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Presence of maternal antibodies to human immunodeficiency virus 1 envelope glycoprotein gp120 epitopes correlates with the uninfected status of children born to seropositive mothers

P. ROSSI*†, V. MOSCHESE*, P. A. BROLIDEN*‡, C. FUNDARÓ§, I. QUINTI¶, A. PLEBANI‖, C. GIAQUINTO**, P. A. TOVO††, K. LJUNGGREN*, J. ROSEN‡‡, H. WIGZELL*, M. JONDAL*, AND B. WAHREN‡

*Department of Immunology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden; ‡Department of Virology, Statens Bakteriologiska Laboratoriet, S-10521 Stockholm, Sweden; †Department of Pediatrics, University of Rome "Tor Vergata," 00100 Rome, Italy; §Catholic University of Rome, 00100, Rome, Italy; ‖University of Milan, 20122, Milan, Italy; **University of Padua, 35128 Padua, Italy; ††University of Turin, 10126, Turin, Italy; ¶Department of Allergy and Clinical Immunology, University of Rome, "La Sapienza," 00100, Rome, Italy; and ‡‡Johnson & Johnson Biotechnology Center, La Jolla, CA 92037

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ABSTRACT **The present study demonstrates that maternal antibodies to certain epitopes of human immunodeficiency virus 1 (HIV-1) proteins are associated with a defined outcome for at-risk pregnancies of HIV-infected women. An initial retrospective analysis of antibodies to synthetic peptides and recombinant proteins representing *env*, *pol*, and *gag* regions of HIV-1 was carried out. Sera studied were from 33 children who were born to HIV-infected mothers and whose clinical outcome was known at the time of analysis. Sera, collected within the first 6 months of life, of uninfected at-risk children were found to selectively contain maternal antibodies to certain peptides containing epitopes of the HIV envelope glycoprotein gp120. To confirm the predictive role of maternal antibodies to defined HIV-1 epitopes, a prospective analysis was then performed on sera from 21 HIV-seropositive mothers and their infants, whose clinical and immunological status was then followed up for a period of at least 15 months. As expected, antibodies to the same envelope protein peptides were detected almost exclusively in sera from mothers of uninfected children. Our data suggest that antibodies against select epitopes of HIV envelope protein gp120 might play an important role in preventing mother-to-child transmission of HIV-1 infection. Accordingly, site-directed serology might be used to predict the outcome of an at-risk pregnancy of an HIV-infected woman.**

Infants born to human immunodeficiency virus 1 (HIV-1)-infected mothers account for most pediatric cases of AIDS. The rate of mother-to-child transmission is estimated to be between 30 and 65%, depending on the particular cohort, length of follow-up, and criteria used to define pediatric HIV-1 infection (1–3). The conditions favoring vertical and/or perinatal transmission of HIV, as well as progression of the disease, are still largely unclear.

Analysis of mother-to-child transmission of HIV-1 infection in relation to transferred maternal HIV antibodies constitutes a natural model for the study of epitope-specific antibodies and their potential protective and/or predictive value by correlating the reactivity pattern and the clinical outcome.

Mapping of functional and immunogenic sites of HIV-1 structural proteins is essential for optimal vaccine development. Synthetic peptides and recombinant proteins representing selected regions of HIV *env*, *gag*, and *pol* gene sequences have been studied extensively in several experimental systems to define the functional properties of various antibodies (4–7). Thus, antibodies have been raised against a number of putative neutralizing sites on the envelope protein gp120 and some have been found to block viral infection *in vitro* (7–12). One particular immunodominant target of such antibodies consists of a 36-amino acid (aa) sequence (aa 296–331) predicted to form a loop between two cross-linked cysteine residues (13).

In the search for a possible association between the presence of defined antibodies and mother-to-child viral transmission, we analyzed the antibodies that bound to HIV peptides and recombinant proteins in sera from two at-risk cohorts. The first cohort consisted of infants born to HIV-infected mothers and whose HIV status was known at the time of analysis. Tested sera were collected in the first 6 months of life when antibodies were mainly transferred maternal antibodies. The other cohort consisted of seropositive mothers whose infants have then been monitored for a mean period of 15 months after delivery.

Peptides, 15 residues long, representing the major B-cell immunodominant regions of *env*, *gag*, and *pol* gene-encoded proteins were tested for antibody binding as were recombinant proteins including PB1, a polypeptide made in *Escherichia coli* containing the central part of the envelope gene (env aa 286–467) (4); pENV9, a protein containing the C-terminal part of gp120 and the N-terminal part of gp41 (env aa 468–768; DuPont) (4); p121 representing a small immunogenic part at the N-terminal end of gp41 (14); p24/15 a polypeptide containing most of p24 and part of p15 sequences (15).

Our results indicate that presence of maternal antibodies to certain epitopes of HIV gp120 is correlated with an uninfected status of children born to seropositive mothers.

MATERIALS AND METHODS

Study Group. Sera enrolled in the retrospective study were obtained in the first 6 months of life (when transferred maternal antibodies predominate) from 33 children born to HIV-infected mothers. The children, who have been monitored for periods of 6–24 months, were classified according to the 1987 CDC criteria for HIV infection (16): 19 children were diagnosed as uninfected and 14 were classified as infected children. Five children died of AIDS before the age of 6 months.

Sera were also obtained at delivery from 21 HIV-seropositive mothers. The corresponding infants were then monitored for a period of 15 months, during which a complete laboratory and clinical evaluation was carried out every third month. The status of children and mothers at present in this prospective study is shown in Table 1.

All sera were identified with a code number and stored at −20°C until tested. Seropositivity to HIV was determined by

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Abbreviations: HIV-1, human immunodeficiency virus 1; aa, amino acid(s).

Table 1. Clinical status of mother–child pairs

Status of HIV-seropositive mother	Children, no.		
	Uninfected (13)	Non-AIDS (5)	AIDS (3)
Asymptomatic (11)	6	4	1
Non-AIDS (7)	6	1	0
AIDS (3)	1	0	2

Numbers in parentheses are *n*.

an HIV IgG ELISA (Organon Teknika, The Netherlands) and confirmed by Western blot analysis (Biotech/DuPont, Rockville, MD).

Synthetic Peptides and Recombinant Proteins. Small linear peptides containing 15 aa and overlapping 10 aa were prepared according to Houghten (17). Synthetic peptides and recombinant proteins had sequences of the BH10 clone derived from the HTLV-IIIB isolate (18). Amino acid residues were numbered in accordance with the data base for human retroviruses and AIDS (19). The sequences of the peptides are given in Table 2.

Expression and purification of recombinant proteins have been described (4, 14, 15). The following recombinant proteins were used: PB1, (4), pEnv9 (4), p121 (14), and p24/15 (15).

Peptide ELISA Assay. Micro-Elisa plates (Nunc, Odense, Denmark) were coated with 100 μl containing the selected synthetic peptide (20 μg/ml) or recombinant protein (1 μg/ml) and stored at 4°C. Before use the plates were washed five times with distilled water/0.05% Tween 20. Sera were diluted stepwise (1:100, 1:1000, and 1:10,000) in a neutral buffer containing 0.5% bovine serum albumin, 2% (wt/vol) normal goat serum, and 0.05% Tween 20, and 100 μl of the diluted serum was added to each well. After incubation at 37°C for 1 hr, the plates were washed five times with distilled water/0.05% Tween. Horseradish peroxidase-conjugated rabbit anti-human IgG (Dakopatts, Copenhagen, Denmark) diluted 1:15,000 in isotonic phosphate-buffered saline containing 0.5% bovine serum albumin, 2% normal goat serum, and 0.05% Tween 20 (100 μl) was added. After incubation at 37°C for 30 min, the washing step was repeated. The substrate *o*-phenylenediamine (100 μl), activated by 30% (vol/vol) H_2O_2, was added. The reaction was stopped after 30 min, at room temperature, by adding 100 μl of 2.5 M H_2SO_4. Values three standard deviations above the mean optical density (OD, 490 nm) of negative controls were considered to be positive.

Statistical Analysis. Fisher's exact test, unpaired Student's *t* test, and one-tail correlation test were used as included in the TADPOLE statistical program.

Table 2. Sequences of synthetic HIV-1 peptides assayed

Peptide	Location		Sequence
	Gene	aa residues	
gp120/C51	*env*	294–308	INCTRPNNNTRKSIR
gp120/C53	*env*	304–318	RKSIRIQRGPGRAFV
gp120/C57	*env*	324–338	GNMRQAHCNISRAKW
gp120/C58	*env*	329–343	AHCNISRAKWNNTLK
gp120/C90	*env*	489–503	VKIEPLGVAPTKAKR
SP22 gp120	*env*	497–511	APTKAKRRYYQREKR
p17/9	*gag*	13–27	LNRWEKIRLRPGGKK
p24/56	*gag*	248–262	GWMTNNPPIPVGEIY
p24/59	*gag*	63–77	KRWIILGLNKIVRMY
p15/94	*gag*	438–452	WPSYKGRPGNFLQSR
pol/B98	*pol*	944–958	DSRNPLWKGPAKLLW

Residues were numbered as described (17) according to the sequence of strain HTLV-IIIB clone B10 (18). The single-letter aa code is used.

RESULTS

Retrospective Study. Specificities of HIV antibodies in sera of newborns from HIV-infected mothers were first analyzed. These sera are expected to mainly contain passively transferred maternal antibodies. A total of 33 HIV-positive sera of newborns of HIV-infected mothers and 5 sera from age-matched control newborns was tested by standard ELISA. The seropositivity was further confirmed by Western blot. All sera were then tested against a panel of HIV proteins and peptides (Table 3). The results are expressed as reactive sera within each group (uninfected or infected). Using this approach, peptide gp120/C57 gave a distinct pattern of reactivity. Five of 19 sera from the uninfected population were reactive with peptide gp120/C57 in contrast to 0 of 14 sera of children from the infected group ($P < 0.05$). Such a skewed distribution was, however, not observed against PB1 protein, which, among other regions, contains the gp120/C57 sequence. Moreover, in peptide gp120/C57-reactive sera, no correlation was found between pEnv9 and peptide gp120/C57 antibody titers. Neither was any relation seen to generally high anti-HIV titers and anti-gp120/C57 titers.

Prospective Study. To further confirm any predictive or protective role of epitope-specific maternal antibodies, a prospective study was performed on 21 HIV-seropositive mothers (Table 4). All 21 sera were HIV-antibody-positive by standard ELISA and confirmed by Western immunoblot assays. Immunoblot analysis also showed that the pattern of reactivity to HIV antigens was the same in serum from the mother and serum from her child, with no substantial differences between the groups (Fig. 1). This supports the view that IgG antibodies against HIV detected in neonatal sera reflect maternal antibody production.

Sera from the 21 seropositive mothers were divided into two groups according to the HIV status of their children—i.e., 13 mothers who gave birth to an uninfected child and 8 seropositive mothers who gave birth to an infected child.

In accordance with our retrospective study, antibodies to peptides containing epitopes of the hypervariable loop of PB1 region were almost exclusively found in sera from mothers who gave birth to an uninfected child (Table 4). Likewise, IgGs to peptide gp120/C57 were ($P = 0.0162$) detected in 9 of 13 sera (69%) from mothers of an uninfected child whereas only 1 of 8 sera (12%) from mothers of an HIV-infected child contained antibodies to this peptide.

Evaluation of seroreactivity to the overlapping peptide gp120/C58 showed the same clustered response in sera from mothers of an uninfected child, although at a lower frequency (positive reactions from 6 of 13 mothers of an uninfected child and 1 of 8 mothers of infected children). Peptides gp120/C57

Table 3. Children less than 6 months of age: Antibody reactivity to specific HIV peptides and proteins

Peptide tested	Children with seropositive reactions, no.	
	Uninfected	Infected
gp120/C51	2 (11)	0
gp120/C53	2 (11)	0
gp120/C57	5 (26)*	0*
gp120/C58	2 (11)	0
SP22	4 (21)	2 (14)
p17/9	1 (5)	2 (14)
pol/B98	5 (26)	3 (21)
PB1	7 (37)	5 (35)
pENV9	15 (78)	14 (100)

Sera from 19 uninfected and 14 infected children were tested. Numbers in parentheses are percentages of total sera tested. All controls ($n = 5$) were negative.
*$P < 0.05$ by Fisher's test.

Table 4. HIV-seropositive mothers of an uninfected or an infected child: Reactivity to HIV synthetic peptides and recombinant proteins

Peptide tested	No. of sera with reactive antibodies/ total no. of sera tested	
	MUC	MIC
gp120/C51	8/13*	1/8*
gp120/C53	6/13	2/8
gp120/C57	9/13†	1/8†
gp120/C58	6/13	1/8
PB1	10/13	3/8
gp120/C90	5/7	4/4
SP22	6/7	3/4
p121	7/7	4/4
pENV9	7/7	4/4
p17/9	6/7	2/4
p24/56	4/7	2/4
p24/59	3/7	3/4
p15/94	6/7	4/4
pol/B98	3/7	1/4
p24/15	5/7	1/4

MUC, mothers with an uninfected child; MIC, mothers with an infected child. All controls (n = 8) were negative.
*P = 0.0375 by Fisher's exact test.
†P = 0.0162 by Fisher's exact test.

and gp120/C58 share the conserved C-terminal cysteine residue, predicted to define the hypervariable domain of gp120 as a loop-like structure. Then, we investigated the seroreactivity to the adjacent peptides gp120/C51 and gp120/C53 containing the N-terminal cysteine residue and the central Gly-Pro-Gly-Arg sequence of the loop, respectively, all features reported to be conserved (13). As shown in Table 4, the same pattern of seroreactivity was found with peptide gp120/C51 (P = 0.0375).

pEnv9 is almost universally recognized in immunoassays of sera from HIV-infected individuals (4). Also in our prospective and retrospective studies, all sera reacted to this recombinant protein with no significant differences when the mean antibody titers against total viral lysate (Organon Elisa) and PB1 in sera from uninfected and infected subjects were compared. Furthermore, no skewed distribution of reactivity against any other tested peptide was detected.

DISCUSSION

The humoral immune response can be an important component of protective immunity against infectious agents. In this report, using the model of mother-to-child transmission of HIV infection, we have demonstrated a positive association between serum antibodies to selected epitopes of gp120 envelope protein—in particular, peptides gp120/C57 and gp120/C51—and birth of an uninfected child. Thus, in the initial retrospective study, the presence of antibodies to peptide gp120/C57 in newborn sera of at-risk children correlated in an exclusive manner with the healthy status of the child at the age of 2 years. This was also strengthened by the facts that gp120/C57 antibody titer was not related to pEnv9 titer and antibodies to total viral lysate or to the larger polypeptide pB1, which includes the gp120/C57 sequence. Thus, the uniqueness of the antibody pattern is not due to a general increased antibody titer but reflects the quality of maternal humoral immune response. Although the number of sera from uninfected children reacting with gp120/C57 was small, the total absence of antibodies to gp120/C57 in the sera of infected newborns suggested that the corresponding region might elicit a protective immune response *in vivo*. Indeed, this skewed distribution of reactivity was further confirmed and extended by findings of the prospective study. Antibodies to gp120/C57 and other select sequences of the loop region, represented by peptides gp120/C51 and gp120/C58, clustered significantly in the population of mothers of uninfected children.

Envelope glycoprotein gp120 plays a central role in the mechanisms of infectivity and pathogenicity of HIV (21–23). In experimental systems discrete epitopes of HIV gp120 are able to elicit antibodies that neutralize virus or that inhibit CD4 binding or cell fusion (4, 6–12). All these mechanisms may operate *in vivo* to prevent the virus from spreading (24, 25), although evidence of strong correlations between the presence of functional antibodies and defined stages of HIV infection is conflicting (26–31). The presence of antibodies directed against the so-called hypervariable loop of the PB1 region of gp120 in sera of HIV-infected mothers and their uninfected children was significant and suggests that, as a consequence of this epitope-specific reactivity, the *in vivo* spread of HIV from mother to fetus may be blocked. This is further strengthened by the fact that this loop contains the dominant neutralizing epitopes, including the RP135 se-

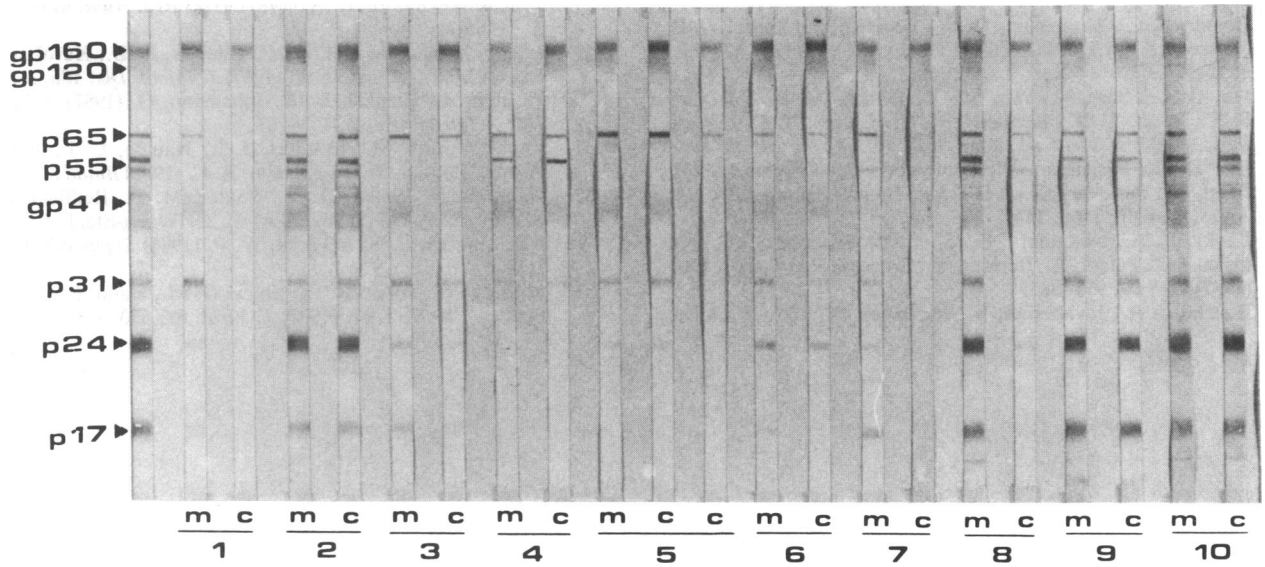

FIG. 1. Western blot analysis of antibodies in sera from 10 mother (m)–child (c) pairs; sera was collected at delivery. Positions of HIV proteins are indicated to the left.

quence, shown (2) to elicit antibodies that can type-specifically neutralize virus and inhibit cell fusion. Although this region shows substantial genomic heterogeneity among isolates, several amino acids are strongly conserved, including the flanking cysteine residues (32, 33). It is thus intriguing that the three peptides gp120/C57, gp120/C58, and gp120/C51, to which only sera of uninfected children and almost exclusively sera of mothers with an uninfected child react, contain these highly conserved cysteine residues. It is likely that the cysteines play a role in maintaining the tertiary structure of the region. Thus, antibodies directed to these peptides might affect protein conformation, essential for biological activity. The coherent data obtained from two different cohorts (Tables 3 and 4) strongly suggest that presence of antibodies against the s.c. hypervariable loop of gp120 is linked with protection against infection. This might be explained by a protective role for maternal antibodies in preventing vertical (intrauterine) transmission of HIV.

The present data suggest that peptide site-directed serology may be useful to predict the outcome of an at-risk pregnancy of an HIV-infected woman and to predict whether at-risk newborns were infected. In fact, the maternal antibodies that hamper early diagnosis of neonatal HIV infection in a standard serological analysis may be helpful predictive markers in a site-directed serological analysis. Assays for antibodies to selected epitopes that are associated with a healthy status and molecular techniques to identify the presence of HIV genomic sequences might be the tools needed to clarify such clinical dilemmas.

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