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Comparison of Cord Blood and Peripheral Blood Mononuclear Cells as Targets for Viral Isolation and Drug Sensitivity Studies Involving Human Immunodeficiency Virus Type 1

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We have shown that umbilical cord blood mononuclear cells (CBMC) are at least as sensitive as peripheral blood mononuclear cells (PBMC) for isolation of human immunodeficiency virus type 1 from the PBMC of infected individuals. Viral replication was more efficiently monitored by a p24 antigen capture assay than by a viral reverse transcriptase test, regardless of whether CBMC or PBMC were employed. We also found that CBMC and PBMC yielded similar results with regard to the susceptibility profiles of both wild-type and drug-resistant variants of human immunodeficiency virus type 1 for 3′-azido-3′-deoxythymidine, 2′,3′-dideoxycytidine, and the (−) enantiomer of 2′,3′-dideoxy-3′-thiacytidine. Finally, viruses isolated on CBMC could be routinely grown on PBMC and vice versa.

Consensus protocols have recently been published for the isolation of human immunodeficiency virus type 1 (HIV-1) from peripheral blood mononuclear cells (PBMC) of patients infected with this virus and for assessment of HIV susceptibility to antiviral drugs (5). Such research commonly depends on the use of PBMC from healthy donors, which serve as targets for HIV infection and amplification in coculture with PBMC from patients. However, not all laboratories have regular access to PBMC, although such cells are now available from commercial sources. An alternative to PBMC for viral isolation and susceptibility assays are umbilical cord blood mononuclear cells (CBMC), which are frequently available through departments of obstetrics of large urban hospitals. Our center is a good example, since we handle almost 5,000 births per year. Indeed, we have routinely employed CBMC for routine isolation of HIV-1 from blood samples from patients for at least 5 years (8, 9). Not only are cord blood cells generally available free of charge, but they might also serve as more efficient hosts of HIV replication than PBMC, given that relatively immature lymphocyte populations are represented. Surprisingly, no comparative study of the use of CBMC and PBMC in virus isolation and drug susceptibility protocols has been reported. Our research was performed to address this issue.

Cells and viruses. Umbilical CBMC and normal PBMC from a wide array of HIV-seronegative donors were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. In each instance, yields of 1×10^6 to 2×10^6 cells per ml of whole peripheral or cord blood were obtained. Volumes of cord blood procured by the staff of our department of obstetrics varied between 3 and 10 ml per patient.

Cells were collected, washed, and stimulated with phytohemagglutin in (PHA) as previously described (4). Cells were then seeded into 30-cm^2 tissue culture flasks and maintained for 3 days in RPMI 1640 medium supplemented with 10% fetal calf serum (ICN Chemicals, Montreal, Quebec, Canada), 2 mM glutamine, 200 U of penicillin (ICN) per ml, and 200 μg of streptomycin (ICN) per ml. After 3 days, pools of cells from three CBMC donors or three PBMC donors were established and used as feeder cultures in wells of 96-well plates as previously described (5). Such pools usually contained between 60×10^6 and 90×10^6 cells. The percentages of CD4$^+$ cells in these targets were determined by flow cytometry as previously described (7).

Blood samples were obtained from HIV-infected individuals, and PBMC were also isolated from such populations by Ficoll-Hypaque separation (8). Clinical isolates of HIV-1 were obtained from patients by means of a cocultivation procedure in which non-PHA-stimulated PBMC (2×10^6/ml) from patients were coincubated with either PHA-stimulated normal PBMC or CBMC (2×10^6/ml) in a total volume of 0.2 ml. Cultures were maintained in complete, serum-supplemented RPMI 1640 medium supplemented with recombinant interleukin-2 (5%, vol/vol; Boehringer Mannheim Inc., Montreal, Quebec, Canada) in 96-well plates (Falcon Plasticware, Oxnard, Calif.).

The presence of the HIV-1 p24 antigen in culture fluids was monitored at regular intervals by enzyme-linked immunosorption assay (Abbott Laboratories, North Chicago, Ill.) (8). Every 7 days, when the medium was changed, culture fluids were sampled and additional feeder CBMC or PBMC were added. Cultures were considered to be HIV positive when two consecutive samples collected on two different days contained ≥20 pg of p24 antigen per ml and when the second reading was at least three times as high as the first. Viral production was also

TABLE 1. Comparison of CBMC and PBMC as targets for primary isolation of HIV-1

Result obtained with samples cocultured with PBMC	No. of samples cocultured with CBMC that were:		Total
	Positive	Negative	
Positive	48	1	49
Negative	1	12	13
Total	49	13	62

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TABLE 2. Detection of HIV-1 p24 antigen in coculture supernatants of CBMC and PBMC

Sample	p24 antigen concn (pg/ml)[a]							
	Day 4		Day 7		Day 11		Day 14	
	CBMC	PBMC	CBMC	PBMC	CBMC	PBMC	CBMC	PBMC
3675	18.0		260	>400	38,000	10,000	21,000	8,000
3676	48.0	78.0	>400	25.0	115,000	260	31,000	9,000
3678-0	45.0		120	27.0	10,000	400	58,000	33,000
3674			>400		42,500		18,000	

[a] Blank spaces indicate negative results.

monitored by reverse transcriptase (RT) assay as previously described (2).

Direct infection of PHA-stimulated CBMC and PHA-stimulated PBMC was also performed with the HIV-III$_B$ laboratory strain (kindly supplied by Robert Gallo, National Institutes of Health, Bethesda, Md.) and HXB2 recombinant viruses engineered for resistance to certain drugs by site-directed mutagenesis (1, 4). We also studied both drug-susceptible and -resistant clinical isolates obtained by coculture of PBMC from patients with CBMC or PBMC from donors. A variety of multiplicities of infection, ranging from 100 to 0.1 50% tissue culture infective doses per cell, as determined in CBMC on the basis of p24 determinations, were used with an adsorption period of 1 h at 37°C. Cells were then centrifuged, washed twice, and resuspended in complete medium containing interleukin-2. Medium was changed every 3 to 4 days. Viral production was assessed by measuring p24 antigen concentrations and RT activities in aliquots of clarified culture supernatants on days 3, 6, and 10 postinfection.

Drugs. The antiviral drugs used in this research were 2′,3′-dideoxycytidine (ddC) (purchased from Sigma Chemical Co., St. Louis, Mo.), the (−) enantiomer of 2′,3′-dideoxy-3′-thiacytidine (3TC) (kindly provided by Glaxo Group Research, Greenford, Middlesex, United Kingdom), and 3′-azido-3′-deoxythymidine (AZT) (a gift of Burroughs-Wellcome Inc., Research Triangle Park, N.C.). We established the 50% effective dose of each drug on the basis of p24 antigen levels in culture fluids of both CBMC and PBMC after inoculation with both wild-type and drug-resistant variants of HIV-1 by using a variety of drug concentrations as previously described (2).

Viral isolation. We began by comparing PBMC and CBMC as targets for primary isolation of HIV-1 from the PBMC of 62 infected patients. Table 1 summarizes the results and shows that approximately 80% of the samples were ultimately positive by both methods. No discordance between the two methods was noted; each yielded either positive or negative results with the same specimens. The 20% of samples that tested negative for viral isolation had been obtained from asymptomatic individuals with CD4 counts above 500/mm^3; conversely, subjects whose blood yielded HIV possessed CD4 counts of 400/mm^3 or less and ranged from asymptomatic persons to those with advanced disease (data not shown).

However, CBMC often yielded positive results faster than did PBMC. This is illustrated for each of four additional clinical specimens, not included in Table 1, studied by p24 antigen capture and RT assays (Tables 2 and 3). One sample, number 3674, from an asymptomatic individual with CD4 counts of 450 to 500/mm^3, became positive in CBMC after 7 and 11 days, respectively, as studied by the p24 and RT assays, but did not become positive in PBMC over 30 days. In general, p24 antigen detection proved to be more sensitive than the RT assay for assessment of culture positivity. We also compared CBMC and PBMC with regard to amplification of HIV-III$_B$ and several clinical isolates of HIV by using multiplicities of infection of 0.1 to 100 50% tissue culture-infective doses per cell. Viruses first isolated on CBMC grew well in PBMC in each case and vice versa. Generation of progeny virus was at least as high in CBMC as in PBMC (data not shown). Percentages of CD4$^+$ cells in pooled CBMC and PBMC populations were 30.0% ± 17.7% and 42.5% ± 9.1%, respectively (data not shown).

Drug susceptibility and resistance. Finally, we compared CBMC and PBMC with regard to the drug susceptibilities of recombinant viruses or clinical isolates known to possess diminished susceptibility to either AZT, ddC, or 3TC by using a multiplicity of infection of 0.5 50% tissue culture-infective dose (6, 9). This study included HIV-III$_B$ and its derivative recombinant clone HXB2-D (1) as controls along with clinical isolates 124, 298, and 176, which had been obtained from HIV-infected subjects both prior to and following prolonged antiviral chemotherapy. We also used viral recombinants HXB2-D65 (3) and HXB2-D184 (4), which are resistant to both ddC and 3TC, as well as viruses selected for resistance to either ddC or AZT in tissue culture through use of concentration gradients of these drugs (2, 3). The data in Table 4 show that the 50% effective doses of all of the drugs were similar with either PBMC or CBMC as the viral targets.

These findings also demonstrate that viruses isolated by using CBMC can be easily grown in PBMC. Thus, CBMC are an appropriate substitute for PBMC for laboratories that choose or need to use the former in HIV isolation and drug susceptibility assays.

We recognize, of course, that CBMC may not be readily available at many centers and do not suggest that they be used in place of PBMC for the types of studies described here. Drawbacks include the need to perform routine HIV serology tests on CBMC donors, a procedure that is performed anonymously within our jurisdiction but may require informed consent elsewhere. Finally, while we have been fortunate to secure the cooperation of members of our hospital's Department of Obstetrics, we understand from virologists at other institutions that cord blood may not always be easy to procure.

TABLE 3. Detection of HIV-1 RT activity in coculture supernatants of CBMC and PBMC

Sample	RT activity (cpm/ml)[a]							
	Day 4		Day 7		Day 11		Day 14	
	CBMC	PBMC	CBMC	PBMC	CBMC	PBMC	CBMC	PBMC
3675				64,745	1,473,354	305,870	485,131	150,215
3676			114,311		4,189,604	105,466	1,065,368	71,083
3678-0					516,705	123,247	243,283	135,136
3674					1,930,856		580,360	

[a] Blank spaces indicate negative results.

TABLE 4. Susceptibilities of HIV-1 grown in CBMC or PBMC to antiviral agents

Variant	50% effective dose (μM) of:					
	AZT		ddC		3TC	
	PBMC	CBMC	PBMC	CBMC	PBMC	CBMC
HIV-III_B	0.02 ± 0.001	0.03 ± 0.004	0.51 ± 0.07	0.42 ± 0.06	0.91 ± 0.07	1.1 ± 0.12
HIV-III_B-AZT[a]	0.94 ± 0.07	1.12 ± 0.21	0.46 ± 0.08	0.53 ± 0.08	0.72 ± 0.05	0.86 ± 0.09
124[b]	0.04 ± 0.006	0.02 ± 0.005	0.72 ± 0.06	0.58 ± 0.04	1.2 ± 0.17	0.92 ± 0.11
124-AZT[c]	1.22 ± 0.14	0.85 ± 0.13	0.41 ± 0.03	0.68 ± 0.07	1.4 ± 0.20	0.85 ± 0.07
HXB2-D[d]	0.01 ± 0.002	0.02 ± 0.004	0.65 ± 0.09	0.36 ± 0.06	0.85 ± 0.09	1.3 ± 0.19
HXB2-D65[e]	0.02 ± 0.003	0.03 ± 0.002	6.4 ± 0.51	5.2 ± 0.45	14.0 ± 0.9	16.8 ± 2.1
HXB2-D184[f]	0.02 ± 0.004	0.03 ± 0.005	ND[g]	ND	847 ± 96	963 ± 114
298[h]	0.01 ± 0.001	0.02 ± 0.004	0.36 ± 0.05	0.50 ± 0.06	0.80 ± 0.09	1.2 ± 0.20
298-ddC[i]	0.003 ± 0.005	0.04 ± 0.005	6.2 ± 0.75	4.6 ± 0.50	ND	ND
176[j]	0.04 ± 0.002	0.03 ± 0.002	0.39 ± 0.03	0.58 ± 0.04	1.4 ± 0.15	1.5 ± 0.24
176-AZT[k]	1.26 ± 0.09	0.90 ± 0.09	0.48 ± 0.03	0.60 ± 0.08	1.0 ± 0.11	1.3 ± 0.19

[a] HIV-III_B selected for resistance to AZT in tissue culture as previously described (6).
[b] Virus from subject 124, inoculated onto CBMC.
[c] Clinical isolate 124 selected for resistance to AZT in tissue culture.
[d] Recombinant viral clone used as a reference reagent.
[e] Recombinant HXB2-D containing a Lys→Arg substitution at site 65 of the *pol* gene, conferring resistance to both ddC and 3TC. This construct was generated as previously described (8).
[f] Recombinant HXB2-D containing a Met→Val substitution at site 184 of the *pol* gene, conferring high-level resistance to 3TC. This construct was prepared as previously described (4).
[g] ND, not determined.
[h] Virus from subject 298, isolated on CBMC prior to antiviral chemotherapy
[i] Virus from subject 298, isolated on CBMC after 6 months of therapy with ddC.
[j] Virus from subject 176, isolated on CBMC prior to antiviral chemotherapy.
[k] Virus from subject 176, isolated on CBMC after 16 months of therapy with AZT.

Indeed, even within our own hospital, it has been difficult to persuade nurses and residents to collect more than one tube of cord blood per patient, although they have cheerfully provided us with samples from as many as 15 obstetric patients per day.

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