Optimal Expansion of HIV-1 Field Isolates Using Human CD4+Cell Substrate Derived from Selected Blood Donors

C. Lai Hipp [1,2], M. Leong [1,2], E.P. Scott [3], and G.N. Vyas [1]
From 1. University of California, San Francisco, CA, 2. San Francisco State University, CA, and 3. Lifeblood Biological Services, Memphis, TN.

Introduction

Clonally-derived HIV DNA or proteins are genetically limited to induce broadly neutralizing antibodies (NAB) capable of preventing HIV infection. We postulate that NAB against HIV-1 prevalent in the population (pHIV) can be elicited using inactivated virions' proteins, which represent the genetic diversity of viral quasi-species of the field isolates co-cultivated in primary CD4+cell substrate (CD4+CS). Prerequisite to testing this concept is the selection of blood donors whose CD4 cells have a biological capacity for uniformly replicating different pHIV-1 isolates and thus provide an optimal pool of CD4+CS for ultimately making an inactivated HIV vaccine candidate (HIVACC).

Materials and methods

Five pHIV-1 isolates (clade B), derived from infected plasma of donations testing positive for HIV nucleic acid test (NAT) but negative for anti-HIV, were individually cultured in pooled peripheral blood mononuclear cells (PBMC) from four random blood donors. Multiple 50ul aliquots of the seed lots were stored in liquid nitrogen for a single use in subsequent co-culture experiments. Fifteen samples from Leukapheresis donations that tested negative for HIV, HCV or HBV infection were shipped overnight from Memphis to San Francisco. The Ficoll-separated PBMC were depleted of CD8+ T lymphocytes by magnetic beads coated with anti-CD8 (Dynal Biotech, Brown-Deer, WI). Every CD4+CS was stimulated with PHA for 3 days and then infected with 50 ul each of the seed isolates of pHIV-1 for evaluating *in vitro* expansion. The aliquots of $1x10^{6}$ cells were inoculated with each pHIV-1 and co-cultured for 10 days in 2mL of RPMI, supplemented with 10% FBS and IL-2. The cell-free supernatants were tested for p24 antigen by ELISA (Perkin Elmer, Boston, MA) as a measure of virus expression.

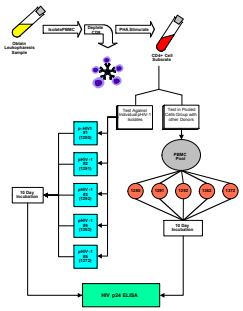

Figure 1: Experimental Design

Results

Donor ID	1280	1291	1292	1362	1372	Donor Mean	Range
25	84777	77594	84883	81804	65833	78978	65833-84777
26	63939	73043	77910	76752	49231	68175	49231-77910
27	7396	54098	34075	35470	36864	33581	7396-54098
86	28473	29072	26127	34003	10830	25701	10380-34003
87	25969	24188	28306	22010	1302	20355	1302-28306
88	37567	33884	43077	58302	17749	38116	17749-58302
90	41904	60433	59020	68476	15211	49009	15211-68476
48	61461	50317	47347	78870	28291	53257	28291-78870
49	41142	23733	21851	9442	37672	26768	9442-41142
50	82478	38201	33643	58109	31379	48762	31379-82478
52	80988	85869	64167	83046	43112	71436	43112-85869
89	87839	87574	87545	81164	71460	83116	71460-87839
91	87251	86604	87309	79546	87015	85545	79546-87309
96	86339	83987	85692	75753	75782	81511	75753-86339
97	87457	87045	87309	82487	77370	84334	77370-87457

Table 1: Raw data collected from p24 ELISA.
Each donor was tested against the 5 isolates of pHIV-1 (1280-1372). The mean and range was calculated to evaluate the capability of each donor to produce HIV *in vitro*. All measurements are in pg/mL.

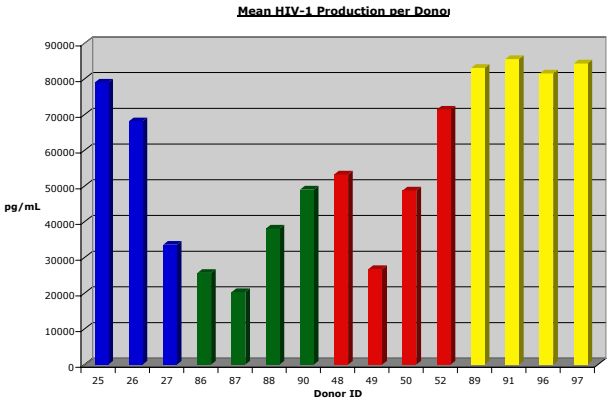

Figure 2: Graph of mean p24 production.
The graph shows the marked variability between each donor's individual ability to produce pHIV-1 as measured by the amount of p24 in culture supernatant.

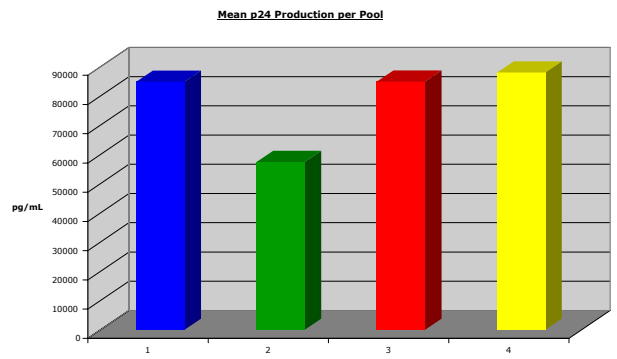

Figure 3: Graph of mean p24 production of donor pools.
The data shows the decrease in variability of p24 production when the donors PBMC's are in pool culture. Interestingly, pool #1 contains only 3 donors, yet it is able to produce approximately the same amount of virus as the pools of four donors.

Pools	1280	1291	1292	1362	1372	Pool Mean	Pool Range
#1-25,26,27	83199	85488	85488	85435	85172	84956	83119-85488
#2-86,87,88,90	68261	66561	64048	62755	26080	57541	26080-68261
#3-48,49,50,52	87192	86398	87309	85810	78488	85039	78488-87309
#4-89,91,96,97	88750	88780	87251	88250	87486	88103	87251-88780

Table 2: Raw data of PBMC pool P24 ELISA.
Like the individual donors, the pools were tested against each isolate. The mean and range is given. All values are in pg/mL.

Conclusions

It is feasible for blood services to provide CD4+CS from donors pre-selected for leukapheresis on the basis of their biologic capacity to uniformly propagate different pHIV-1 isolates. Yields of pHIV-1from the 15 CD4+CS showed considerable variation ranging between 2.6 – 174.6 ng per million cells. Donors #89, 91, 96, and 97 uniformly produced high mean virus yields, viz. 166, 171, 168, and 169 ng, respectively. In contrast, donors #86, 87, 88, and 90 produced relatively poor mean yields, viz. 51, 41, 76, and 98 ng, respectively. The pool of CD4+CS from donor #89, 91, 96, and 97 was optimal for highest yields of each of the 5 pHIV-1 isolates, i.e. 177.5, 177.5, 174.5, 176.5, and 175.0 [mean 176.2] ng per million cells. Since leukapheresis can be performed at weekly intervals on 4 selected blood donors, the blood service can provide the CD4+CS for HIVACC R&D. Thus, it is possible that $4x10^{10}$ CD4+CS at the rate of 175 ng/million cells can yield 7000 ug of pHIV-1. Such a service for optimal cell substrate would enable advancement of research and development of a HIVACC designed to induce broadly neutralizing antibodies, as well as providing large amounts of intact pHIV-1 for other fields of HIV research.

References

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For further information

Dr. Girish N. Vyas,
Transfusion Medicine Research Program
Department of Laboratory Medicine
University of California, San Francisco
San Francisco, California, 94143-0134
vyas@itsa.ucsf.edu
(415) 476-4678, fax: (415) 476-6322