

# *In vitro* synthesis of human immunodeficiency virus-specific antibodies in peripheral blood lymphocytes of infants

(perinatal infection/antibodies)

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**ABSTRACT** An assay system was developed for the analysis of antibodies secreted *in vitro* against human immunodeficiency virus (HIV) by cultured peripheral blood lymphocytes of HIV-infected individuals. Cultures of peripheral blood lymphocytes were established with medium alone or with medium containing Epstein-Barr virus (EBV) or pokeweed mitogen. HIV antibodies were determined by an ELISA performed with commercial kits in which a whole virus extract served as antigen. Optimal antibody secretion was detected in 7-day peripheral blood lymphocyte cultures to which EBV had been added to provide polyclonal B-cell activation. Pokeweed mitogen-induced antibody secretion and spontaneous antibody secretion were less consistent. With EBV as a stimulus, the sensitivity and specificity of this assay for determining HIV infection status were each 100% in adults. When the assay was applied to infants and children, 23 of 24 symptomatic HIV-seropositive children (class P-2) and 11 of 33 asymptomatic seropositive infants aged  $\leq 15$  months (class P-0) tested positive for EBV-induced antibody secretion. Six of the 11 P-0 patients who tested positive have progressed to develop symptomatic disease, while the remainder are still seropositive at ages 2-15 months. Of the infants who were negative in this assay, all have remained asymptomatic. Treatment with 3'-azido-3'-deoxythymidine in infected adults and children has resulted in transient suppression of the *in vitro* antibody response in some instances. Thus EBV-induced synthesis of HIV-specific antibodies *in vitro* is a sensitive and specific indicator of HIV infection and is of help in determining infection status of asymptomatic seropositive infants who are classified as having "indeterminate" infection.

Children with human immunodeficiency virus (HIV) infection present a spectrum of clinical manifestations and may be classified as asymptomatic (class P-1) or symptomatic (class P-2) (1). In the vast majority of infants, HIV infection occurs perinatally from infected mothers. The perinatal transmission rate is approximately 30% (2), but all infants born to infected women are seropositive because passive transfer of maternal antibodies occurs. Seropositive asymptomatic infants  $\leq 15$  months of age are classified as P-0, which signifies an "indeterminate" status (1). A cutoff age of 15 months was selected as the age at which antibody of maternal origin would no longer be detectable. However, infants with passive antibody beyond 15 months have recently been reported (2). Serologic testing for HIV in infants is thus of limited value. At the present time, diagnosis of HIV infection in class P-0 infants and children cannot be made unless HIV is cultured, antigen is present in blood, or the infant develops symptomatic disease. Viral cultures are labor intensive, and anti-

genemia is infrequent (3, 4). Alternative assays have been proposed for diagnosis of HIV infection (5-9) in P-0 infants; none have yet been validated or achieved widespread acceptance. Development of reliable assays for diagnosis of HIV infection in offspring of seropositive women has become a matter of urgency, since clinical trials to test efficacy of antiviral drugs in newborns have already been initiated and infants are being enrolled regardless of their infection status.

We applied knowledge about immunologic effects of HIV and developed an assay to help in the diagnosis of infection in such infants. B cells are activated *in vivo* relatively early in the course of HIV infection (10). Peripheral blood lymphocytes (PBL) of HIV-infected individuals secrete HIV antibodies spontaneously or after pokeweed mitogen (PWM) stimulation (10, 11), which might help with the diagnosis of HIV infection in P-0 infants (11-14). Herein we optimized and standardized culture conditions for *in vitro* HIV antibody secretion by cultured lymphocytes of HIV-infected individuals and ascertained that immunologic memory and B-cell activation are present. PBL of HIV-infected infants may thus be induced to secrete HIV-specific antibodies *in vitro*.

## MATERIALS AND METHODS

**Human Subjects.** Eighteen HIV-seropositive adults, 65 HIV-seronegative control subjects (children and adults), and 57 HIV-seropositive infants and children (from New York and Florida) were tested for HIV antibody secretion *in vitro*. Thirty-three infants were asymptomatic and were  $\leq 15$  months of age (class P-0) when tested, and 24 children were symptomatic (class P-2) and aged 5 months to 9.5 years.

**Cell Cultures.** PBL were isolated on sodium metrizoate gradients. Cells were washed four times and then suspended in RPMI 1640 containing 15% (vol/vol) fetal calf serum and streptomycin and penicillin. To determine optimal conditions, cultures were established in triplicate in 96-well round-or flat-bottomed microtiter plates, at cell densities of  $0.5-1.0 \times 10^6$  cells per ml in medium alone, or in medium containing Epstein-Barr virus (EBV) ( $5.0 \times 10^5$  transforming units/ml) or PWM ( $10 \mu\text{g/ml}$ ) for up to 14 days. Supernatants were pooled and stored at  $-20^\circ\text{C}$  until assayed.

B-cell precursor frequencies for the cells producing HIV-specific IgG antibody and total IgG were determined by limiting dilution of multiple microcultures in 96-well microtiter plates (Nunc) as described (15). Purified B cells were cultured in limiting dilutions from  $1.0 \times 10^4$  to 50 cells per well with  $1 \times 10^5$  irradiated autologous T cells (3000 rad; 1 rad = 0.01 Gy) in final volumes of 0.2 ml. At least 24 replicate

Abbreviations: AZT, 3'-azido-3'-deoxythymidine; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; PBL, peripheral blood lymphocytes; PWM, pokeweed mitogen.

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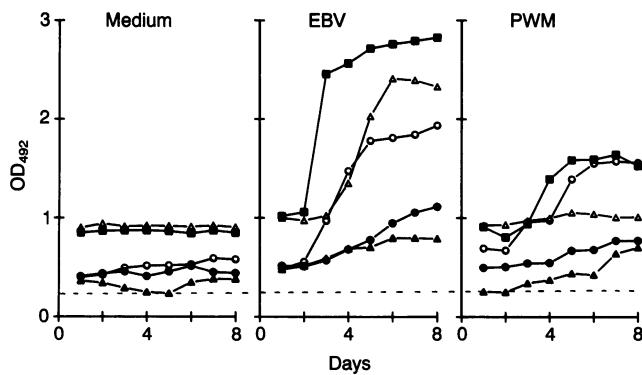


FIG. 1. IgG HIV antibody secretion *in vitro* in supernatants of PBL cultured with medium, EBV, or PWM for 1–8 days. Five known seropositive individuals were tested. Open symbols, asymptomatic adults; closed symbols, children ages 5 and 8 years. The cutoff of 0.170 for negative and positive results is depicted as a dashed line.

cultures were established with EBV for each dose of B cells; 24 wells were established with T cells alone. The cultures were harvested after 10 days, and supernatants were stored as before.

**Assays.** HIV-specific antibodies were determined in commercial ELISA plates (Electro-Nucleonics) coated with whole virus extract. The plates were incubated at 37°C, first with test supernatants (1:10 dilution in duplicate wells) for 30 min and then with goat anti-human IgG labeled with peroxidase for an additional 30 min. The reaction was stopped by the addition of *o*-phenylenediamine dihydrochloride. Total IgG in supernatants was determined by “sandwich” ELISA (15). Results, expressed as the mean OD of duplicate assays at 492 nm, were analyzed by a FUSIONWARE computer software program (16).

**Data Analysis.** Values obtained with culture supernatants of 65 seronegative healthy individuals were used for determining a cutoff for distinguishing “positives” from “negatives”. The mean and SD of the OD for each of the three culture conditions (medium, EBV, and PWM) were calculated, and a value of 3 SDs above the mean, 0.170, was chosen as a cutoff value. A test supernatant was positive if the mean OD of duplicate measurements was greater than 0.170 and negative if the mean OD was lower. As internal controls for culture conditions, cryopreserved lymphocytes from a known HIV-infected individual and PBL of uninfected subjects were cultured concurrently with test samples. These controls and the positive and negative serum controls provided in the ELISA kits were used.

For the limiting dilution experiments, a cutoff of 3 SDs above the mean OD of supernatants of T cells cultured in the

absence of B cells was used. The cultures scored negative for HIV-specific IgG or total IgG were plotted against B-cell number, and precursor frequency of antibody or immunoglobulin secreting cells was calculated by Poisson analysis (17) with the STATPAK computer program.

**RESULTS**

**Optimal Culture Conditions for Secretion of HIV Antibody *in Vitro*.** Optimal culture period, cell culture density, stimulus, and culture plate geometry were investigated with PBL of seropositive adults. Of the cell concentrations tested, production of anti-HIV IgG antibody was optimal at  $1 \times 10^6$  cells per ml. Of medium, EBV, or PWM, maximal and most consistent antibody secretion was with EBV stimulation. Spontaneously secreted antibody remained almost constant over the entire culture period; increased HIV antibody secretion occurred with EBV and PWM stimulation, which peaked during 4–7 days of culture (Fig. 1). Equal response was obtained in flat- or round-bottom plates (data not shown). All subsequent cultures were performed at cell densities of  $1 \times 10^6$  cells per ml for 7 days. Cell viability after 7 days of culture was approximately 80%.

**Sensitivity and Specificity.** A cutoff value of mean plus 3 SDs (0.170) was determined as described in *Materials and Methods*. In tests with 65 HIV-negative and 18 HIV-infected adults, the sensitivity of assay was 66.66% for spontaneously secreted antibody, 100% for EBV-induced antibody, and 83.33% for PWM-induced antibody. Specificity was 100% for all the three conditions. In contrast to the results for HIV-specific antibody secretion, total IgG in EBV- or PWM-stimulated culture supernatants was less in HIV-infected persons as compared to healthy HIV-seronegative control subjects (Fig. 2).

By changing the cutoff value, the sensitivity and/or specificity of the assay was potentially altered. To evaluate how cutoff points affected these two parameters, receiver operating characteristic curves (18) of sensitivity plotted against 100 minus the specificity for each of the three stimuli were determined (Fig. 3). Receiver operating characteristic curves falling to the far left in the figure are indicative of greater sensitivity and specificity than those toward the right. EBV was the best stimulus for this assay.

To eliminate detection of passively transferred antibody adhering to test cells despite exhaustive washing prior to culture, cells were treated with the protein synthesis inhibitors puromycin and cycloheximide; these inhibitors were added, at 10  $\mu\text{g}/\text{ml}$ , to cultures 24 hr prior to harvesting. Cell viability was minimally reduced, but HIV-specific antibody secretion was abrogated (Fig. 4).

**Limiting Dilution Analysis.** The frequencies of B cells secreting HIV-specific IgG in response to EBV showed a

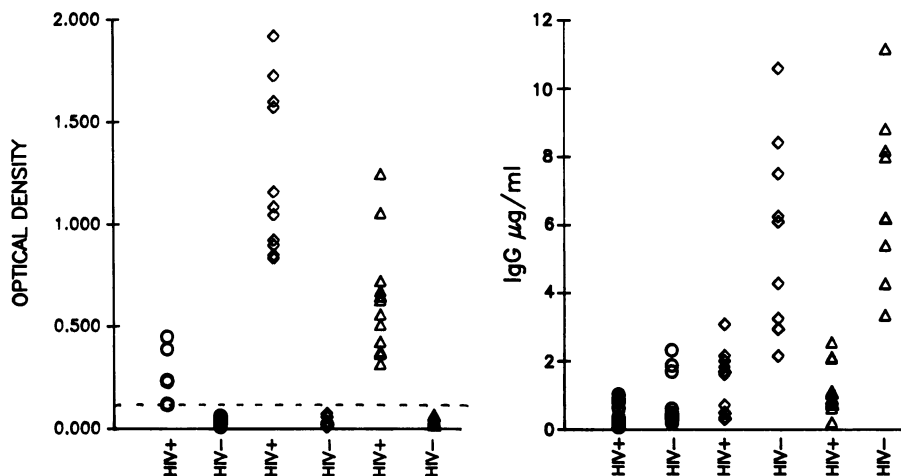


FIG. 2. *In vitro* HIV antibody and total IgG secretion in known seropositive patients (HIV+) and seronegative individuals (HIV-). PBL were cultured in the presence of medium (○), EBV (◇), or PWM (△) for 7 days. Anti-HIV IgG (Left) and total IgG (Right) in culture supernatants were determined by ELISA.

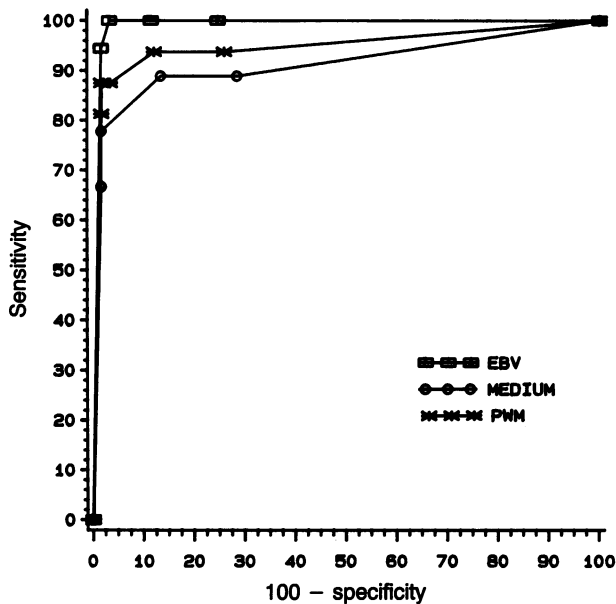


FIG. 3. Receiver operating characteristic curves. Sensitivity and specificity were calculated for various levels of SD (1.5–3) for PBL cultured with medium, EBV, or PWM.

wide variation: from 1 in 567.85 to 1 in 7263.41 (mean, 1 in 2374; median, 1 in 2763) (Fig. 5). Precursor frequencies of total IgG secreted by these patients were approximately 5-fold greater and ranged from 1 in 147 to 1 in 1161 (mean, 1 in 504; median, 1 in 568).

**Assay Results in Children.** Of the 24 symptomatic infants and children from class P-2, 23 (96%) were initially positive for HIV-specific antibody secretion with EBV stimulation. Spontaneous antibody secretion was positive in 10 of 24 patients (42%), and 17 of 24 (71%) were positive in PWM-stimulated cultures (Fig. 6). Of 33 asymptomatic seropositive P-0 infants, 11 tested positive for HIV antibody with EBV stimulation; 6 also tested positive by PWM-stimulated cultures, and 4 made antibody spontaneously. These 11 patients are listed in Table 1. One infant (NS01) whose PBL secreted HIV antibody with EBV stimulation at 3 weeks of age progressed into full-blown AIDS at 4 months and died at 5 months. Another asymptomatic infant (NS02) who tested positive with EBV on day 5 became symptomatic at 7 months. Patients NS04, NS05, NS06, and MS10 have also become symptomatic. The other

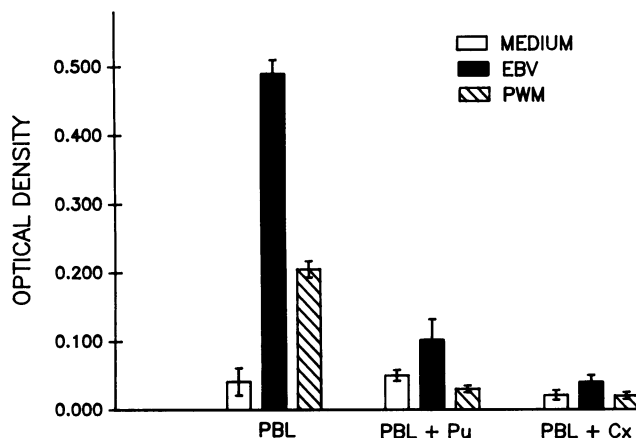


FIG. 4. Protein synthesis inhibitors puromycin (Pu) and cycloheximide (Cx) inhibit HIV antibody synthesis. Puromycin or cycloheximide at 10  $\mu$ g/ml was added to PBL cultures 24 hr prior to harvesting the supernatants.

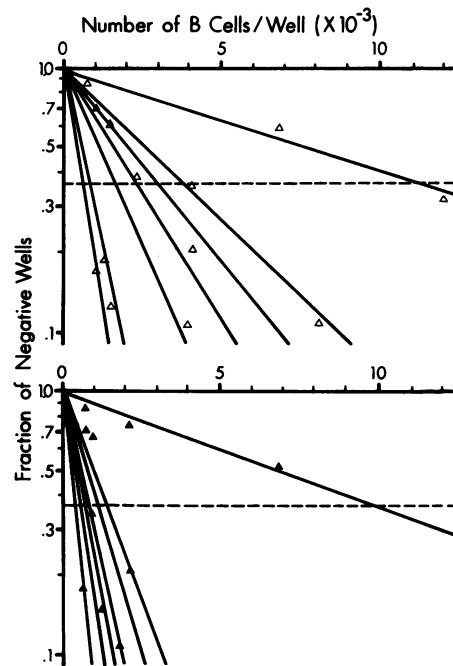


FIG. 5. Precursor frequencies of B cells secreting HIV-specific IgG (Upper) and total IgG (Lower) in response to EBV in seven HIV-infected patients. The number of B cells per well was plotted against the logarithm of the fraction of negative wells, yielding a straight line.

five infants, positive in one or more of the assays, remain asymptomatic and seropositive.

**Sequential Testing and Effect of 3'-Azido-3'-deoxythymidine (AZT) Treatment.** Children and their mothers were tested sequentially 2–8 times over a follow-up period of 1–20 months. Infants in the P-0 class who initially tested negative (22 patients) continue negative for *in vitro* HIV antibody secretion. In patients who became seronegative, testing was stopped when they were 15 months old. Some P-2 children were treated with AZT at doses of 360–720 mg/m<sup>2</sup> per day at six hourly intervals, and some mothers were on 1200 mg of AZT per day. Among the 15 children not treated with AZT, *in vitro* antibody response remained positive throughout the follow-up period; 5 children died and 1 of these turned negative in all three assays approximately 3 months prior to death. Adults not on AZT treatment remained consistent in *in vitro* antibody responses. Results of *in vitro* antibody

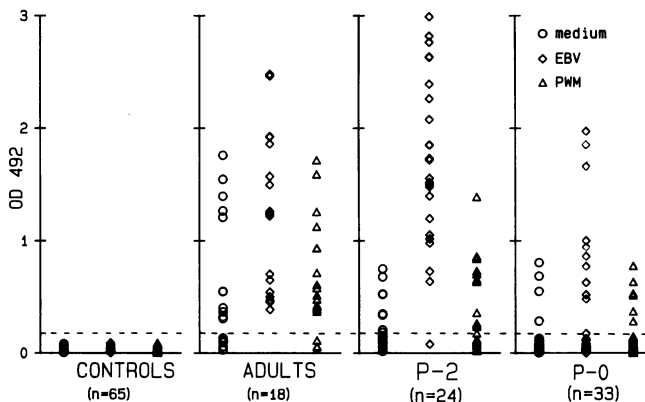


FIG. 6. *In vitro* HIV antibody secretion in uninfected controls, seropositive adults, infected children (class P-2), and infants with indeterminate infection (class P-0). PBL ( $1 \times 10^6$  per ml) were cultured in the presence of medium, EBV, or PWM, and HIV-specific IgG was determined by ELISA. The cutoff of 0.170 for negative and positive results is depicted by a dashed line.

Table 1. Results of P-0 infants who were positive for HIV-specific antibody secretion *in vitro*

Patient	Age	HIV-specific IgG <i>in vitro</i>			Serum antibody	Absolute CD4 no.	Ratio of CD4 to CD8	Current age and status
		Medium	EBV	PWM				
NS01	3 weeks	<i>0.017</i>	0.172	<i>0.043</i>	+	2621	2.10	Died at 5 months; symptoms at 4 months; PCP, candidiasis, disseminated CMV
	3 months	0.197	0.259	0.196	+	368	3.30	
	5 months	0.229	0.479	0.229	-	224	1.71	
NS02	5 days	<i>0.106</i>	0.218	<i>0.160</i>	+	7185	3.80	12 months; symptoms at 7 months; FTT, LAD, thrush
	3 weeks	0.747	0.714	0.695	+	ND	ND	
	10 months	1.248	1.971	1.991	+	1081	0.96	
MS10	3 weeks	0.806	0.760	0.775	+	3278	2.60	6 months; LAD
NS04	10 months	<i>0.111</i>	0.392	0.244	+	3133	2.20	20 months; symptoms at 12 months; FTT, LAD, thrush
NS05	8 months	<i>0.137</i>	0.235	<i>0.167</i>	+	2604	1.20	12 months; symptoms at 10 months; LAD, FTT
NS06	15 months	<i>0.111</i>	0.409	<i>0.163</i>	+	1262	0.90	20 months; symptoms at 18 months; LAD
MS08	1 month	<i>0.109</i>	0.310	0.340	+	ND	ND	13 months; asymptomatic
MS09	7 months	0.284	0.428	0.275	+	3027	1.30	15 months; asymptomatic
MS11	3 days	0.686	0.770	0.761	+	1816	2.70	6 months; asymptomatic
NS03	4 weeks	<i>0.129</i>	0.173	<i>0.145</i>	+	2131	3.10	3 months; asymptomatic
NS07	13 days	0.578	0.605	0.530	+	1079	3.60	2 months; asymptomatic

Italic boldface numbers for *in vitro* antibody secretion represent negative tests based on a cutoff of 0.170. LAD, lymphadenopathy; FTT, failure to thrive; CMV, cytomegalovirus; ND, not determined; PCP, *Pneumocystis carinii* pneumonia.

responses of individuals treated with AZT are shown in Fig. 7. Of 8 children treated with AZT, 2 became transiently negative at 4 and 5 months in all three assays. Both children had <100 CD4<sup>+</sup> T cells per mm<sup>3</sup> at that time. One child became negative only for spontaneous antibody secretion at the same time. One child who previously tested negative became positive after starting AZT. Of 6 AZT-treated adults tested 2 weeks to 20 months after starting AZT, only 1 showed a decreased spontaneous *in vitro* antibody response at 7 months.

**DISCUSSION**

This study examines characteristics of an assay system for *in vitro* HIV antibody production in HIV-infected individuals. The concept that HIV-infected individuals have circulating activated B cells, which could secrete HIV antibody *in vitro*, was first tested by Amadori *et al.* (11) who demonstrated spontaneous secretion of HIV antibody in PBL cultures. This assay was extended to infants in the P-0 class with promising results (12). Subsequently, others found spontaneous HIV

antibody-secreting B cells in PBL of infected individuals (13, 14, 19). Criticisms have included questions of specificity and sensitivity. Passive antibody carryover rather than active antibody secretion has raised doubts about assay specificity. Negative test results in subsets of infected individuals have raised questions about assay sensitivity. The present study defined optimal culture conditions of *in vitro* antibody secretion in HIV-infected individuals to maximize specificity and sensitivity and determined the potential of the assay for discriminating between infected and uninfected P-0 infants.

First, spontaneous antibody secretion was compared with that induced in culture by two polyclonal B-cell activators, PWM and EBV. Although spontaneous antibody secretion was observed in a majority of cases, a significant proportion (33%) were negative. This assay system might be made more sensitive for detecting spontaneous antibody with improved antigen coats for the ELISA plates. With EBV as a stimulus, however, the sensitivity of the present assay was 100%. That not all HIV-infected individuals were positive for spontaneous antibody secretion indicates that "activated" circulating B cells spontaneously secreting HIV antibody are not universally present and that the numbers of such activated cells in circulation may vary from individual to individual and from time to time in the same individual. The 100% sensitivity obtained in EBV-stimulated cultures suggests that memory B cells can consistently be amplified to secrete antibody *in vitro* by polyclonal B-cell activators. That PWM was a less efficient stimulus than EBV is not surprising. With progressive T-helper cell depletion, PWM, a T-cell dependent stimulus, might not be expected to be as potent and consistent a stimulus as EBV, which is T-cell independent. The T-cell dependent nature of PWM makes it an unsuitable stimulus for other reasons [e.g., it could potentially activate T suppressor cells (20)]. Activation of T cells also might lead to viral replication in infected cells with consequent viral protein-mediated suppression of B cells (21). Precursor frequency analyses indicated that of all immunoglobulin-secreting B cells, approximately 20% were secreting HIV antibodies. Thus EBV proved by these analyses to be the ideal stimulus for induction of HIV antibody in culture supernatants of HIV-infected individuals.

Specificity of the observed response was addressed in two ways. First, protein synthesis inhibitors, puromycin and

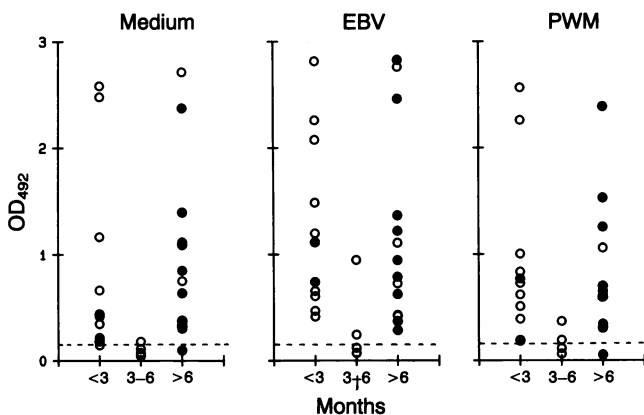


FIG. 7. Effect of AZT treatment on *in vitro* HIV antibody synthesis. PBL of children (○) and adults (●) treated with AZT were cultured in the presence of medium, EBV, or PWM for 8 days. HIV-specific IgG in the culture supernatants was assessed by ELISA. The x axis denotes the duration of AZT treatment. The cutoff of 0.170 for negative and positive results is depicted by a dashed line.

cycloheximide, abrogated the antibody response. Second, kinetic determinations revealed a consistent increase in antibody in the supernatants of EBV-stimulated cultures over 4–7 days. These observations indicate that active synthesis and secretion of HIV antibody occurred *in vitro*. Moreover, although total immunoglobulin secretion was poor in infected individuals in comparison to uninfected controls, HIV-specific antibody was detected only in culture supernatants of infected individuals (22–24).

The ELISA assay kits used for the current study are marketed for serum antibody determination. To apply the kits for antibody determination in culture supernatants, a few modifications had to be made in the assay. In addition to negative and positive serum controls provided in the kit, negative and positive culture-supernatant controls were also assayed with the test supernatant. These control supernatants were derived from PBL cultures performed simultaneously with test cultures. Cryopreserved PBL of a known seropositive individual served as positive control. The principle of 3 SDs above mean cutoff recommended for the negative control serum was applied to the negative culture supernatant in each assay. Data of all negative supernatants was subsequently pooled to provide a standard cutoff value. With these modifications, receiver operating characteristic curves proved the assay to be highly sensitive and specific with EBV as a test stimulus.

Children with established HIV infection were tested serially in this assay to evaluate the response over an extended period of time in symptomatic children. Serial testing revealed the majority of the children to be consistently positive over test periods of 1–20 months. Failure to secrete antibody was observed in a terminally ill child during profound antigenemia, but other terminally ill patients did not manifest this phenomenon. It is possible that with extreme antigenemia the inhibitory effect of HIV proteins shuts off antibody secretion.

Treatment with AZT reduces viral replication and might decrease antigenic load and influence antibody secretion. Six adults and eight children who were on AZT for periods of 1–18 months were tested for *in vitro* antibody secretion. Except for a transient decrease in some patients 3–5 months after starting treatment (at which time some of whom tested negative), the responses were positive at periods of <3 months and >6 months after starting treatment, especially to EBV stimulation. These data would suggest that most AZT-treated patients continue to secrete HIV-specific antibody *in vitro*. Instances in which AZT shuts off antibody secretion need investigation, in relation to the dose and duration of treatment, and to determine the biological significance of a transient shutting-off of the response. With emerging evidence of AZT-resistant HIV strains (25), tests that reveal *in vitro* influences of the drug on the immune system need careful scrutiny.

The utility of the *in vitro* antibody assay in discriminating between infected and uninfected infants was tested with a sample size of 33 asymptomatic, seropositive infants in the P-0 class. Eleven infants tested positive. Six positive infants subsequently developed symptomatic disease at ages of 4–18 months; the remainder are still persistently seropositive and thus potentially infected. Thus the positive predictive value of this assay is high. The ability of newborn B cells to secrete IgG anti-HIV antibody has been questioned because of the known immaturity of newborn B cells (26). The positive results obtained within the first week of life would suggest that the B cells of the newborn do have this ability. The negative predictive value of this assay also appears to be high as none of the infants who tested negative have developed disease and they have not remained seropositive beyond 15 months of age. However, a large population of P-0 cases needs testing to fully validate the assay.

HIV-specific IgM antibody in culture supernatants was also tested (data not shown). Most seropositive adults and the P-2 class of children were positive for IgM antibody secretion *in vitro*, but results were uninterpretable in the asymptomatic P-0 class of infants. Twelve infants were tested, and 7 were positive for the IgM HIV antibody responses. Four of these were negative for the IgG HIV antibody response and subsequently became negative for secreted IgM antibody as well. The phenomenon of *in vitro* IgM HIV antibody synthesis and its relationship to “infection” versus “antigenic exposure” is unclear. That this could represent an anti-anti-idiotypic response to antibodies of maternal origin is an attractive explanation. Based on this initial analysis, the IgM antibody response *in vitro* does not appear to be diagnostically useful. EBV-induced secretion of HIV-specific IgG antibody is a sensitive and specific indicator of HIV infection that does appear to be useful for distinguishing between seropositive infected and uninfected infants in the P-0 class. This test is potentially of value and may prove specially to be useful when applied in conjunction with other assays such as the polymerase chain reaction (27, 28).

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- Brunell, P., Chin, J., Cooper, L., Oleske, J., Epstein, L., Luban, N., Mailoux, S., Pahwa, S., Scott, G., Stiehm, R., Thomas, P., Wara, D., Williams, D. & Witte, J. (1988) *Morbid. Mortal. Wkly. Rep.* **36**, 91–96.
- Peckham, C. S., Senturia, Y. D., Ades, A. E. & Newell, M. L. (1988) *Lancet* **ii**, 1039–1042.
- Goudsmit, J. J., Lange, M. A., Paul, D. A. & Dawson, G. J. (1987) *J. Infect. Dis.* **155**, 558–560.
- Popovic, M., Sargadharan, M. G., Read, F. & Gallo, R. C. (1984) *Science* **224**, 497–500.
- Gaetano, C., Scano, G. & Carbonari, M. (1987) *Lancet* **i**, 631.
- Johnson, J. P. & Nair, P. (1987) *N. Engl. J. Med.* **316**, 273–274.
- Pyun, K. H., Ochs, H. D., Dufford, M. T. W. & Wedgewood, R. J. (1987) *N. Engl. J. Med.* **316**, 272–273.
- Tovo, P. A., Gabino, C. & Riva, C. (1987) *Lancet* **i**, 1201.
- Slade, H., Pica, R. & Pahwa, S. (1989) *J. Infect. Dis.* **160**, 126–130.
- Fauci, A. (1988) *Science* **239**, 617–622.
- Amadori, A., DeRossi, A., Faulkner-Valle, G. P. & Chieco-Bianchi, L. (1988) *Clin. Immunol. Immunopathol.* **46**, 342–351.
- Amadori, A., DeRossi, A., Giaquinto, C., Faulkner-Valle, G., Zacchello, F. & Chieco-Bianchi, L. (1988) *Lancet* **i**, 852–854.
- Nahmias, A., Lee, F., Nesheim, S., Jones, D., Lower, S., O’Neil, C. & Slade, B. (1989) *Pediatr. Res.* **25**, 185 (abstr.).
- Pollack, H., Moore, T., Krasinski, K., Ming Xia, Z. & Borkowsky, W. (1989) *Pediatr. Res.* **25**, 167 (abstr.).
- Yarchoan, R., Tosato, G., Blase, M., Simon, R. & Nelson, D. L. (1983) *J. Exp. Med.* **157**, 1–14.
- Slade, H. B., Lopatin, D. & Schwartz, S. (1986) *J. Immunol. Methods* **94**, 169–179.
- Taswell, C. (1981) *J. Immunol.* **126**, 1614–1629.
- Turner, D. A. (1978) *J. Nucl. Med.* **19**, 213–220.
- Teeuwssen, V. J., Logtenberg, T., Siebelink, K. H., Langer, J. M., Goudsmit, J., Uytdehaag, F. G. & Ofterhaus, A. D. (1987) *J. Immunol.* **139**, 2929–2935.
- Pahwa, S., Good, R. A. & Hoffmann, M. J. (1981) *J. Clin. Immunol.* **1**, 101–105.
- Pahwa, S., Pahwa, R., Good, R. A., Gallo, R. C. & Saxinger, C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9124–9128.
- Pahwa, S., Quilop, M. T. J., Lange, M., Pahwa, R. & Greico, M. (1984) *Ann. Intern. Med.* **101**, 757–763.
- Lane, H. C., Masur, H., Edgar, L. C., Whalen, G., Rook, A. H. & Fauci, A. S. (1983) *N. Engl. J. Med.* **309**, 453–458.
- Birx, D. L., Redfield, R. R. & Tosato, G. (1986) *N. Engl. J. Med.* **314**, 874–879.
- Larder, B. A., Darby, G. & Richman, D. D. (1989) *Science* **243**, 1731–1734.
- Tosato, G., Magrath, I. T., Koski, I. R., Dooley, N. J. & Blaese, M. R. (1980) *J. Clin. Invest.* **66**, 383–388.
- Ou, C.-Y., Kwok, S., Mitchell, S. W., Mack, D. H., Sninsky, J. J., Krebs, J. W., Feorino, P., Warfield, D. & Schochetman, G. (1988) *Science* **239**, 295–297.
- DeRossi, A., Amadori, A., Chieco-Bianchi, L., Giacchino, C., Zacchello, F., Buchbinder, A., Wong-Staal, F. & Gallo, R. C. (1988) *Lancet* **ii**, 336.