

Transfer of antibodies across the placenta and in breast milk from mothers on intravenous immunoglobulin

Palmeira P, Costa-Carvalho BT, Arslanian C, Pontes GN, Nagao AT, Carneiro-Sampaio MMS. Transfer of antibodies across the placenta and in breast milk from mothers on intravenous immunoglobulin. *Pediatr Allergy Immunol* 2009.

© 2009 The Authors

Journal compilation © 2009 Blackwell Munksgaard

We studied the levels of immunoglobulins in colostrum, milk and sera from two common variable immunodeficiency (CVID) mothers (M1 and M2), and in sera from their newborn infants. During pregnancy they continued intravenous immunoglobulin therapy (IVIG). Antibody levels from maternal and cord blood collected at delivery and colostrum and milk, collected on the 3rd and 7th post-partum days, respectively, were analyzed. Although cord/maternal blood ratios of total immunoglobulins and subclasses, as well as specific antibodies differed between M1 and M2, both showed good placental transfer of anti-protein and anti-polysaccharide antibodies, despite lower cord/maternal blood ratios in M2. Anti-*Streptococcus pneumoniae* antibody avidity indexes were similar between paired maternal and cord serum. Both mothers' colostrum and milk samples showed only traces of IgA, and IgM and IgG levels in colostrum were within normal range in M1, whereas M2 presented elevated IgG and low IgM levels, when compared with healthy mothers. The study of colostrum and milk activity showed that they strongly inhibited enteropathogenic *Escherichia coli* adhesion *in vitro*. CVID patients must be informed about the relevance of regular IVIG administration during pregnancy, not only for their own health but also for their immune immature offspring. Breast-feeding should be encouraged as colostrum from these CVID patients strongly inhibited *E. coli* adhesion to human epithelial cells thus providing immunological protection plus nutritional and psychological benefits for the infant.

Patricia Palmeira¹, Beatriz T. Costa-Carvalho², Christina Arslanian³, Gerlândia N. Pontes^{1,3}, Aparecida T. Nagao^{3,4} and Magda M. S. Carneiro-Sampaio¹

¹Department of Pediatrics, Faculdade de Medicina and ²Department of Pediatrics, Universidade Federal de São Paulo, São Paulo, Brazil, ³Department of Immunology, Universidade de São Paulo, Brazil, ⁴Department of Clinical Analysis and Toxicology, Universidade Federal do Ceará, Fortaleza, Brazil

Key words: primary immunodeficiency; placental transfer; breast-feeding

Patricia Palmeira, Department of Pediatrics, Medical School, University of São Paulo, Av. Dr. Enéas Carvalho de Aguiar, 647, Cerqueira César CEP: 05403-000, São Paulo, SP, Brazil
Tel.: +55 11 3069 8606
Fax: +55 11 3091 7224
E-mail: patpalm@usp.br

Accepted 4 October 2008

Common variable immunodeficiency (CVID) is the second most common primary immunodeficiency disease, affecting males and females equally with an incidence of 1:10,000 to 1:50,000 (1), and is characterized by reduced serum levels in the immunoglobulin classes, impaired antibody response, with variable T cell numbers and function (2). CVID includes a heterogeneous group of immunologic disorders, and its genetic heterogeneity has become clear in the last years, with the identification of four genetic defects underlying: inducible co-stimulator (ICOS), B cell activating factor receptor (BAFF-R), transmembrane activator and cal-

cium-modulator and cyclophilin ligand interactor (TACI) and CD19 deficiencies, which together account for approximately 10% of the patients (3, 4). It is well known that the transplacental transfer of IgG antibodies from the mother to the fetus is important for the protection of the infant in the first months of life. Placental transfer of IgG antibodies reactive to polysaccharide antigens from *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (5, 6), to lipopolysaccharide (LPS) from *E. coli* O16, O6, O111, and O157 (7–9) and to protein antigens (10) was already demonstrated in term and preterm neonates.

Although CVID is not an extremely rare disorder, many patients reach childbearing age in reasonably good health and become pregnant. There are only a few reports on total immunoglobulin placental transfer, whereas no descriptions about specific antibody placental transfer or immunological factors in colostrum and milk of affected patients have been found (2, 11–14). Here we describe two parturient patients with CVID, whose blood and cord blood from their respective newborns were collected at delivery, from whom colostrum was collected on the 3rd post-partum day and milk on the 7th post-partum day, all of which were analyzed for passive transfer of immunoglobulin classes and subclasses, specific anti-protein and anti-polysaccharide antigens IgG antibodies, *in vitro* inhibitory activity of colostrum and milk on the adhesion of enteropathogenic *E. coli* (EPEC) to human epithelial cells, a pathogen responsible for acute infant diarrheal disease which is highly disseminated in the Brazilian population.

Case report

Mother 1 (M1). A 22-yr-old patient with CVID gave birth to a normal healthy (weight 3330 g and height 49 cm), male at 38 wk gestation, by caesarean section. Throughout the pregnancy she received 400–500 mg/kg of IVIG every 4 wk and did not present with any significant infectious problems, her IgG levels, monitored monthly, varied from 421.0 to 684.0 mg/dl. Diagnosis of CVID was established when she was 18-yr-old, 4 yr before the pregnancy. On diagnosis lab exams showed IgG = 30.0 mg/dl (normal 739.0–1390.0 mg/dl), IgM = 14.0 mg/dl (normal 81.0–167.0 mg/dl), IgA was undetectable (normal 84.0–354.0 mg/dl), a negative antibody response, T lymphocytes CD4⁺ and CD8⁺ were 752.0 and 849.0 cells/mm³, respectively, CD19⁺ cells = 105.0 cells/mm³ and CD16⁺56⁺ = 35.0 cells/mm³. She improved clinically with IVIG treatment.

Mother 2 (M2). A 21-yr-old patient with CVID gave birth to a full term, normal healthy male (weight 2800 g and height 48 cm). Throughout her pregnancy she received 600–700 mg/kg of IVIG every 4 wk. As this patient presented with bronchiectasis she has been treated with IVIG in doses of 600–700 mg/dl. During the pregnancy she did not present any significant infectious problems, and her IgG levels, monitored monthly, varied from 661.0 to 1308.0 mg/dl. Diagnosis of CVID was established 6 yr before the pregnancy when she was 15-yr-old with the following lab

results: IgG = 469.0 mg/dl (normal 739.0–1390.0 mg/dl), IgM = 8.0 mg/dl (normal 81.0–167.0 mg/dl), IgA < 6.0 mg/dl (normal 84.0–354.0 mg/dl), T lymphocytes CD4⁺ and CD8⁺ were 663.0 and 531.0 cells/mm³, respectively, CD3⁺ cells = 1314.0 cells/mm³, CD19⁺ cells = 78.0 cells/mm³ and CD16⁺56⁺ = 270.0 cells/mm³.

Methods

Maternal and cord blood were collected during delivery. Colostrum and milk samples were obtained on the 3rd and 7th post-partum days. Appropriate informed consent and institutional ethics committee approval were obtained, and all guidelines for human experimentation were followed throughout this study.

Immunoglobulins and subclasses levels

Total IgG, IgA and IgM, and IgG subclasses concentrations (mg/dl) in sera were measured by nephelometry. The presence of IgG, IgA and IgM in colostrum and milk samples was evaluated by enzyme-linked immunosorbent assay (ELISA), as previously described by Nagao et al. (15).

Anti-tetanus toxoid levels

Anti-toxoid levels were determined by ELISA (16), using purified tetanus toxoid at 5.0 mg/l as antigen. An anti-tetanus immunoglobulin (Tetanogamma, Aventis Behring, Germany), which presents 250.0 IU/ml of anti-tetanus IgG antibodies, was used as a reference standard. The results were expressed in IU/ml.

Anti-Hib levels

The ELISA for Hib was performed using the technique modified by Takano (17) to detect anti-polyribosyl-polymers (PRP) (Polyoside *Haemophilus influenzae* type b, kindly donated by Pasteur Mérieux Laboratory, Lyon, France) IgG antibodies' concentrations, in the maternal and cord sera and anti-PRP IgA and IgG antibodies' concentrations in colostrum and milk. The Hib pool (prepared and donated by Pasteur Mérieux, Lyon, France), containing 77.0 mg/l of anti-PRP IgG antibodies was used as standard curve. The results were expressed in mg/l.

Anti-*Streptococcus pneumoniae* IgG levels

Anti-pneumococcal IgG to serotypes (Ps) 1, 3, 5, 6B, 9V, and 14 were determined by modified

ELISA protocol, described by Sorensen et al. (18) and the results expressed in mg/l.

Anti-*Streptococcus pneumoniae* IgG antibodies' avidity indexes

Avidities of anti-*S. pneumoniae* serotypes 1, 3, 5, 6B, 9V, and 14 IgG antibodies in maternal and cord sera were determined by elution with different molarities (M) of potassium thiocyanate (0.0–3.0 M) according to technique described by Jones et al. (19). The results were expressed as the molarity of potassium thiocyanate necessary to elute 50% of the bound antigen-antibody complexes.

Inhibition of the EPEC adhesion by colostrum and milk

EPEC O111:H⁻ adhesion assay to HEP-2 cells was performed as described by Silva & Giampaglia (20). The inhibition of EPEC adhesion was tested when colostrum and milk samples were added to the mixture of bacterial suspension and tissue culture, and compared with the adherence positive control, performed without colostrum or milk. A human colostrum pool, prepared with 50 colostrum samples collected from healthy mothers, was used as positive control of the inhibitory activity. At least 300 cells were observed in each preparation under a light microscope. The amount of adhesion was determined by calculating the percentage of cells with six or more adhering bacteria on the cell surface. The inhibitory effects of colostrum and milk were calculated as the difference between the percentage of bacterial adhesion in the control well without colostrum and milk and that in the well containing the colostrum or the milk samples $\times 100\%$.

After the first incubation for 30 min, antigen-antibody interactions were analyzed using monoclonal antibodies conjugated to fluorescein-isothiocyanate (FITC). Briefly, the mixtures containing EPEC and colostrum samples from M1 or M2 were incubated with anti-human IgG, IgM or IgA antibody conjugated to FITC (Sigma, St. Louis, MO, USA), diluted 1:200 in phosphate-buffered saline (PBS) pH 7.4 for 1 h at 37°C. The suspensions were washed two times for 10 min with PBS pH 7.4 and then pipetted onto microscope slides and examined at $\times 1,000$ final magnification under fluorescent microscopy.

Immunoblotting

Immunoblotting assays were performed as previously described (21). Protein antigens from EPEC strain O111:H⁻ were separated by SDS-PAGE in 10% polyacrylamide gels. Molecular

weight standards (Pharmacia, Uppsala, Sweden) were run in each gel. After transferring to nitrocellulose membranes for 40 min at 14 V in a semidry Trans-Blot S.D., apparatus (Bio-Rad, Hercules, CA, USA), strips were incubated overnight with appropriate dilutions of serum or colostrum samples. A human colostrum pool and a human serum pool were used as controls of the assay. Subsequently, strips were incubated with a 1:1000 dilution of peroxidase conjugated anti-human IgG, IgM or IgA, for 2 h (Sigma). Reactions were developed using the substrate in 3,3'-diaminobenzidine (Sigma).

Results

Total immunoglobulins and subclasses levels in serum

The analysis of immunoglobulin levels in serum demonstrated low IgG concentrations in M1, and normal IgG levels in M2, but both neonates had IgG levels from placental transfer similar to the healthy population. Both mothers presented low IgM and IgA levels in serum, as expected with this immunodeficiency. IgG subclasses were low for M1 but within normal range for M2, the placental transfer of these subclasses was effectively performed for both neonates, which can be observed from the cord/maternal serum ratios. Although M1 presented lower immunoglobulin and subclasses levels than M2, her cord/maternal ratios to IgG3 and IgG4 were higher than for M2 (Tables 1 and 2).

Table 1. Serum immunoglobulin levels, specific antibody concentrations and avidity indexes in the maternal and cord serum and cord/maternal serum ratios for IgG antibodies from mother 1 (M1)

	Maternal serum	Cord serum	Cord / maternal serum ratio
IgG (mg/dl)	473.0	912.0	1.9
IgM (mg/dl)	< 6.0	12.0	—*
IgA (mg/dl)	< 3.0	< 3.0	—
IgG1 (mg/dl)	362.0	752.0	2.1
IgG2 (mg/dl)	249.0	192.0	0.8
IgG3 (mg/dl)	10.0	20.0	2.0
IgG4 (mg/dl)	6.0	21.0	3.5
IgG anti-tetanus toxoid (UI/ml)	1.6	3.1	1.9
IgG anti-Hib PRP (mg/l)	3.8	3.7	1.0
IgG anti-PS1 (mg/l)/avidity (M)	2.3/> 3.0	2.6/> 3.0	1.1
IgG anti-PS3 (mg/l)/avidity (M)	2.4/> 3.0	2.8/> 3.0	1.2
IgG anti-PS5 (mg/l)/avidity (M)	7.3/2.7	8.0/2.7	1.1
IgG anti-PS6 (mg/l)/avidity (M)	6.9/2.7	6.6/> 3.0	1.0
IgG anti-PS9 (mg/l)/avidity (M)	4.4/2.9	4.7/> 3.0	1.1
IgG anti-PS14 (mg/l)/avidity (M)	12.8/2.5	15.0/2.8	1.2

*IgM and IgA maternal/cord blood ratios were not performed.

PRP, polyribosyl-ribitolphosphate polymers; (M), avidity index in molarity; anti-PS, anti-*Streptococcus pneumoniae* polysaccharide.

Table 2. Serum immunoglobulin levels, specific antibody concentrations and avidity indexes in the maternal and cord serum and cord/maternal serum ratios for IgG antibodies from mother 2 (M2)

	Maternal serum	Cord serum	Cord/maternal serum ratio
IgG (mg/dl)	988.0	1,404.0	1.4
IgM (mg/dl)	20.0	29.0	—*
IgA (mg/dl)	3.2	<3.0	—
IgG1 (mg/dl)	612.0	1,068.0	1.7
IgG2 (mg/dl)	308.0	262.0	0.8
IgG3 (mg/dl)	60.0	28.0	0.5
IgG4 (mg/dl)	22.0	18.0	0.8
IgG anti-tetanus toxoid (UI/ml)	1.4	1.8	1.3
IgG anti-Hib PRP (mg/l)	2.7	1.2	0.5
IgG anti-PS1 (mg/l)/avidity (M)	3.5/1.6	2.6/1.4	0.7
IgG anti-PS3 (mg/l)/avidity (M)	2.4/1.5	1.9/1.5	0.8
IgG anti-PS5 (mg/l)/avidity (M)	3.3/1.5	2.4/1.4	0.7
IgG anti-PS6 (mg/l)/avidity (M)	4.9/1.2	3.3/1.1	0.7
IgG anti-PS9 (mg/l)/avidity (M)	2.3/1.2	1.9/1.0	0.8
IgG anti-PS14 (mg/l)/avidity (M)	9.7/1.2	7.5/1.1	0.8

*IgM and IgA maternal/cord blood ratios were not performed.

PRP, polyribosyl-ribitolphosphate polymers; (M), avidity index in molarity; anti-PS, anti-*Streptococcus pneumoniae* polysaccharide.

Antibody concentrations and avidity indexes

Both mothers presented normal anti-tetanus toxoid, anti-Hib and anti-*S. pneumoniae* IgG levels and transferred these antibodies efficiently to their newborns. As expected, cord/maternal ratios for anti-tetanus toxoid IgG were higher than one for both patients, and these ratios to anti-polysaccharide IgG were approximated to one for both. The avidity indexes of anti-*S. pneumoniae* IgG antibodies were equivalent when mother and her respective newborn were compared (Tables 1 and 2).

Total immunoglobulin levels and anti-Hib IgA and IgG antibodies in colostrum and milk

Total IgG, IgM and IgA levels in colostrum and milk samples are represented in (Table 3). Both mothers presented only traces of IgA in both

colostrum and milk samples. Concerning IgM, a normal level was found only in colostrum from M1. Low levels of IgG were found in colostrum and milk from M1, whereas M2 presented high levels of this immunoglobulin in colostrum and milk. Anti-Hib IgA antibody concentrations were very low in colostrum and milk from both mothers and anti-Hib IgG was found in colostrum from M1 (Table 3).

Inhibition of EPEC adhesion by colostrum and milk

The colostrum and milk samples exhibited the same inhibitory activity on the EPEC adhesion to HEp-2 cells (99.3 and 99.2% for M1, and 98.9 and 98.1% of inhibitory activity for M2, respectively) compared with the control assay performed without colostrum or milk that showed 100% of EPEC adhesion to HEp-2 cells. The human colostrum pool, prepared with 50 colostrum samples from healthy mothers used as positive control, demonstrated inhibitory activity equivalent to these two CVID mothers' colostrum and milk (99.2% of inhibitory activity) (Fig. 1). The antigen-antibody interactions using monoclonal antibodies conjugated to FITC demonstrated that colostrum from M1 only presented IgM antibodies bound to EPEC, visualized by immunofluorescent staining with anti-IgM FITC-conjugated antibody. However, in the colostrum from M2, it was observed only IgG antibodies bound to these bacteria, visualized when anti-IgG FITC-conjugated antibody was used (Fig. 2).

Immunoblotting assays

Immunoblotting assays demonstrated a strong recognition of EPEC antigens by serum IgG from M1 and M2 and identical recognition profiles were obtained when their respective newborns' serum were tested. Colostrum IgM from M1 and colostrum IgG from M2 showed

Table 3. Total immunoglobulin levels and anti-Hib IgA and IgG antibody concentrations in colostrum and milk from the two CVID mothers

	M1		M2		Control – healthy population+
	Colostrum	Milk	Colostrum	Milk	Colostrum
IgG (mg/dl)	1.95	1.37	112.10	9.10	0.9 – 53.0
IgM (mg/dl)	50.00	0.11	0.51	0.43	11.0 – 3,184.0
IgA (mg/dl)	2.60	0.09	0.15	0.07	92.0 – 5,500.0
IgA anti-Hib PRP (mg/l)	0.05	0.03	< 0.005	< 0.005	—*
IgG anti-Hib PRP (mg/l)	0.28	< 0.015	< 0.015	< 0.015	—

+Range of immunoglobulin concentrations (mg/dl) in colostrum from healthy mothers of our population (42).

*Not available.

PRP, polyribosyl-ribitolphosphate polymers.

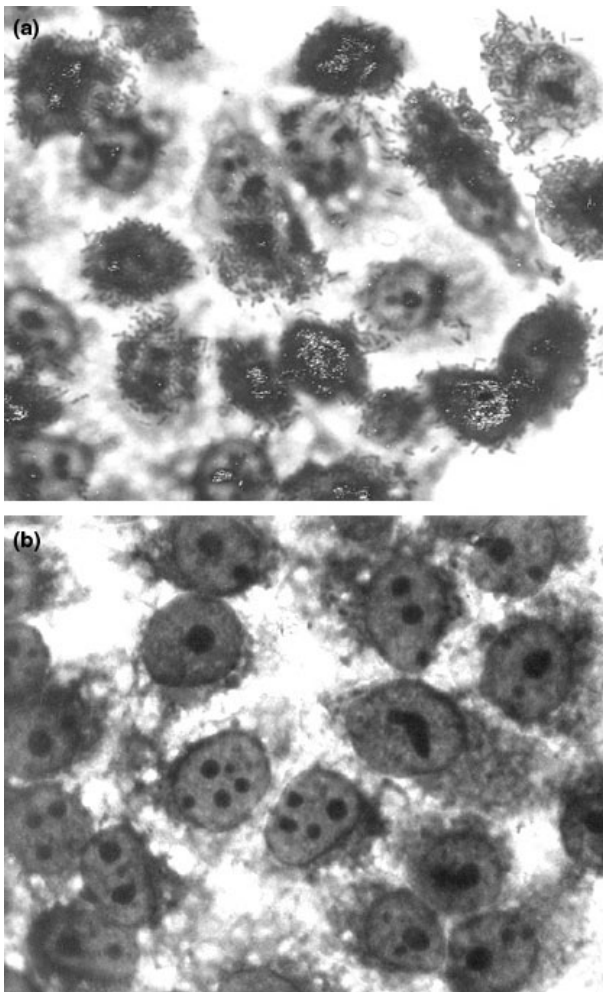


Fig. 1. Micrograph showing the adhesion of EPEC O111:H⁻ to HEp-2 cells. (a) control adhesion without colostrum; (b) adhesion inhibition in the presence of colostrum.

the recognition of few and weak antigenic bands of EPEC, but no bacterial antigenic recognition was observed by IgA when colostrum samples from M1 and M2 were tested. No bacterial recognition was visualized when milk samples were tested (Fig. 3).

Discussion

The study of CVID mothers having IVIG therapy and their newborns demonstrates that exogenous IgG crosses the placenta in similar patterns as endogenous immunoglobulins showing: (a) cord blood IgG levels higher than maternal levels in term babies; (b) a preferential transfer of IgG1, IgG3 and IgG4 by comparison with IgG2, and (c) anti-protein IgG antibody levels equivalent to or higher than maternal ones in cord serum and a good anti-polysaccharide IgG antibodies trans-

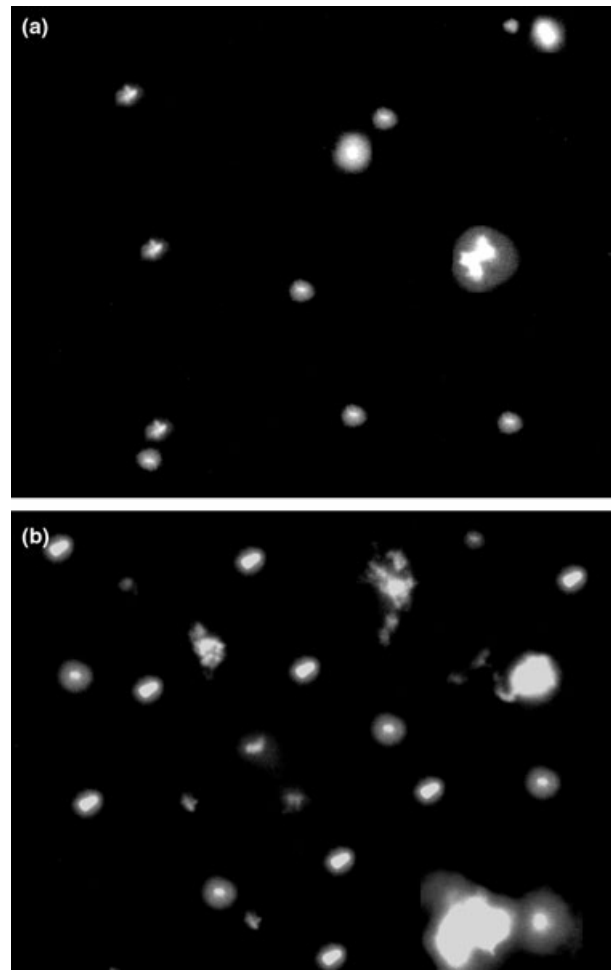


Fig. 2. Antigen-antibody interactions of colostrum IgM from M1 (a) and colostrum IgG from M2 (b) incubated with viable EPEC for 30 min. These activities were visualized with anti-human IgM or IgG antibodies conjugated to FITC. The suspensions were analyzed at 1000× magnification under a fluorescent microscope light.

fer, with neonatal levels usually not exceeding the maternal ones, even in term babies (5, 22–25).

In the present report, cord/maternal serum ratios of total IgG and subclasses, except for IgG2, from M1 were higher than those usually observed in immunocompetent mothers and even in the few other CVID mothers not having treatment or IVIG or subcutaneous immunoglobulin replacement therapy already described (11, 14, 22, 23). Maternal serum IgG was approximately twice as high in M2 compared with M1 which could partially explain this ratio difference between these mothers. Another point is that the cord/maternal serum ratio can vary between different mothers, because in our own series, we have seen some healthy newborn/mother pairs with IgG ratios as high as 2.00⁽⁹⁾.

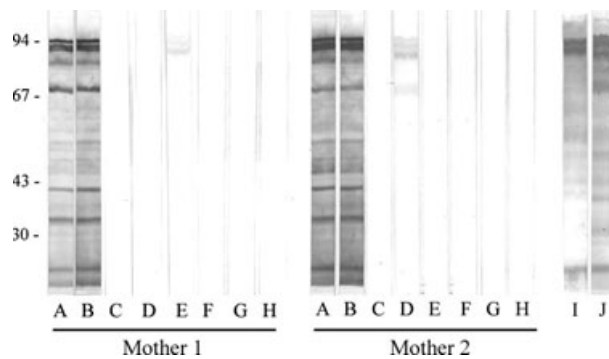


Fig. 3. Immunoblotting of anti-EPEC O111:H⁻ IgG, IgA and IgM antibodies in the paired maternal and cord serum samples, and in colostrum and milk from mothers 1 and 2 (M1 and M2). Bacterial proteins were separated by 10% polyacrylamide SDS-PAGE. Reaction developed with anti-human IgG, IgA or IgM peroxidase conjugate diluted 1:1000. (a) maternal serum IgG; (b) cord serum IgG; (c) colostrum IgA; (d) colostrum IgG; (e) colostrum IgM; (f), milk IgA; (g) milk IgG; (h) milk IgM. A human colostrum pool (i) and a human serum pool (j) were used as controls.

The high IgG levels observed in the cord serum from the newborn of M2, are probably not due to fetal synthesis, because the fetus synthesizes little IgG, so that the concentrations in cord serum are considered as reflecting solely maternally derived antibody (26). The high IgG4 level, which is a late acquired function in life, certainly reinforces this idea. Regarding specific antibody concentrations, antibodies to protein antigens (anti-tetanus toxoid) are mainly IgG1, while part of anti-polysaccharide antigen's antibodies may be IgG2, which could explain the differences in cord/maternal serum ratios seen between anti-protein and anti-polysaccharide IgG antibodies in these mothers (5, 27). On the whole, the ratios of antibody transfer in these mother/newborn pairs, particularly in M1, clearly indicate that IgG placental transfer must be due to an active transport phenomenon, made against the gradient of concentration.

IgG is detected in the fetus by 17 wk of gestation, after which IgG in fetal circulation rises steadily, reaching half of the term concentrations at about 30 wk. The fetal levels increases rapidly after 35 wk, and at term, cord blood IgG levels are usually equivalent to or exceed those of the mothers (22, 25). Different to other IgG subclasses, IgG2 levels are typically lower in cord blood, even in term neonates, which appears to reflect a relatively low affinity of the receptors involved in the IgG trophoblastic uptake for IgG2 (25, 26, 28).

Avidity, as well as antibody specificity, is determined by Fab' fragment of IgG while placental transfer seems to be Fc-related, so it

is unexpected, that antibody avidity could be different between mother/newborn pairs. However, higher avidity indexes to tetanus toxoid and pneumococcal antibodies were found in cord blood compared with mothers' serum (27). Our results showed similar anti-*S. pneumoniae* avidity indexes between patients and neonates.

One interesting point, well explored in murine models but not in humans yet, is that placental-derived IgG exert long-life immunoregulatory functions, including the one of imprinting the fetal immune network (29). Thus, by crossing the placenta, exogenous IgG, in addition to giving anti-infectious protection to the infant, could have other active immunoregulatory long-term effects, as does IgG from healthy mothers. This mechanism of transplacental antibodies could also be involved in the recognition of allergens and priming of small populations of allergen-specific T cells in the newborn during intra-uterine life, which could represent a normal stimulatory signal (30).

The present work revealed that the *in vitro* protective colostrum and milk ability was somewhat surprising, as, although the materials presented only IgA antibody traces, they were able to strongly inhibit *in vitro* EPEC adhesion to HEp-2 cells.

Several factors in human milk, including antibodies and non-specific soluble factors, may be important in conferring the protective role of breast-feeding (31, 32). Among the nonspecific soluble factors, human milk has oligosaccharides and complex carbohydrate structures like glycoconjugates. These soluble components act by competitively inhibiting the ability of pathogens to bind to receptors in the gut, thus increasing the resistance of breast-fed infants (33). Human milk contains secretory IgA (SIgA) antibodies reactive to a variety of pathogens, to which the mother has been exposed during her lifetime, which will protect the offspring against the prevalent pathogens in the environment (34). Several studies have already demonstrated that SIgA antibodies of colostrum obtained from Brazilian women strongly inhibited the *in vitro* adhesion of enteropathogenic and Shiga toxin-producing *E. coli* to human epithelial cells (21, 35).

Among those mothers studied by our group, we found one with selective IgA deficiency. Surprisingly her colostrum inhibited both EPEC adherence and enteroinvasive *E. coli* (EIEC) invasion to HEp-2 cells at an equivalent level to normal controls (unpublished data). Measuring immunoglobulin levels in her colostrum, we found IgM concentrations much higher

compared with normal mothers, which recognized the same EPEC and EIEC bands as do IgA antibodies from healthy mothers' colostrum samples. We have previously described elevated colostral IgM levels in another IgA-deficient puerperae, which may represent a compensatory phenomenon for IgA deficiency (36).

These observations do not seem to occur in this study because only M1 presented normal IgM level in colostrum, when compared with healthy mothers. The immunofluorescent staining performed with anti-IgG, anti-IgM and anti-IgA conjugated to FITC showed that only IgM antibodies of colostrum from M1 recognized EPEC, visualized under fluorescent microscopy light, while in the assay performed with colostrum from M2, it was detected high levels of IgG antibodies bound to EPEC, indicating that there is a possible role for IgM and IgG in the inhibitory ability of these colostrum samples.

The recent identification of FcRn expression in numerous epithelial cell types in humans during adult life (37), together with the recognition that FcRn mediates the bidirectional transport of IgG *in vitro* and *in vivo* across epithelial barriers, has directed attention to other potential extensions of FcRn function in immunity (38, 39). The study of Yoshida et al. (40) showed that the presence of FcRn in the intestinal epithelium is the primary means by which IgG reaches the lumen, as demonstrated by the absence of luminal IgG in the context of FcRn deficiency. Albeit the study of this mechanism has not been performed yet in the mammary gland, this could be a possible explanation for the transport of exogenous IgG into human milk.

It was recently reported a study with an individual with CVID with persistent neurovirulent poliovirus infection of vaccine-origin, who was submitted to *in vitro* and *in vivo* therapeutic studies using oral immunoglobulin, breast milk (as a source of SIgA), ribavirin and the anti-picornaviral agent, pleconaril. No treatment was successful at clearing the virus, although in one trial, breast milk significantly reduced excretion levels temporarily *in vivo* and showed a 2–3 log₁₀ pfu/g feces reduction in viral titer using a 1 in 10 dilution of breast milk *in vitro*. These reductions were greater than those obtained with intravenous immunoglobulin (41).

Thus, CVID patients must be informed about the relevance of regular IVIG administration during pregnancy, not only for their own health but also for their immune immature offspring. They should also be encouraged to breast-feed their babies, thus providing anti-infectious prop-

erties as well as the nutritional and psychological benefits.

Acknowledgments

Financial support was provided by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, grant no. 02/05880-4). The authors are grateful to the two mothers who kindly agreed to participate in this study.

References

1. HAMMARSTROM L, VORECHOVSKY I, WEBSTER D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol* 2000; 120: 225–31.
2. CUNNINGHAM-RUNDLES C, BODIAN C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin Immunol* 1999; 92: 34–48.
3. BACCHELLI C, BUCKRIDGE S, THRASHER AJ, et al. Translational mini-review series on immunodeficiency: molecular defects in common variable immunodeficiency. *Clin Exp Immunol* 2007; 149: 401–9.
4. SCHAFER AA, SALZER U, HAMMARSTROM L, et al. Deconstructing common variable immunodeficiency by genetic analysis. *Curr Opin Genet Dev* 2007; 17: 201–12.
5. COSTA-CARVALHO BT, CARNEIRO-SAMPAIO MMS, SOLÉ D, et al. Transplacental transmission of serotype-specific pneumococcal antibodies in Brazilian population. *Clin Diagn Lab Immunol* 1999; 6: 50–4.
6. QUIAMBAO BP, NOHYNEK HM, KÄYHTY H, et al. Immunogenicity and reactogenicity of 23-valent pneumococcal polysaccharide vaccine among pregnant Filipino women and placental transfer of antibodies. *Vaccine* 2007; 25: 4470–7.
7. NAGAO AT, MARTINEZ CC, VIEIRA VS, et al. Placental transfer of IgG and IgG subclass antibodies anti-purified *Escherichia coli* LPS O16, O6 and O111. *Scand J Immunol* 1998; 47: 609–14.
8. NAGAO AT, FRIEDLANDER-DEL NERO D, ARSLANIAN C, et al. Elevated levels and different repertoire profile of colostral anti-LPS antibodies may have a significant role in compensating newborn immunity. *Scand J Immunol* 2001; 53: 602–9.
9. PALMEIRA P, YU ITO L, ARSLANIAN C, et al. Passive immunity acquisition of maternal anti-enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 IgG antibodies by the newborn. *Eur J Pediatr* 2007; 166: 413–9.
10. LEINEWEBER B, GROTE V, SCHAAD UB, et al. Transplacentally acquired immunoglobulin G antibodies against measles, mumps, rubella and varicella-zoster virus in preterm and full term newborns. *Pediatr Infect Dis J* 2004; 23: 361–3.
11. HOLLAND N, HOLLAND P. Immunological maturation in an infant of an agammaglobulinemic mother. *Lancet* 1966; 26: 1152–5.
12. OLATUNBOSUN OA. Hypogammaglobulinemia associated with pregnancy. *Int J Gynaecol Obstet* 1993; 40: 162–3.
13. VITORATOS N, BAKAS P, KALAMPANI H, et al. Maternal common variable immunodeficiency and pregnancy. *J Obstet Gynaecol* 1999; 19: 654–5.
14. GARDULF A, ANDERSSON E, LINDQVIST M, et al. Rapid subcutaneous IgG replacement therapy at home for pregnant immunodeficient women. *J Clin Immunol* 2001; 21: 150–4.

15. NAGAO AT, RAMOS OL, PEREIRA AB. Immunoassays of secretory IgA and secretory component. *Braz J Med Biol Res* 1990; 23: 211–24.
16. SIMONSEN O, BENTZON MW, HERON I. ELISA for the routine determination of antitoxic immunity to tetanus. *J Biol Stand* 1986; 14: 231–9.
17. TAKANO AO. Resposta imune humoral sistêmica e secretora de crianças eutróficas à vacina de polissacarídeo capsular de *Haemophilus influenzae* tipo b conjugado com toxóide tetânico. PhD Thesis. São Paulo, Brazil: Faculdade de Medicina, Universidade de São Paulo, 1997: 138.
18. SORENSEN RU, HIDALGO H, MOORE C, et al. Post-Immunization pneumococcal titers and IgG subclass levels. *Pediatr Pulmonol* 1996; 2: 167–73.
19. JONES CL, MACDONALD RA, HOSKING CS, et al. Estimating the relative avidity of mucosal IgA for antigen. *J Immunol Methods* 1987; 105: 111–7.
20. SILVA MLM, GIAMPAGLIA CMS. Effect of colostrum and human milk on the adherence to HeLa cells of common and rare enteropathogenic *Escherichia coli* serotypes found in Brazil. *Rev Microbiol* 1992; 23: 211–6.
21. CARBONARE SB, SILVA MLM, PALMEIRA P, et al. Human colostrum IgA antibodies reacting to enteropathogenic *Escherichia coli* antigens and their persistence in the faeces of a breastfed infant. *J Diarrhoeal Dis Res* 1997; 15: 53–8.
22. COSTA-CARVALHO BT, VIEIRA HMS, CARBONARE SB, et al. Niveles de inmunoglobulinas y lisozimas en sangre de cordón umbilical en recién nacidos de diversas edades gestacionales. *Rev Latinoam Perinatol* 1988; 8: 98–105.
23. COSTA-CARVALHO BT, BERTIPAGLIA A, SOLÉ D, et al. Detection of immunoglobulin (IgG and IgA) anti-outer-membrane proteins of enteropathogenic *Escherichia coli* (EPEC) in saliva, colostrum, breast milk, serum, cord blood and amniotic fluid. Study of inhibition of localized adherence of EPEC to HeLa cells. *Acta Paediatr* 1994; 83: 870–3.
24. DE MORAES-PINTO MI, VERHOEFF F, CHIMSUKU L, et al. Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Arch Dis Child Fetal Neonatal Ed* 1998; 79: F202–5.
25. SIMISTER NE. Placental transport of immunoglobulin G. *Vaccine*. 2003; 21: 3365–9.
26. LEWIS DB, WILSON CB. Developmental immunology and role of host defenses in fetal and neonatal susceptibility to infection. In: REMINGTON JS, KLEIN JO, WILSON CB, BAKER CJ, eds. *Infectious diseases of the fetus and newborn infant*. 6th edn. New York: Elsevier Saunders, 2006: 87–210.
27. AVANZINI MA, PIGNATTI P, CHIRICO G, et al. Placental transfer favors high avidity IgG antibodies. *Acta Paediatr* 1998; 87: 180–5.
28. COSTA-CARVALHO BT, VIEIRA HMS, DIMANTAS RBR, et al. Transfer of IgG subclasses across placenta in term and preterm newborns. *Braz J Med Biol Res* 1996; 29: 201–4.
29. LEMKE H, COUTINHO A, LANGE H. Lamarckian inheritance by somatically acquired maternal IgG phenotypes. *Trends Immunol* 2004; 25: 180–6.
30. CASAS R, BJÖRKSTEN B. Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers. *Pediatr Allergy Immunol* 2001; 12: 59–64.
31. TAYLOR HP, DIMMOCK NJ. Mechanism of neutralization of influenza virus by secretory IgA is different from that of monomeric IgA or IgG. *J Exp Med* 1985; 161: 198–209.
32. MAZANEC MB, NEDRUB JG, KAETZEL CS, et al. A three-tiered view of the role of IgA in mucosal defense. *Immunol Today* 1993; 14: 430–5.
33. NEWBURG DS, RUIZ-PALACIOS GM, MORROW AL. Human milk glycans protect infants against enteric pathogens. *Annu Rev Nutr* 2005; 25: 37–58.
34. KOLB AF. The prospects of modifying the antimicrobial properties of milk. *Biotechnol Adv* 2001; 19: 299–316.
35. PALMEIRA P, CARBONARE SB, AMARAL JA, et al. Colostrum from healthy Brazilian women inhibits adhesion and contains IgA antibodies reactive with Shiga toxin-producing *Escherichia coli*. *Eur J Pediatr* 2005; 164: 37–43.
36. BARROS MD, PORTO MHO, LESER PG, et al. Study of colostrum of a patient with selective IgA deficiency. *Allergol Immunopathol* 1985; 13: 331–4.
37. BITONTI AJ, DUMONT JA, LOW SC, et al. Pulmonary delivery of an erythropoietin Fc fusion protein in non-human primates through an immunoglobulin transport pathway. *Proc Natl Acad Sci USA* 2004; 101: 9763–978.
38. DICKINSON BL, BADIZADEGAN K, WU Z, et al. Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. *J Clin Invest* 1999; 104: 903–11.
39. SPIEKERMANN GM, FINN PW, WARD ES, et al. Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. *J Exp Med* 2002; 196: 303–10.
40. YOSHIDA M, KOBAYASHI K, KUO TT, et al. Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest* 2006; 116: 2142–51.
41. MACLENNAN C, DUNN G, HUISSOON AP, et al. Failure to clear persistent vaccine-derived neurovirulent poliovirus infection in an immunodeficient man. *Lancet* 2004; 363: 1509–13.
42. STRIKER GA, CASANOVA LD, NAGAO AT. Influence of type of delivery on A, G and M immunoglobulin concentration in maternal colostrum. *J Pediatr* 2004; 80: 123–8.