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The Blood Donor: Detection and Magnitude of Cytomegalovirus Carrier States and the Prevalence of Cytomegalovirus Antibody

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Interest in transfusion-associated Cytomegalovirus (CMV) infections began with the recognition that the overwhelming majority of cases of late-onset postperfusion mononucleosis were heterophile negative (1, 2). In 1965 Klemola and Kääriäinen (3) associated heterophile-negative mononucleosis with CMV infection, and 1 year later the same group documented the association of CMV infection with heterophile-negative posttransfusion mononucleosis (4). Those patients experiencing CMV infection had not undergone extracorporeal perfusion but had been transfused with large volumes of fresh blood, which implied that asymptomatic carriage of CMV might be quite common. This supposition seemed to be borne out by Diosi *et al.* (5) in work published in 1968 which reported CMV isolation from the peripheral leukocytes of 2 out of 35 healthy blood donors. Since that time peripheral leukocytes from over 1500 blood donors have been inoculated into cell culture for virus isolation, but no CMV has been recovered. This work has been done in various parts of the world including Bristol, England (6), Pittsburgh (7), Cleveland (8), Atlanta (9), Houston (10), Kansas City (11), and Seattle (12).

Prospective studies have examined the incidence of CMV infection by serology in patients receiving multiple blood transfusions. Table 1, taken from Randall and Plotkin (13), lists these studies which have shown posttransfusion infection frequencies ranging from 21 to 38%. It is noteworthy that 10 to 15% of those infected developed the postperfusion syndrome. At this point it is important to emphasize the difference between infection and disease. "Disease" is a very simple English word that means just what it says. . . . dis - ease, or loss of comfort. "Infection", on the other hand, means the establishment of a host–parasite interaction and need not lead to damage to the host. There are many ways of detecting this interaction, ranging from isolation of the agent to evidence of the immunological response in the host. This table shows evidence of infection.

Table 2 is taken from a paper by Krech (18) and shows the distribution of CMV complement Fixing (CF) antibodies in healthy blood donors in different parts of the world. Antibody prevalences range from 40% in highly industrialized areas to 100% in developing countries. It is interesting that antibody prevalences in Japan and Hong Kong are in excess of 90%, even though they cannot be considered developing areas.

Table 3 is a summary of CMV CF antibody prevalence studies in blood donors living in Europe, Australia, and North America. The low prevalence reported in the study of Monif *et al.* (24) can be explained by the fact that the majority of the donors in Gainesville are college students, thus young and likely to be middle class. Additional studies reporting low antibody prevalences are those of Jack and McAuliffe

TABLE 1
Incidence of CMV Antibody Rise (CF) in Patients Receiving Multiple Blood Transfusions from Randall and Plotkin (13)

Study	Number of patients	Number of patients with rise	Percentage
Prince *et al.*, 1971 (14)	72	15	21
Henle *et al.*, 1970 (15)	72	17	23
Stevens *et al.*, 1970 (16)	41	13	32
Perham *et al.*, 1971 (6)	55	21	38
Paloheimo *et al.*, 1968 (17)	63	19	30

(19) and Embil *et al.* (21). Only the study of Mirkovic *et al.* (10) reported relatively high antibody prevalence. A skewed age distribution of donors does not appear to be the explanation for the low prevalences cited in the two former studies, nor is it responsible for the high prevalence reported in the Houston study. Socioeconomic factors may explain these differences.

From June, 1972 to February, 1973 we conducted a study at the Community Blood Center aimed at defining the epidemiology of CMV infections among blood transfu-

TABLE 2
Distribution of Cytomegalovirus Complement-Fixing Antibodies among Healthy Blood Donors in Different Parts of the World from Krech (18)

Place where blood was collected	Country code	Number of serum samples tested	Samples with complement-fixing antibody	
			Number[a]	Percentage
Lyon	F	98	39	40
Freiburg	D	89	37	42
St. Gallen	CH	105	47	45
Albany	USA	98	44	45
Melbourne	AUS	99	54	54
Stockholm	S	99	60	60
Manchester	GB	94	58	61
Honolulu	USA	145	97	67
Johannesburg (whites)	SA	96	72	75
Houston	USA	98	77	79
Buenos Aires	RA	43	35	81
Bratislava	CS	100	83	83
Port of Spain	TT	99	86	86
Mauritius	MS	93	83	89
Anchorage	USA	100	94	94
Hong Kong	HK	99	94	94
Sendai	J	99	96	96
Greenland	DK	90	88	98
Dar es Salaam	EAT	117	114	98
Morocco	MA	109	107	98
Fiji Islands	GB	95	95	100
Entebbe	EAU	143	143	100
Ibadan	WAN	95	95	100
Johannesburg (Bantu negroes)	SA	112	112	100
Manila	PH	89	89	100
Chandigarh	IND	68	68	100

[a] $\geq$ 1 in 4. The tests were performed by the coordinating laboratory.

TABLE 3
Prevalence of Complement-Fixing Antibodies to CMV in Blood Donors

Study	Year	Reciprocal of lowest serum dilution tested	Geographic region	Prevalence (%)
Jack and McAuliffe (19)	1968	8	Melbourne, Australia	24
Baron *et al.* (20)	1969	10	Pittsburgh, Pennsylvania	60
Diosi *et al.* (5)	1969	8	Timisoara, Rumania	65
Embil *et al.* (21)	1969	8	Nova Scotia, Canada	38
Klemola *et al.* (22)	1969	4	Helsinki, Finland	66
Collaborative (23)	1970	NS[a]	Manchester, England	56
Mirkovic *et al.* (10)	1971	10	Houston, Texas	75
Perham *et al.* (6)	1971	8	Bristol, England	55
Wentworth and Alexander (12)	1971	8	Seattle, Washington	52
Monif *et al.* (24)	1973	8	Gainesville, Florida	21
Kane *et al.* (11)	1975	8	Kansas City, Missouri	59

[a]NS, not stated.

sion recipients (11). In the Kansas City metropolitan area our 100% volunteer donor base is really from the mainstream of society. It had a mean family income of $14,000 in 1972 which has obviously increased with inflation. Our donors are 97% Caucasian. Our black donor base is similar to our white donor base when compared in terms of HB_sAg and anti-HB_s prevalence.

Attendant to this study we examined the prevalence of CMV antibodies, viremia, and viruria among 223 volunteer blood donors and followed the recipients of blood from infected donors. The donors ranged in age from 19 to 62 with a mean of 37 years. They were 98% Caucasian and 77% were males. No CMV isolations were made from any of the 223 leukocyte-rich plasmas or washed leukocytes inoculated

TABLE 4
Laboratory Data on Seven Blood Donors with Cytomegaloviruria from Kane *et al.* (11)

Donor	Age/sex	Time	Urine culture	Reciprocal antibody titer	
				CF[a]	IHA[b]
012	25-M	Donation	+[d] (23)[c]	16	160
		6 weeks later	0[e]	16	160
025	38-M	Donation	+ (22)	16	40
		10 weeks later	0	16	40
029	28-M	Donation	+ (13)	32	80
		9 weeks later	0	32	80
123	37-M	Donation	+ (27)	8	40
		14 weeks later	0	16	40
162	51-M	Donation	+ (21)	64	160
190	56-F	Donation	+ (21)	8	80
		8 weeks later	0	16	80
198	53-M	Donation	+ (31)	32	40
		8 weeks later	0	32	40

[a]Complement fixation.
[b]Indirect hemagglutination.
[c]Number of days in culture when viral cytopathogenicity first observed.
[d]+, CMV isolated.
[e]0, CMV not isolated.

TABLE 5
Complement Fixing Antibody Titers of 223 Blood Donors from Kane *et al.* (11)

Age	Number of donors	Positive[a] (%)	Number of donors with titer				
			< 8	8	16	32	64
19–24	26	19	21	1	4	0	0
25–29	46	48	24	2	10	8	2
30–39	68	57	29	7	22	10	0
40–49	45	76	11	8	13	9	4
50–62	38	79	8	9	9	10	2
Total	223	58	93	27	58	37	8

[a]Antibody titer ≥ 1:8.

directly onto Wentworth's human fetal tonsil fibroblasts. Seven donors (3%) were cytomegaloviruric, and Table 4 summarizes the laboratory data on these donors. Follow-up blood and urine cultures were taken from six of the seven cytomegaloviruric donors at times ranging from 6 to 14 weeks after donation; none of the cultures was positive for CMV. All viruric donors were antibody positive by CF and indirect hemagglutination (IHA) and their titers remained unchanged in those who were followed after donation. Their titers did not differentiate them from the nonviruric donors. Table 5 shows that 58% of all the donors had CMV CF titers of 1:8 or greater, while Table 6 documents that 59% of this group had IHA antibody titers of 1:10 or greater. When the CF and IHA titers from each donor were compared, the direct correlation illustrated in Table 7 was found. Six of the seven units taken from viruric donors were transfused, but only three of the six recipients lived long enough to be followed for evidence of CMV infection. Table 8 summarizes the laboratory data on the recipients of blood from the viruric donors. Two underwent coronary artery bypass surgery, while the third was transfused during a gunshot wound repair. Each of the recipients showed serologic evidence of CMV infection by a fourfold rise in IHA titer 13 to 16 weeks after transfusion. CMV-specific IgM antibodies were detectable in the posttransfusion sera of two of the recipients. No posttransfusion serum was available for testing from the third recipient. Somewhat puzzling was the fact that CF antibody rises were not detected in the two recipients on whom sufficient quantities of serum were available for testing. CMV was isolated from the urine of one of the recipients 14 weeks after transfusion. No disease attributable to CMV infection was seen in any of the recipients of blood from the viruric donors.

TABLE 6
Indirect Hemagglutination Antibody Titers of 223 Blood Donors from Kane *et al.* (11)

Age	Number of donors	Positive[a] (%)	Number of donors with titer							
			< 10	10	20	40	80	160	320	≥ 640
19–24	26	23	20	0	2	0	3	1	0	0
25–29	46	50	23	0	4	2	7	6	2	2
30–39	68	59	28	0	3	9	16	8	2	2
40–49	45	76	11	0	1	7	10	7	7	2
50–62	38	76	9	0	0	5	8	7	7	2
Total	223	59	91	0	10	23	44	29	18	8

[a]Antibody titer ≥ 1:10.

TABLE 7
Distribution of 223 Donors by Indirect Hemagglutination (IHA) and Complement Fixing (CF) Antibody from Kane *et al.* (11)

Reciprocal IRA titer	Reciprocal CF titer				
	< 8	8	16	32	64
≥ 640	0	0	1	4	3
320	0	1	8	6	3
160	0	2	14	11	2
80	0	10	23	11	0
40	0	9	9	5	0
20	4	4	2	0	0
10	0	0	0	0	0
< 10	89	1	1	0	0

The observation of CMV infection in three recipients of blood from cytomegaloviruric donors prompted us to continue our search for viruric donors in order to see if viruria rather than viremia might be a more reliable indicator of ability to transmit CMV infection via blood. We have followed an additional 420 donors for evidence of CMV infection by urine culture and serology. Only four donors (1%) were excreting CMV in their urine at the time of donation. Antibody prevalence to CMV by CF and IHA was 65% in this cohort. Two of the four units from viruric donors were transfused to patients who could be followed, and the recipients had pre-existing CMV CF antibody titers of 1:8 and 1:16, respectively. Neither recipient showed evidence of infection by virus isolation from the urine or by fourfold or greater rise in antibody titer. Thus, three of five recipients of blood from viruric donors have shown evidence of CMV infection subsequent to transfusion. This study is still in progress and will continue until larger numbers of recipients have been studied. We are also following recipients of blood from nonviruric donors who are matched by age, sex, hospital, and medical history to the patients receiving blood from cytomegaloviruric donors.

The large number of studies cited previously (6–11) which have unsuccessfully attempted to isolate CMV from the leukocytes of normal donors stands in sharp

TABLE 8
Laboratory Data on 3 Recipients of Blood from Cytomegaloviruric Donors from Kane *et al.* (11)

Recipient	Age/sex	Time	Virus culture		Reciprocal CMV antibody titer		
			Blood	Urine	CF[a]	IHA[b]	IHA-IgM[c]
025R	61-F	Pretransfusion	—[d]	—	32	320	< 8
		9 weeks later	0	0	32	640	32
		16 weeks later	0	0	32	1280	64
162R	48-M	Pretransfusion	—	—	16	80	< 8
		13 weeks later	—	—	32	320	QNS
198R	38-M	Pretransfusion	—	—	QNS	40	QNS
		14 weeks later	0	+	128	320	8

[a] Complement fixation.
[b] Indirect hemagglutination.
[c] IHA antibody titer of serum fraction containing IgM.
[d] +, CMV isolated; 0, CMV not isolated; —, not performed.

contrast to the report of Diosi *et al.* (5) who identified two asymptomatic donors with CMV viremia out of 35 tested. This may well have been a chance observation, but Diosi's methods were sufficiently different from those reported in the other studies that this factor could be responsible for the disparate results. In order to test this hypothesis, we obtained blood and urine samples from 120 donors and attempted to isolate CMV by closely following the procedures described in Diosi's paper. The protocol involved culturing the leukocytes in suspension for 72 hr prior to inoculating them onto cultures of human fetal fibroblast cell cultures. In addition, we cultured three aliquots of cells from each donor in Diosi's medium which contained 10 μg/ml dexamethasone, 10 μg/ml azathioprine, and 2% antilymphocyte serum, respectively, for 72 hr and then inoculated the treated leukocytes onto human fetal tonsil cell cultures. The rationale for doing this was that drug treatment might unmask latent CMV resident in the leukocytes of the donors. Of 120 donors, 71 (59%) had CMV IHA antibody titers of 1:10 or greater, but no CMV was isolated from the untreated or drug-treated leukocytes of any donor. Three antibody-positive donors (2.5%) were excreting CMV in their urine at the time of donation. Recipients of the blood from these donors were not followed. In short, our data support the contention that methodologic differences are not responsible for the failure of numerous investigators to isolate CMV from the leukocytes of normal donors.

Although specifically charged with discussing the blood donor, we want to present some data on a group of open-heart patients who were prospectively followed for evidence of CMV infection. The number of open heart surgeries performed in Kansas City in 1972 was 827. This figure increased to 1199 in 1973 and climbed to 1289 in 1974; however, the average number of units used per case has declined from a figure of six in 1973 to four in 1974, and is about three units per case this year. Two units of fresh blood are made available for each surgery, but actual transfusion of units less than 2-days-old occurs only 15% of the time. Most of the blood transfused in heart surgery is 3- to 8-days-old, and approximately 18% of the transfused units are over 10 days old. Table 9 shows the results of our first study on open-heart patients which was done in Hospital A. The 35 patients in this study received an average of 5.8 units of blood. Throat, urine, and blood samples were collected for virus isolation and serology prior to surgery and at 6 and 12 weeks after surgery. Clinical data were obtained from the patient's cardiologist or his family physician. Three of 35 patients (9%) showed serologic evidence of CMV infection both by IHA and CF tests. Although CMV was isolated from the urine of one of these three patients at 12 week follow-up, no virus isolations were made from any other specimens. No illnesses at-

TABLE 9
Serologic Response of Open Heart Patients to CMV as Monitored by CF and IHA Tests, Hospital A

Serologic Status	Number of patients	Percentage
Negative,[a] no change in antibody titer	2	6
Pre-existing antibodies,[b] no change in titer	30	86
Conversion[c]	1	3
Pre-existing antibodies, fourfold or greater rise in antibody titer	2	6
Totals	35	100

[a] Negative means a serum antibody titer of $< 1{:}8$ by CF or $< 1{:}10$ by IHA.
[b] Pre-existing antibodies mean a serum antibody of $\geq 1{:}8$ by CF or $\geq 1{:}10$ by IHA.
[c] Conversion means a change in titer from $< 1{:}8$ to $\geq 1{:}16$ by CF or $< 1{:}10$ to $\geq 1{:}20$ by IHA.

TABLE 10
Serologic Response of Open Heart Patients to CMV as Monitored by CF and IHA Tests, Hospital B

Serologic Status	Number of patients	Percentage
Negative,[a] no change in antibody titer	5	25
Pre-existing antibodies,[b] no change in titer	15	75
Conversion[c]	0	0
Pre-existing antibodies, fourfold or greater rise in antibody titer	0	0
Totals	20	100

[a] Negative means a serum antibody titer of $< 1:8$ by CF or $< 1:10$ by IHA.
[b] Pre-existing antibodies mean a serum antibody of $\geq 1:8$ by CF or $\geq 1:10$ by IHA.
[c] Conversion means a change in titer from $< 1:8$ to $\geq 1:16$ by CF or $< 1:10$ to $\geq 1:20$ by IHA.

tributable to CMV were seen in any of the three patients experiencing CMV infection.

Our second study of open-heart patients, still in progress at Hospital B, has the same design as the one previously described. Table 10 displays the preliminary results of this study. Twenty patients have been followed, and none has shown evidence of CMV infection. An average of 2.5 units of blood was used for each case.

To evaluate the relative risk of CMV infection after transfusion with antibody-positive and antibody-negative blood, we have been studying the recipients of one and two unit transfusions (where both donors are either antibody positive or antibody negative). The data accumulated to date are presented in Table 11. The number of patients followed is small, and therefore no conclusions can be drawn. Only 1 of 32 recipients has shown evidence of CMV infection. The patient, who had a presurgical CMV CF titer of 64, received two CMV antibody-positive units during hysterectomy and showed a fourfold rise and viruria at 12 weeks follow-up. No disease was seen in this patient.

In summary, transfusion-associated cytomegalovirus disease is not an obvious problem in this community, at least in terms of adult transfusions. Two factors which are responsible for the apparent absence of disease and the low frequency of CMV infections are excellent surgeons who use very little blood in their operative procedures and the high quality of the blood donor base in the Kansas City metropolitan area.

TABLE 11
Serologic Response to CMV of Single and Double Unit Transfusion Recipients

Number of recipients	Percentage	Pretransfusion antibody status	Antibody status of donor blood	Conversions or fourfold rises	No change in antibody status
17	53	Positive[a]	Positive	1[c]	16
11	34	Positive	Negative[b]	0	11
3	9	Negative	Positive	0	3
1	3	Negative	Negative	0	1
Totals 32	100			1	31

[a] Positive is defined as a serum antibody of $\geq 1:8$ by CF or $\geq 1:10$ by IHA.
[b] Negative is defined as an antibody titer of $\geq 1:8$ by CF and $\geq 1:10$ by IHA.
[c] CMV was isolated from the recipient's urine at 8 weeks follow-up.

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