Prognostic value of immunological data, in vitro antibody production, and virus culture in vertical infection with HIV-1

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Abstract

The prognostic value of immunological indices, in vitro antibody production, and virus culture pattern at 3 months of age was estimated in 35 infants infected by HIV-1 from a cohort of 298 babies born to HIV-1 seropositive mothers and followed up from birth. At 1 year old, 15 of these infants were classified as stage P-1 (according to the Centers for Disease Control classification) seven were P-2A, and seven had AIDS. Significantly higher CD8 percentages, lower percentages and absolute value of CD4, and lower CD4/CD8 ratios at 3 months were observed in infants with severe symptoms at 1 year of age when compared with those who were asymptomatic at this age. Seventy seven per cent of infants with a 'rapid' virus culture when 3 months old had developed AIDS or had died by 1 year of age and only 8% of those with 'slow' virus culture had AIDS when 1 year old. Moreover, 100% of those who were asymptomatic at 1 year had a slow virus culture at 3 months. Significant statistical association was found between the virus replication pattern at 3 months and the clinical stage at 1 year of age.

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(d) the mother's clinical stage of infection during pregnancy^{11 12}; and (e) the possible role of cofactors activating HIV-1 replication.¹³ In adults the rate of decline of CD4 lymphocytes and virological parameters, such as high level persistent replication,^{14 15} may be good predictive markers of clinical outcome.¹⁶ In children little information is available on the prognostic value of these and other markers on the clinical outcome.

We studied a cohort of infants born to HIV-1 infected mothers and followed up from birth. We investigated the prognostic value of immunological data and the in vitro biological properties of the virus in order to identify at 3 months of age those who will develop severe immunodeficiency with serious infections in the first year of life and those in whom the disease will progress slowly and is probably similar to that observed in adults.

Subjects and methods

STUDY POPULATION

Two hundred and ninety eight infants born to HIV seropositive mothers and attending the paediatric department of La Paz Hospital, Madrid form the basis of this study. All the infants were assessed clinically and immunologically during the first 72 hours of life and every three months thereafter. Only the 268 infants born 12 months or more before the date of analysis are included in this study. Thirty five of them had HIV infection diagnosed based on two of the following criteria: positive HIV-1 antigen test, positive HIV-1 virus culture, or in vitro HIV-1 antibody production on more than two samples. The infected infants were classified according to the Centers for Disease Control classification.¹⁷ At 1 year of age, 15 infants had infection but no symptoms (P-1) and 20 had infection and were symptomatic (P-2). Seven of the patients classified as P-2 had only non-specific clinical manifestations (P-2A subclass) and in 13 P-2 patients these manifestations were associated with the more severe symptoms that are included in the other P-2 subclass (AIDS). Six of these 13 patients with severe symptoms died of HIV related illness at the median age of 5 months. The remaining 233 infants were considered to be uninfected. All these children are enrolled in the European Collaborative Study.^{5 18}

In this study we analysed the immunological data and virus culture patterns at 3 months of age to investigate an association between immunological alterations or the biological

HIV-1 infection in children is usually due to vertical transmission during pregnancy and/or delivery. The rate of HIV-1 transmission from infected mothers to their offspring varies from 7–45% with higher rates reported in African studies.^{1–4} In our hospital the rate of vertical transmission is 13%, not much different from the 14.4% in the European Collaborative Study.⁵ The results of several retrospective studies indicate that HIV-1 infection can take two clinical forms in children.^{6 7} Some children present with symptoms in the first weeks of life and develop severe immunodeficiency during the first year with early and severe illness. Other children show a late presentation of symptoms followed by a slow clinical course. This double pattern can be explained by several factors: (a) a different time for infection (in utero or perinatal)⁸; (b) the biological properties of the virus with higher or lower viral replication capacity⁹; (c) the cellular tropism for CD4 T cells and/or monocytes¹⁰;

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Immunological data at 3 months of age in infants infected with HIV-1; results are mean (SD)

	P-1*	P-2A*	AIDS*	p Value (by ANOVA)
IgG (g/l)	7.52 (4.07)	8.54 (5.11)	8.59 (5.27)	NS
IgA (g/l)	0.55 (0.57)	0.69 (0.65)	0.63 (0.49)	NS
IgM (g/l)	0.95 (0.6)	0.99 (0.49)	1.34 (1.06)	NS
CD4 (%)	37 (15)	34 (10)	21 (8)	0.006
CD8 (%)	25 (9)	27 (13)	41 (9)	0.0006
CD4/CD8	1.80 (1.24)	1.64 (1.13)	0.56 (0.27)	0.0053
Lymphocytes (mm ³)	6699 (3129)	6612 (2568)	4447 (2849)	NS
CD4 (mm ³)	2455 (1544)	2083 (522)	1035 (792)	0.0092
CD8 (mm ³)	1811 (1234)	2097 (1871)	1971 (1562)	NS
Pokeweed mitogen (cpm)	15793 (11545)	8110 (2796)	10079 (8109)	NS
Spontaneous production of immunoglobulins (µg/l)				
IgG	627 (655)	1276 (943)	1025 (1217)	NS
IgA	364 (655)	637 (587)	1571 (3210)	NS
IgM	991 (2185)	228 (130)	772 (804)	NS

*Clinical status at 1 year of age. NS=not significant, cpm=counts/minute.

properties of the virus and the disease outcome. At this age 21 of the 35 infected infants were classified as P-1, seven P-2A, and seven had developed AIDS.

METHODS

Serum immunoglobulins concentrations were measured by laser nephelometry (Array Protein System, Beckman Instruments). Lymphocyte subsets were assessed using monoclonal antibodies conjugated to either fluorescein isothiocyanate or phycoerythrin. Flow cytometric analysis was carried out in a FACScan Cytometer (Becton Dickinson).

Peripheral blood mononuclear cells (PBMC) were isolated from heparinised fresh blood by Ficoll-Hypaque gradient centrifugation. The proliferative response of PBMC was performed by a standard technique¹⁹ using pokeweed mitogen (Gibco) at 1 µg/ml.

In vitro immunoglobulin production

This was measured by culturing 2.5×10^5 PBMC in a final volume of 250 µl of supplemented RPMI culture medium (Flow Laboratories) in the absence of pokeweed mitogen. Culture supernatants were harvested on the seventh day of culture and the immunoglobulins produced were measured by an enzyme linked immunosorbent assay (ELISA).²⁰

In vitro HIV-1 antibody production

PBMC washed twice were cultured at 2.5×10^6 /ml²¹ in RPMI medium containing 10% fetal calf serum (Flow Laboratories), 1% L-glutamine, and penicillin-streptomycin. Subsequently, supernatants were recovered at seven days, diluted 1/2, and tested for the presence of HIV-1 antibody according to the commercial recommendations of the western blot kit (Sanofi Diagnostics Pasteur). As this test has a low specificity in the first two months of age,²² only the results obtained after this period were considered for diagnosis.

Virus culture

In brief,²³ 5×10^6 PBMC from healthy donors previously stimulated by phytohaemagglutinin

during 48 hours, were cultured with an equal number of patient cells for one month in RPMI medium with 10% T cell growth factor (Cellular Products), 1% glutamine, 10% fetal calf serum, and 1 µg/ml Polybrene (Sigma). Supernatants were removed twice a week and complete medium was replaced. These samples were investigated for the presence of p24 antigen using a commercial enzyme immunoassay (Ag I, Sanofi Diagnostics Pasteur).

Statistical analysis

Quantitative data are expressed as mean (SD) and qualitative data as a percentage. A one way analysis of variance (ANOVA) was performed to compare quantitative data between P-1, P-2A, and AIDS groups as a parametric test and the Kruskal-Wallis test as a non-parametric test. We found similar results in both tests therefore results from ANOVA are reported. Pairwise comparisons were studied by Fisher's PLSD test ($\alpha=0.05$). Association between the virus replication pattern at 3 months of age and the clinical stage at 1 year was studied by the χ^2 test. All tests used were two tailed and the threshold of significance was 0.05.

Results

The table shows immunological data at 3 months of age by clinical status at 1 year for 35 infected infants. Significantly lower CD4 percentages ($p=0.006$) were observed in infants who subsequently, at 1 year, had severe symptoms (AIDS) compared with those that at this age had mild symptoms or remained asymptomatic (P-1). The percentages of CD8 were significantly higher ($p=0.006$) in those who at 1 year had AIDS when compared with those who were classified as P-2A or asymptomatic. We found similar differences ($p=0.005$) in the CD4/CD8 ratio. At 3 months the CD4 absolute values were significantly lower ($p=0.009$) in those infants who developed AIDS in the first year compared with those who at 1 year were diagnosed as P-1 or P-2A.

The bands found on western blot of in vitro HIV-1 antibody production by PBMC at 3 months of age were similar in infants classified as P2 and P1.

In only 25 infected infants was it possible to perform virus culture. In virus culture, antigen concentrations higher than 30 pg/ml in three successive supernatants were considered as positive. We found two patterns in relation to p24 antigen values in the supernatants (figure): 'rapid' with high p24 antigen concentrations (>100 pg/ml) from the first supernatant and 'slow', with constantly low values of p24 antigen during the month of culture. Ten of 13 (77%) of the infants with rapid virus culture at 3 months had AIDS or had died before age 1 year, but only 1/12 (8%) of those with a slow virus culture pattern had been diagnosed with AIDS when 1 year old. Ten of 11 (91%) of those who at 1 year old had AIDS diagnosed had a rapid culture virus at 3 months of age. Moreover, all infants who were asymptomatic

HIV-1 replication patterns from two infected children.

at 1 year had a slow virus culture at 3 months of age. The association between virus replication pattern at 3 months of age and the clinical stage at 1 year was statistically significant ($p=0.0003$).

Discussion

In a previous report we found IgG and IgA hypergammaglobulinaemia, increased spontaneous production of immunoglobulins in vitro, and a decreased percentage of CD4 circulating cells in most of our HIV-1 infected patients at the age of 3 months when compared with uninfected infants.²⁴ Further follow up of these infants showed that these immunological abnormalities were early, persistent, and generally progressive. Hutto *et al* found similar results in a prospective study.⁴

In the present study the CD4 and CD8 percentages and the CD4/CD8 ratio at 3 months of age were associated with clinical progression at 1 year. We also observed a significant decrease in the CD4 absolute value at 3 months in infants who during the first year of life had severe symptoms (AIDS) compared with infected asymptomatic infants. However, although these immunological alterations at 3 months are closely linked with the clinical stage, their prognostic value is low. So, when we considered all 35 infected infants at 3 months of age, 11 had a CD4 absolute count below the fifth centile,²⁵ but when those with severe symptoms at 3 months were excluded only two infants in the P-2A and two in the P-1 category had a CD4 count below the fifth centile. Our results confirm other reports,^{18 26} that immunological abnormalities at 3 months of age have a low prognostic value and are not useful to predict which asymptomatic infants will develop early and severe symptoms before 1 year.

The results contradict those of an Italian study in children where early immunological abnormalities preceded the onset of symptoms,²⁷ suggesting that early alterations have predictive prognostic value rather than diagnostic value. This discrepancy could be explained by the fact that the immunological data in that study referred to measurements between 1 and 6 months of age, while in our present work all examinations were performed when the infants were 3 months old.

In HIV-1 infected adults a low response to pokeweed mitogen before the decrease of CD4 T cells has been considered to be a predictor for disease progression.^{27 28} It has also been suggested that rapid viral replication in asymptomatic adults may be a prognostic factor¹⁶ as in cohorts of adults with a widely variable clinical outcome the viruses isolated from asymptomatic patients replicate slowly, while viruses from symptomatic ones replicate rapidly.²⁹ Similar results have been recorded in children. Those who already had symptomatic HIV-1 infection showed a rapid viral growth pattern in vitro and those asymptomatic had mostly a slow replication.^{9 23} In a recent paper, de Rossi *et al* reported 18 infants from 4 to 8 weeks of age who were identified as infected with HIV-1. At this time, those infected children who had rapid and high virus replication, progressed faster to AIDS than those with a slow replication pattern.¹⁰ In their cohort no patient with a slow replication pattern at this early age had AIDS at 1 year, but 5/7 (71%) of those with rapid replication had developed AIDS by the time they were 1 year old.

Our results confirm and extend these findings and we found a significant association between the virus replication pattern at 3 months of age and the presence or absence of symptoms during the first year of life. Seventy seven per cent of the infants with a rapid replication pattern at 3 months old developed symptomatic HIV-1 infection during the first year of life and 100% of those who were asymptomatic (P-1) by 1 year old had a slow virus culture at 3 months. When we exclude those infants who already had severe symptoms at 3 months in order to know the true predictive value of the culture pattern, this association persists. Therefore we think that the virus culture pattern at 3 months is associated with the clinical stage. Moreover, this viral characteristic has prognostic value for identifying the 30% of infected infants who will develop severe immunodeficiency (AIDS) in the first year of life.¹⁸ In agreement with these results Rogers *et al* found in a retrospective study a strong association between the early and severe course of HIV-1 infection and the viral DNA burden at birth.³⁰ Burgard *et al* found a less strong association with virus culture at birth. However, birth is not the best moment to establish prognosis, as only 30–50% of infected children can be diagnosed by the polymerase chain reaction or virus culture at this time.^{13 32}

We considered that 3 months of age is the appropriate time for a prognostic evaluation. At this age, virus culture and in vitro HIV-1 antibody production showed, in our laboratory, a sensitivity and specificity near to 100%. Currently we are also using the polymerase chain reaction for early diagnosis of HIV-1 vertical transmission and have found a similar sensitivity and specificity at this age. These techniques are less reliable when used before 2 months of age. The assay for in vitro HIV-1 antibody production could give false positive results during the first 2 months by possible persistence of maternal cells in the

infant's blood.²² Both virus culture and the polymerase chain reaction could give false negative results at this age because it is possible that infants infected just before or during delivery have not yet a sufficient viral burden.^{33 34}

In conclusion, the significant association found between the viral replication pattern at 3 months and clinical stage at 1 year increases understanding about the prognosis of vertically acquired HIV infection and could be of help in future therapeutic trials.

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