplantation, the incidence of CMV infection varies from 52% (18) to 91% (2). There are a number of putative risk factors which contribute to cytomegalovirus infection after transplantation (Table 1). It is not clear how important each one is. The virus may either be introduced from an outside source to produce a primary infection, or an endogenous infection may be activated to produce a secondary infection. The latter type of infection occurs more frequently than the former type. For example, Craighead *et al.* (8) found that 90% of the seropositive recipients became infected, while only 47% of the seronegative became infected. Our own rates, 80% and 59% (see below) are consistent with this experience. Kanich and Craighead (18) pointed out in a retrospective autopsy study that only patients who had received immunosuppressive agents (azathioprine, prednisone, azaserine, and actinomycin C, in various combinations) became infected. Immunosuppressive drugs may contribute to infection by facilitating reactivation or primary infection, but their relative importance in these two types of infections is unknown.

In this paper we will review what is known about each risk factor (Table 1), present some data from our laboratory regarding the role of transfused blood, the donated kidney, and the host versus graft reaction (factors A3, A4, and B4), and estimate quantitatively their roles in producing CMV infection. The role of immunosuppressive drugs (factor B2) is treated in greater detail in a separate communication (11).

SOURCE OF VIRUS

There is no consensus as to how cytomegalovirus is usually transmitted. The ubiquity of this infection, the higher rates of infection in lower socioeconomic strata (27, 22), and the rapid acquisition of infection in young children and general increase with age (29) are all observations consistent with transmission by close person-to-person contact. Familial contacts of known virus excretors, or persons housed in an institution as contrasted to those living at home, are at greater risk for infection (33).

Weller (36) distinguishes the following modes of viral transmission: (i) Prenatal transmission of the virus occurs from the mother transplacentally to the fetus, usually but not exclusively following a primary infection of the mother. The pregnant

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TABLE 1
Risk Factors in Cytomegalovirus Infection

A. Source of virus (primary infection)
1. Outside environment
2. Contact with persons
3. Transfused blood
4. Donated kidney
B. Depression of host resistance
1. Renal failure
2. Immunosuppression by drugs
3. Immunosuppression by other methods
4. Host versus graft reaction
5. Graft versus host reaction

woman has been found to be a more frequent carrier of the virus than the nonpregnant (25, 29). The fetus may also be infected by intrauterine transfusion. (ii) Perinatal transmission may be acquired by passage through an infected birth canal or shortly thereafter. Not only do pregnant women carry the virus in the cervix, but so do postpartum women (9, 1). (iii) The immediate postnatal period is one of high rate of acquisition of infection. Potential sources of infection include the cervical secretions, milk (29, 9) and respiratory tract (4) of the mother and other contacts of the infant. More recently CMV in the cervix was associated with venereal disease (15). It has also been detected in semen of young men (20), in stools (6), and in transfused blood (10). These findings suggest numerous additional potential sources for CMV and that its modes of transmission may be protean. Like infectious mononucleosis it may be a "kissing disease", transmitted by respiratory secretions. It may also be transmitted as a venereal infection analogous to Herpes simplex Type II, or it may be transmitted by contaminated urine or feces like an "enteric disease." Finally, cytomegalovirus mononucleosis has been observed to occur in the adult frequently following transfusions (16, 17).

The foregoing emphasizes the many possible human reservoirs for CMV. There is no known environmental reservoir of CMV, nor has there been described an outbreak associated with a suspected contaminated environmental source. This possibility exists whenever an agent is dispersed in large quantities from a bodily source, such as urine, where the virus may be excreted for long periods of time in concentrations as high as 10^6 infectious particles/ml. Significant environmental reservoirs are made less likely by the fact that this virus is quite labile.

No specific information is available concerning the infectivity of carriers of CMV for the renal transplant patient just as there are no precise data for natural infection. Coulson *et al.* (5) describe an "epidemic" of "40-day fever" due to CMV in a renal transplant population which lasted for 18 months. The likely source was considered to be the close person-to-person contact in the renal dialysis unit, although the precise site was not identified.

BLOOD AS A SOURCE OF VIRUS

Since the description of posttransfusion CMV mononucleosis (16, 17, 28, 12, 21) and the report of Diosi *et al.* (10) that CMV was isolated from the leukocytes of two out of 35 healthy donors, transfused blood has been implicated as a source of infective virus. This hypothesis is tested in various high risk populations elsewhere in this

volume. The pertinent facts are: (i) Contrary to Diosi's findings (10), the frequency of CMV isolation from the buffy coats of bloods used in this country is extremely low (probably $<0.1\%$) or nonexistent (26). This "negative" finding of course does not rule out that certain donor populations are a hazard, or that inapparently or latently infected buffy coats may exist, and are infectious in a susceptible subject. (ii) It is possible that transfused blood cells may act in other ways (19). They are foreign cells which incite a host versus graft response in the recipient. Donated lymphocytes may also initiate a graft versus host reaction in the recipient. Either or both reactions may activate or enhance latent virus in the donor cells or in the recipient. These possibilities are discussed below.

The relationship of the amount of blood transfused to CMV infection in a group of 46 renal transplant recipients is shown in Fig. 1. Infection is defined as isolation of CMV from blood or urine and/or a fourfold increase in CF titer within 6 months of transplantation. The mean number of units transfused for the entire group was 3.17 per patient. Figure 1a shows data for the entire group while Fig. 1b considers the subgroup of those who were initially seronegative. The CMV infection rates for the entire group and for the seronegative subgroup were 54% (25/46) and 50% (16/32), respectively. There is no discernable relationship between infection and number of

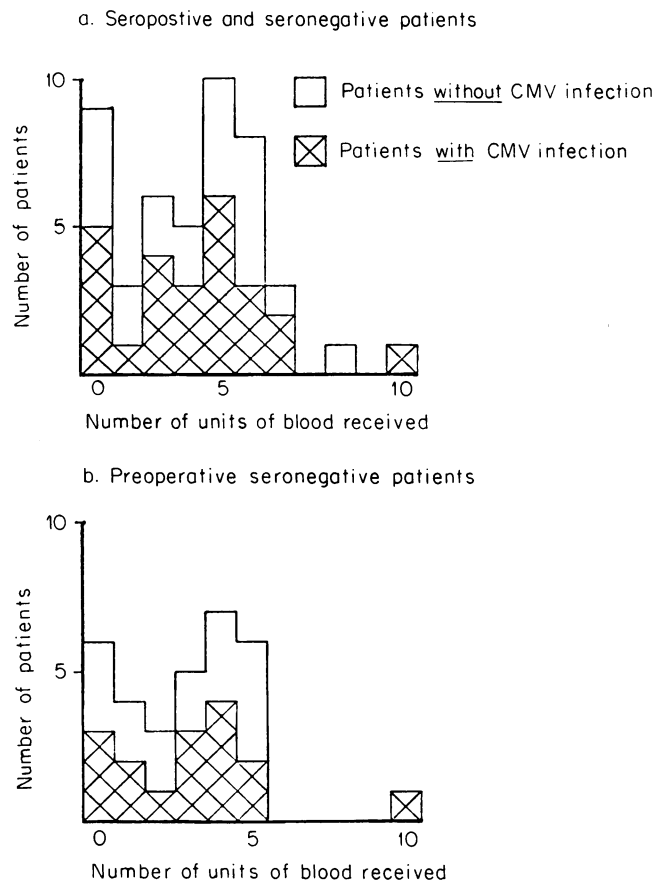

FIG. 1. Relationship between units of blood transfused to subsequent CMV infection in 46 renal transplant recipients (a) and in those who were seronegative preoperatively (b).

units received during or after a transplant operation. Five of nine patients who received no transfusions became infected, a rate (56%) comparable to that of the group as a whole (54%). One patient received 8 units and remained uninfected. Hence we see no evidence that blood was a source of CMV infection in our transplant group. What other contributory role it may have played also could not be detected.

THE DONATED KIDNEY AS A SOURCE OF VIRUS

A suspected but unproven source of exogenous virus infecting the transplant recipient is the grafted kidney itself (13, 23). Sporadic attempts to isolate the virus from the grafted kidney have been unrewarding (3, 23). As a latent virus might not be readily demonstrable, we undertook a study relying on the presence of detectable antibody in the donor as an indicator of latent infection. If the kidney is a source of virus, and its presence is indicated by the donor being seropositive, we would expect a positive correlation between seropositivity of the donor and subsequent CMV infection in the recipient, as demonstrated by virus isolation or serological rise. Such a correlation should be particularly strong in recipients who develop a primary infection, i.e., who were seronegative before transplantation.

Table 2 shows the relationship of the donor's CF titer to the development of CMV infection in the recipient. Out of 32 patients, 21 became infected (66%). Eleven seronegative donors provided kidneys for 12 recipients, of whom five became infected (42%). On the other hand, 20 patients received kidneys from donors with titers $\geq$ 1:4, and 16 became infected (80%). This difference is significant ($P = 0.03$, Fisher's exact test). The infected recipients also included those whose titer's were $\geq$ 1:4 before transplantation, and therefore may not have been at risk for primary infection.

Table 3 considers a recipient subgroup whose titers were all $<$ 1:4 before operation. Within this group, those whose donor titers were $<$ 1:4 became infected 30% of the time (3/10), while those whose donor titers were $\geq$ 1:4 were 83% infected (10/12). This difference is statistically significant ($P = 0.02$) and is consistent with the hypothesis that a source of virus in primary CMV infection is the transplanted kidney.

It has been observed that the incubation period of primary infection with CMV is around 2 months, and that in secondary infection it is longer (2). In our patient group, all 13 seronegative patients who became infected did so within a 3-month postoperative period, while four patients who developed their infection later than this had pre-

TABLE 2
Effect of Donor's CMV–CF Titer on CMV Infection of Recipient

Donor		Recipient				
			CMV infection			
Number	Titer	No CMV infection	Rising titer only	CMV Isolation	Rate	Rate
11	$<$1:4	7	0	5	42%	42%
4	1:4	3	3	1	57%	
1	1:8	0	0	2	100%	
4	1:16	0	0	4	100%	80%
5	1:32	0	0	5	100%	
2	1:64	1	0	1	50%	
27		11	3	18	66%	

TABLE 3
Effect of Donor CF Status on CMV Infection in Seronegative Recipients

Donor's CF titer	Recipient with preoperation titer < 1:4		
	No CMV infection	CMV infection	Total
< 1:4	7	3 (30%)	10
≥ 1:4	2	10 (83%)	12
Total	9	13 (59%)	22

operative titers > 1:4 and were presumably secondarily infected. This gives some assurance, but by no means certitude, that the seronegative patients were actually seronegative, and not patients in whom antibody was undetectable and who may have been harboring latent virus. We realize the limits of inferences from serological studies, and the dangers of over-interpretation. At best these data point to the kidney as a possible source of infection in renal transplantation. This is a reasonable postulate, as the kidney is a frequent site of latent CMV infection in man.

There were three seronegative patients out of ten who became infected, but whose donors' CF titers were < 1:4. The following considerations may be made: (i) The donors may have had low titers, which were not detected. (ii) The recipients may have been infected from outside sources other than the donated kidney, suggesting that the donated kidney is not the exclusive source of exogenous virus. The latter is our present interpretation because "epidemics" of CMV infection in transplant recipients (5) probably could not have been caused by virus in donated kidneys. However, it is interesting that all three kidneys were from seronegative cadavers. Conversely, six seronegative related live donors provided kidneys for six seronegative recipients, and none became infected. This suggests that the poorer tissue matching of cadaver donors (host versus graft reaction) might further increase the susceptibility of the recipient to extrarenal sources of virus.

Two approaches are suggested by our results: (i) Look for latent virus in the kidneys of seropositive individuals. This is now eminently feasible with development of newer techniques of unmasking latent viruses, particularly herpesviruses. (ii) Study the role of immunological rejection responses on the activation of virus infections. Experimental results pertaining to the latter point will be discussed next.

ENCHANCEMENT OF INFECTION BY HOST VERSUS GRAFT REACTION

Enhancement of virus infection by immunological reactions is a topic of increasing interest. Mouse leukemia virus was activated in inapparently infected mice by the transfer of parenteral spleen cells to an F_1 recipient, producing a graft versus host reaction (14). Olding *et al.* (30) noted that virus could be activated when spleen cells from inapparently infected mice were cultured on allogeneic but not syngeneic mouse fibroblasts, and they found that activation occurred in immunologically stimulated B lymphocytes.

We began with the hypothesis that one constant immunological reaction which takes place in all renal transplantation is the host versus graft reaction. To study the effect of this reaction on enhancement of a chronic infection or increasing susceptibility to primary infection apart from the influence of renal failure, transfusions, and immunosuppression, we felt that a rigorous test of the hypothesis could only be achieved in an animal model.

Five-week-old C_3H He mice were inoculated ip with 2×10^5 PFU of Smith strain of mouse CMV. Five weeks later these C_3H mice were given skin allografts from normal 10-week-old BALB/C mice. Controls similarly infected consisted of C_3H mice which received no graft or an autograft. At periodic intervals, organs of each mouse were individually titrated for virus content.

As shown in Fig. 2A, the proportion of infected spleens of allograft animals was not significantly different from controls, but the geometric mean virus titers of positive spleens of grafted animals were consistently higher than the controls from the first through the tenth day after grafting ($P < 0.01$, Wilcoxon rank sum test). Similar data for the proportion of kidneys infected and mean virus titers of infected kidneys are shown in Fig. 2B. Significant elevation of virus titers is again demonstrated. Comparable results were obtained when recipient mice were infected for 2 and 13 weeks prior to grafting.

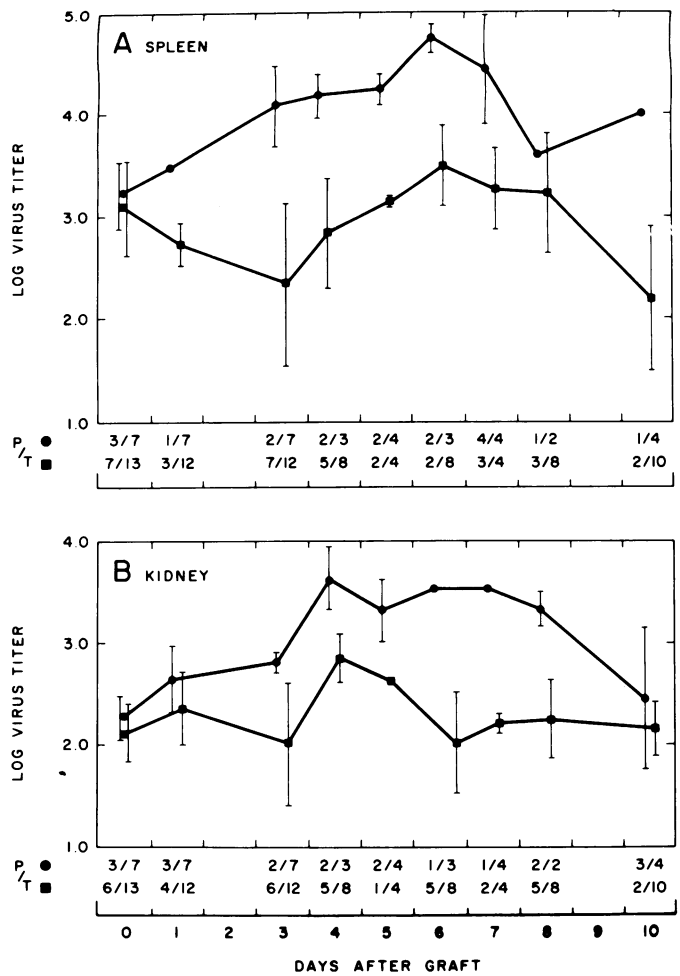

FIG. 2. Effect of skin allograft on CMV titers and proportion of infected spleens and kidneys in C^3H mice infected with CMV for 5 weeks. The points indicate geometric mean titers of infected organs in grafted (●) and control (■) mice. P/T = proportion of infected organs; P = number of positives; T = total number of mice in each group.

TABLE 4
Incidence of CMV Infection after Renal Transplant in Seropositive and Seronegative Recipients

Author	Infection rate					
	Total		Seropositive		Seronegative	
	(No.)	(%)	(No.)	(%)	(No.)	(%)
Craighead *et al.*, 1967 (8)	41	73%	24	91%	17	47%
Craighead, 1969 (7)	53	60%	36	64%	17	53%
Andersen and Spencer, 1969 (2)	36	91%	31	97%	5	60%
Armstrong *et al.*, 1971 (3)	6	100%	2	100%	4	100%
Spencer, 1974 (32)	100	86%	73	92%	27	70%
Luby *et al.*, 1974 (24)	44	73%	12	?73%	13	?73%
Present series	32	66%	10	80%	22	59%

These experiments demonstrate that virus titers may be enhanced by a host versus graft reaction, such as that which takes place following renal transplantation. They do not indicate the mechanism of enhancement. Specific immune responses may be impaired, facilitating viral replication in general, or a more direct reactivation of latent virus in specific cells (such as B cells, as suggested by Olding *et al.* (30)) may take place. In any case, it is not unreasonable to assume that a similar process occurs in man. These experiments also do not differentiate reactivation of a latent or chronic infection (secondary infection) from increased susceptibility to primary infection. Evidence of 30% primary infection in recipients of seronegative cadaver kidneys suggests that the latter mechanisms are also operative. Finally, these animal experiments do not indicate how important the host versus graft mechanism is in producing CMV in humans. They only demonstrate that it may be operative.

DISCUSSION

Primary infection with CMV in an adult can rarely be conclusively proven because its presence is indicated by the *absence* of antibody before infection. A negative finding, particularly in the somewhat controversial arena of CMV serology (35), is tenuous ground on which to diagnose a positive event. Further, authors have assumed that most CMV infections following transplantation are of the secondary or reactivation type (e.g., 2). Nevertheless, a review of literature and our own experience (Table 4) shows that a significant, though smaller, number of infections occurs in initially seronegative subjects, and hence they are of the primary type. Primary infection implies that the virus was transmitted from outside the patient.

An estimate of the importance of various sources is presented in Table 5. From our data, the infection rate for seronegative recipients who did not receive a kidney from a seropositive, or putatively latently infected, donor was 30%. By exclusion, such an individual was infected from a nonrenal exogenuous source. This represents only 23% of the "primary" infections, and 14% of the total, but it may be an important factor in the certain "epidemic" situations (5).

By comparison, when a seronegative recipient received a kidney from a seropositive donor, the risk of infection increased to 83%. Interestingly, the two seropositive donors whose kidneys did not produce an infection in this group both had low CF titers of 1:4. Infection from donated kidneys represented 77% of the "primary infections", or 47% of the total. Kidneys from seropositive donors, particularly those with high antibody titers, must be considered a significant risk.

TABLE 5
Quantitation of Risk Factors in CMV Infection After Renal Transplant

	Risk[a]
A. Source of virus in primary infection	
1. From non-renal source	
Seronegative donor in seronegative recipient (3/10)	30%
Infecting seronegatives (3/13)	23%
Infecting total group (3/21)	14%
2. From donated kidney	
Seropositive donor in seronegative recipient (10/12)	83%
Infecting seronegatives (10/13)	77%
Infecting total group (10/21)	47%
B. Depression of host resistance	
1. Immunosuppression[b]	
Role in total transplant group (RG:43%, TG:66%)[c]	65%
Role in seropositive group (RG:56%, TG:80%)	70%
Role in seronegative group (RG:20%, TG:59%)	34%
2. Steroids—nondetectable effect on CMV infection	
3. Graft reaction—operative but not quantitated	
4. Renal failure—probably none	

[a] Risk is the calculated proportion of infection accounted for by the stated factor.
[b] Immunosuppression means that patients received a cytotoxic immunosuppresive drug such as azathioprine or cyclophosphamide.
[c] RG = rheumatology group; TG = transplant group.

Whether kidney donors should be screened routinely for their CMV CF titer is a moot point. One would like to have more direct evidence that the kidneys of such subjects are infected, and one would like more data on the disease implications of CMV infection which in the majority of cases now appear asymptomatic.

The risk due to immunosuppression can be assessed if we assume that the study of immunosuppressed rheumatology patients (see Dowling *et al.*, elsewhere in this volume) isolated the factor of immunosuppression. CMV infection in the total renal recipient group was 66%, while 43% of prospectively studied immunosuppressed rheumatology patients developed infection. Thus 65% (0.43/0.66) of the infections in the transplant recipients may be accounted for by immunosuppression. By the same reasoning, immunosuppression could account for 70% of the infections in seropositive, and 34% in seronegative, transplant patients.

It is clear that immunosuppression is a predominant but not sufficient factor in CMV infection. In primary infection, its role is less important. Immunosuppression might partially explain why most of the CMV infections after renal transplantation are secondary reactivation types.

The doses of azathioprine used in both groups are comparable. Persistent viruria was common in patients on immunosuppression. None of the rheumatology patients developed viremia, while 52% (12/21) of the infected in the transplant group had viremia. None of our children who developed CMV infection after undergoing open-heart surgery developed viremia either (see Armstrong *et al.*, elsewhere in this volume). The transplant patient may be subject to additional risk factors peculiar to that group of patients.

One of these is immunological enhancement of CMV infection. We recently showed that a host versus graft response enhanced murine CMV infection (37), and Hirsch *et al.* (14) have shown that a graft versus host response enhanced mouse

leukemia virus activity. Presumably these reactions may be mediated by the allograft, although components of transfused blood may participate in these reactions (19). We were, however, unable to demonstrate that risk of CMV infection increases with amounts of blood transfused.

A number of other factors may be contributing to risk of infection, but their role cannot be proven either because they are always present and cannot be studied separately, or because by themselves they do not predispose to CMV, but they may act synergistically with other factors. Two such factors are uremia and administration of steroids. Neither alone has been shown to facilitate CMV infection (32, 18, and elsewhere in this volume). Uremia, however, almost invariably precedes transplant surgery, and steroids are almost invariably administered with immunosuppressants so that it is difficult to rule out their contributory role.

SUMMARY

Cytomegalovirus infection is a frequent occurrence after renal transplantation. Primary infections is caused by transmission of virus from the donated kidney, particularly if the recipient is seronegative (nonimmune), and if the donor is seropositive (harbors virus). It can be shown that a small but significant risk of infection arises from unknown, nonrenal, exogenuous sources. Blood may still be a source, although units transfused could not be related to infection.

In mice chronically infected with mouse cytomegalovirus, skin allograft enhanced virus titers. It can be assumed that host versus graft reaction following renal transplant may similarly enhance or activate human CMV. Immunosuppressive drugs can be shown to be particularly important in producing reactivation or secondary infection. However, it alone cannot explain the high rate of infection.

Note added in proof: Our evidence that the donated kidney may be a source of CMV infection was published in M. Ho *et al., New Eng. J. Med.* **293,** 1109–1112, 1975.

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