Beatriz Mariana Abramczuk

# Linfócitos T, Células Natural Killer, Atividade Citotóxica e Resposta à Vacina em Lactentes Expostos e Não Infectados pelo Vírus da Imunodeficiência Humana

Orientadora: Profª Drª Maria Marluce dos Santos Vilela

Campinas

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Dissertação de Mestrado apresentada ao Curso de Pós-Graduação em Saúde da Criança e do Adolescente da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do Título de Mestre em Saúde da Criança e do Adolescente, na área de concentração Saúde da Criança e do Adolescente.

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Curso de Pós-Graduação em Saúde da Criança e do Adolescente da Faculdade de Ciências Médicas da Universidade Estadual de Campinas.

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***DEDICATÓRIA***

*A meus pais, André e Paulina, com muito amor e carinho.*

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"Nada é mais digno de nosso patrocínio que o fomento da ciência e da literatura. O conhecimento é, em todo e qualquer país, a base mais segura da felicidade pública."

George Washington

(discurso, 8 de janeiro de 1790.)

# Lista de Abreviaturas e Siglas

ARV: Antiretroviral

AZT : Zidovudina/zidovudine/ azidothymidine

BCG: Bacillus Calmette-Guérin

CM: Complete medium

DTP: Diphtheria-tetanus-pertussis/ difteria-tétano-pertussis

DTxd: Diphtheria toxoid

ENI: Exposto não infectado

*E:T* : Effector to target ratio

Fd: Freedom degree

GMTs: Geometric mean titers

HBsAg: Hepatitis B surface antigen

HEU: HIV-exposed uninfected

Hib: *Haemophilus influenzae* type b

HIV: Vírus da imunodeficiência humana/human immunodeficiency virus

MHC: Complexo principal de histocompatibilidade/major histocompatibility complex

NE : Não exposto/not exposed

NK: Natural killer

PBMC: Peripheral blood mononuclear cells

PI: Propidium iodide

SMX: Sulfametoxazol

TMP: Trimetropin

ToBI: Toxin binding inhibition

TTxd: Tetanus toxoid

# SUMÁRIO

# RESUMO

Uso de antiretrovirais pela gestante, parto cesárea na 38ª semana de gestação, administração intravenosa de zidovudina durante o parto e por via oral para o recém-nascido além de não aleitamento materno são medidas empregadas com sucesso para reduzir a transmissão vertical do HIV. Essas recomendações, associadas ao ambiente intrauterino alterado pela infecção materna, interferem no crescimento e desenvolvimento do feto/embrião podendo levar a disfunção mitocondrial e alterações hematológicas e imunológicas. O presente trabalho incluiu, no estudo referido como capítulo I, 33 lactentes com exposição vertical ao HIV não infectados (ENI) e 47 lactentes não expostos ao vírus (NE) e, no estudo referido no capítulo II, 51 ENI e 112 NE, todos com mediana de idade de 7 meses. Comparamos ENI e NE em relação ao peso de nascimento, à contagem de linfócitos TCD3, TCD4+, TCD8+, CD3-CD16+CD56+ (natural killer), atividade citotóxica de células mononucleares do sangue periférico para células tumorais K562 e resposta humoral para as vacinas hepatite B, difteria e tétano (Instituto Butantan-SP, Brasil). Os resultados mostram baixo peso ao nascimento e reduzida contagem de linfócitos TCD3, TCD4+ e TCD8+ entre os lactentes do grupo ENI. Resultados inéditos desse estudo foram uma reduzida resposta protetora à vacina da hepatite B, títulos baixos de anticorpos IgG para o toxóide tetânico e normais para o toxóide diftérico para os lactentes ENI. Além disso, encontramos para esse grupo uma contagem normal de células natural killer e preservada atividade citotóxica de células mononucleares do sangue periférico. Concluímos que o grupo de lactentes jovens ENI apresenta baixo peso ao nascer e alteração no desenvolvimento da imunidade adaptativa, necessitando de orientação específica para o calendário de vacinação.

# ABSTRACT

Use of antiretroviral drugs by the pregnant woman, cesarean delivery at 38 weeks of gestation, intravenous zidovudine during delivery and orally to the newborn, in addition to not breastfeeding, are recommendations used successfully to reduce vertical transmission of HIV. These recommendations, coupled with the intrauterine environment altered by maternal infection, interfere with growth and development of the fetus/ embryo and may lead to mitochondrial dysfunction and hematological and immunological changes. In the study referred to as Chapter I were included 33 HIV-exposed uninfected infants (HEU) and 47 healthy infants not exposed to the virus (NE) and in the study referred to as Chapter II, 51 ENI and 112 NE, all of them with median age of 7 months. We compared HEU and NE with respect to birth weight, TCD3 lymphocyte, CD4+, CD8+ and CD3-CD16+CD56+ (natural killer) counts, cytotoxic activity of peripheral blood mononuclear cells against tumor cells K562 and humoral response to hepatitis B, diphtheria and tetanus vaccine (Instituto Butantan, SP, Brazil). The results show low birth weight and reduced lymphocyte count TCD3, CD4 + and CD8 + among infants of the HEU group. Inedited results of this study were a reduced protective response to hepatitis B vaccine, lower anti-tetanus titres of and normal anti-diphtheria titres for the HEU infants. Furthermore, we observed to the HEU group a normal count of natural killer cells and preserved cytotoxic activity of the peripheral blood mononuclear cells. We conclude that the group of HEU young infants has a low birth weight and changes in the development of adaptive immunity, requiring specific guidance for the vaccination schedule.

# 1- INTRODUÇÃO

O sucesso da gestação é considerado um enigma imunológico, envolvendo uma comunicação bidirecional determinada por um lado pela apresentação de antígenos fetais e por outro lado pelo reconhecimento e reação a esses antígenos pelo sistema imune materno (PANDEY *et al.* 2004).

O primeiro confronto do feto com o sistema imune materno ocorre no momento da implantação, quando células trofoblásticas do blastocisto penetram a membrana basal do epitélio uterino e invadem o estroma endometrial (HOUWERT-DE JONG *et al.* 1989). Isto conduz a um dos maiores paradoxos imunológicos: a sobrevida do feto que possui antígenos de histocompatibilidade (MHC) de origem paterna (SUTTON *et al.* 1986).

A invasão do trofoblasto na mucosa uterina materna (decídua) assegura a ancoragem da placenta na parede uterina e o acesso ao sistema vascular materno para garantir suprimento de oxigênio e nutrientes. No começo do segundo trimestre o sangue materno começa a perfundir o espaço intraviloso e as células do trofoblasto que se desprendem dos vilos da placenta podem ser encontradas no sangue materno. Com isso, o contato local inicial entre células fetais e a decídua materna é expandido para o corpo inteiro e todo o sistema imune materno entra em contato com os imunógenos fetais. A capacidade responsiva materna, ao contrário de ter conseqüências negativas, serve para limitar a invasão do trofoblasto, garantindo a integridade do corpo materno (RANGO 2008).

O sistema imune fetal atua no sentido de evitar respostas pró-inflamatórias prejudiciais que possam induzir reações aloimunes entre mãe e feto, proteger contra infecções bacterianas e virais na interface materno-fetal e mediar uma transição entre um

ambiente intra-uterino estéril para um ambiente externo rico em antígenos. Essa transição é acompanhada por uma maturação gradual do sistema imune (LEVY 2007).

Nos primeiros meses de vida, destaca-se a atuação do sistema imune inato, com a participação de células natural killer (NK) nas respostas antivirais através de mecanismos citolíticos dependentes de contato, como também através da secreção de citocinas. A atividade de citotoxicidade de células NK atinge competência funcional entre o 1° e o 5º mês de vida, representando uma importante defesa antes da geração de anticorpos específicos e células TCD8+ citotóxicas (YABUHARA 1990, O´CONNOR 2005).

Por causa da imaturidade do sistema imune, recém nascidos e lactentes são vítimas de muitas infecções microbianas, resultando em milhões de mortes no mundo todo, sendo importante uma vacinação efetiva para conferir proteção a eles (DEMIRIJIAN & LEVY 2009).

Entretanto, a imunização no início da vida constitui um desafio por diversos motivos, destacando-se a presença de anticorpos maternos potencialmente inibitórios e a própria imaturidade dos leucócitos neonatais, especialmente dos linfócitos T (KOVARIK 1998, ADKINS 1999). Os neonatos têm resposta pobre a antígenos polissacárides T independentes e produzem anticorpos em menor quantidade e menos persistentes em resposta a antígenos protéicos T dependentes (SIEGRIST 2007).

A infecção pelo vírus da imunodeficiência humana (HIV) na criança compromete ainda mais as respostas a antígenos T dependentes. As crianças infectadas apresentam menor índice de proteção sorológica para hepatite B, menores títulos de anticorpos para sarampo e tétano (MOSS *et al.* 2003, MELVIN & MOHAN 2003) e perda de células T efetoras e de memória para as vacinas da varíola e de Bacillus Calmette-Guérin (BCG)

(PUISSANT-LUBRANO 2009). Devido ao risco que as crianças infectadas pelo HIV possuem de disseminação do BCG a partir da vacinação e também pela menor resposta protetora que desenvolvem, é proposto que os recém-nascidos de mães infectadas pelo HIV sejam vacinados apenas após a definição do estado de não infecção. O problema é que na maioria dos países em desenvolvimento, não há garantia de que as crianças vão retornar à clínica para fazer o teste para infecção pelo HIV, ou ele pode nem estar disponível (MANSOOR *et al.* 2009).

Em países desenvolvidos, a profilaxia antiretroviral (ARV) diminuiu drasticamente a transmissão perinatal do HIV, porém os efeitos no desenvolvimento do sistema imune fetal e neonatal não são completamente conhecidos (MOFENSON & MUNDERI 2002).

No Brasil, estratégias para o tratamento da infecção pelo HIV e AIDS datam de 1980s e a terapia ARV tornou-se disponível no Serviço de Saúde Pública em 1991. O Brasil foi o primeiro país em desenvolvimento a adotar uma política de distribuição gratuita de drogas ARV (BRITO *et al.* 2005). A política preventiva para a transmissão vertical do HIV no país inclui a introdução em 1995 de ARV para as gestantes infectadas pelo vírus e a administração de zidovudina (AZT) durante o segundo e terceiro trimestre gestacional. Parto cesárea na 38ª semana de gestação e administração de AZT no momento do parto são também recomendados (CDC 1994, VERMELHO *et al*. 1999, MINISTÉRIO DA SAÚDE 2009A).

No estado de São Paulo temos vivenciado quatro fases distintas de profilaxia da transmissão vertical de HIV: a primeira fase foi de 1990 a 1994, quando não se dispunha de ARV para profilaxia, sendo apenas contra-indicado o aleitamento materno; a segunda fase, de 1995 a 1996, quando se iniciou o uso de ARV oral para a gestante e o recém-nascido; a

terceira fase, de 1997 a 1998, período da utilização do ARV na gestação, no trabalho de parto, no parto e pelo recém-nascido; e a quarta fase, de 1999 a 2000, quando se introduziu ARV em esquema duplo ou triplo para as gestantes, associado à cesárea eletiva. Houve redução na transmissão vertical da primeira até a quarta fase, de 32,3 para 2,9%. A maior queda, observada na terceira fase, ocorreu após a introdução do esquema completo do Aids Clinical Trials Group Ptotocol 076 (ACTG 076) (AMARAL *et al*. 2007).

Tem sido proposto que, na gestante, a infecção pelo HIV e os medicamentos ARV têm um potencial modulatório no padrão de citocinas da placenta, modificando o ambiente intrauterino normal (FAYE *et al*. 2007). Com a crescente expansão da profilaxia com ARV para gestantes infectadas pelo HIV, a exposição às drogas ARV também vai aumentar, o que requer uma vigilância continua sobre os seus efeitos tanto na gestante quanto na criança (MOFENSON & MUNDERI 2002).

O recém nascido de mãe infectada pelo HIV recebe AZT durante as seis primeiras semanas de vida e Sulfametoxazol (SMX) + Trimetropin (TMP) para profilaxia para pneumonia pelo *Pneumocystis jiroveci* da sexta semana até a definição de seu estado de infecção (MINISTÉRIO DA SAÚDE 2009B).

O diagnóstico de AIDS para crianças acima de 18 meses, é feito a partir de duas sorologias para HIV (MINISTÉRIO DA SAÚDE 2009B). O teste sorológico não é recomendado para diagnóstico de crianças abaixo de 18 meses por causa da transferência placentária de anticorpos maternos IgG anti-HIV, os quais decaem nas crianças em média aos 12 meses de idade (READ 2007). Assim, para essa faixa etária o diagnóstico é baseado na determinação da carga de RNA ou DNA viral em duas amostras obtidas com intervalo mínimo de um mês, sendo que duas cargas virais positivas indicam infecção e duas

negativas, provável não infecção, sendo necessária complementação com teste sorológico após o primeiro ano de vida (MINISTÉRIO DA SAÚDE 2009B).

A Organização Mundial da Saúde recomenda a vacinação dos recém nascidos de mães infectadas pelo HIV (MOSS *et al.* 2003), mas não considera as interferências a que o sistema imune em desenvolvimento esteve submetido: transferência materna de IgG anti-HIV e drogas ARV, o não aleitamento materno e o uso de drogas profiláticas mielotóxicas como SMX-TMP e AZT. Apesar da barreira placentária, diversos estudos mostram a presença do HIV no tecido fetal oriundo de aborto espontâneo ou eletivo, além da detecção de resposta específica para o HIV no cordão umbilical, sugerindo que o vírus atravessa a placenta durante a gestação, podendo interferir com o desenvolvimento do sistema imune fetal (KUHN *et al*.2002).

Os recém nascidos de mães com infecção pelo HIV apresentam disfunção mitocondrial (BARRET 2003) e diversas alterações hematológicas (CONNOR *et al*. 1994, SILVA *et al*. 2001, LE CHENADEC *et al*. 2003, FEITERNA-SPERLING *et al*. 2007). No cordão umbilical de neonatos com exposição vertical a HIV, a produção de interleucina-12 é deficiente (CHOUGNET *et al*.2000) e os linfócitos T são mais susceptíveis à apoptose (ECONOMIDES *et al*. 1998) Em crianças expostas ao HIV e não infectadas são relatadas alterações no número absoluto de linfócitos T e das subpopulações TCD4+ e TCD8+, além de uma maior resposta imunossupressora a antígenos policlonais para células T (CLERICI *et al*. 2000, BUNDERS *et al*. 2005, ONO *et al*. 2008, HYGINO *et al*. 2008). Essas crianças também apresentam uma maior ativação imune, possivelmente resultante de uma exposição intra-uterina a antígenos do HIV e/ou exposição a outros antígenos relacionados a infecções oportunistas nas gestantes infectadas pelo HIV (RICH *et al*. 1997).

Apenas mais recentemente os estudos têm explorando a influência do ambiente materno com infecção pelo HIV no desenvolvimento e função de outros componentes do sistema imune, tendo sido observado que a exposição intrauterina ao vírus induz mudanças quantitativas e qualitativas nas células dendríticas dos neonatos (VELILLA *et al*. 2008). Estudos sobre a resposta imune inata e sobre a resposta imunológica à vacinação dos lactentes com exposição ao HIV são raros, especialmente nos casos em que os indivíduos não são infectados.

# 2- OBJETIVOS

O objetivo desta dissertação foi avaliar nos lactentes com exposição vertical a HIV e não infectados e comparar com os lactentes normais não expostos ao vírus:

1. Peso ao nascer
2. A contagem de linfócitos T e das subpopulações TCD4 e TCD8.
3. A contagem de células natural killer.
4. A atividade citotóxica de células mononucleares do sangue periférico.
5. O título sérico de anticorpos IgG às vacinas de hepatite B, tétano e difteria.

# 3- CAPÍTULO I

# T LYMPHOCYTES, NK CELLS AND CYTOTOXICITY ACTIVITY IN HIV-EXPOSED UNINFECTED BRAZILIAN INFANTS

Submetido ao Journal of Tropical Pediatrics

**T lymphocytes, NK cells and Cytotoxicity Activity in HIV-Exposed Uninfected Brazilian Infants**

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**SUMMARY**

With the implementation of antiretroviral (ARV) therapy for HIV-1 infected pregnant women, the mother-to-child transmission had drastically decreased. However, the intra uterine exposure to HIV particles and ARV drugs are known to interfere with the offspring´s development. We studied 33 HIV-exposed uninfected (HEU) Brazilian infants and 47 healthy infants not exposed to the virus (NE), with median age of seven month. We compared both groups with respect to birth weight, TCD3+, TCD4+, TCD8+ lymphocytes and CD3-CD16+CD56+ (natural killer) counts and cytotoxic activity of peripheral blood mononuclear cells against tumor cells K562. We evaluated the effect of the duration of the mother´s ARV therapy in prenatal on the cytotoxic activity of the HEU infants. These infants presented lower birth weight, diminished counts of TCD3+, TCD4+ and TCD8+ lymphocytes and higher CD4/CD8 ratio. The natural killer cell counts and cytotoxicity activity were similar in both groups and it was not affected by the duration of the mother´s ARV therapy. Despite the lower birth weight and the diminished T lymphocytes, our result shows that the intrauterine exposition to HIV and to ARV drugs does not affect the natural killer cell number neither the cytotoxicity activity in the infants.

**Keywords:** cytotoxicity test, HIV, infant, T lymphocytes, natural killer.

## INTRODUCTION

The immune system undergoes a dramatic transition at birth, from a sheltered intra-uterine environment to a distinct environment of the outside world. This transition is followed by a gradual, age-dependent maturation [1].

The innate immune system is particularly important in the first months of life [2]. Natural killer (NK) cells are bone marrow derived and account for 2 to 18% from the peripheral lymphocytes [3]. NK have the capacity to instantly eliminate virally infected and transformed cells without clonal expansion. NK can also influence an adaptive immune response via interaction with dendritic cell and through cytokine secretion [4]. The NK role in the resistance and control of HIV infection is currently unclear [5-8].

The intrauterine exposure to HIV and antiretroviral (ARV) drugs leads to mitochondrial dysfunctions [9], abnormalities in hematological features [10-12] and in the maturation of T lymphocytes [13-18]. Few data exist about NK cells and cytotoxicity activity in HIV-exposed individuals, and they are all focused on adults [6-8].

The present study investigated T lymphocytes, NK and cytotoxicity activity of peripheral blood mononuclear cells from HIV-exposed uninfected (HEU) Brazilian infants and age matched controls infants not exposed (NE) to the HIV. We also evaluate the effect of the mother´s ARV therapy on the cytotoxicity activity.

## METHODS

### Study population

Between January and December 2007, 37 individuals were born from HIV-infected mothers in the University of Campinas (UNICAMP) Medical School Hospital. 33 of these infants, followed at the Pediatrics Immunodeficiency Out-patients Unit, and 47 NE infants recruited in Campinas Public Health Centers, participated in the study. In the HEU group, we studied the subjects when their diagnosis was defined as not infected according to the criteria of the Centers for Disease Control and Prevention experts' group (CDC) [19]. The gestational age and birth weight were accessed in the medical records.

The study was approved by the Committee for Ethics in Research from UNICAMP, São Paulo, Brazil.

### Peripheral blood counts and immunophenotyping

Hemoglobin concentration, hematocrit and counts of whole leukocytes, lymphocytes and red cells were performed in the SE9500 or XE 2100 blood cell counters (Sysmecs Corporation, Japan).

Samples of whole blood were incubated with the anti-human CD3/CD4/CD8 and CD3 /CD16/CD56 fluorescent conjugated monoclonal Abs (Beckman Coulter, USA) for 20 minutes at room temperature. The red blood cells were lysed with ammonium chloride solution ($NH_4Cl$ 0.15M, $KHCO_3$ 10mM, EDTA 4Na 37 mg/L) and washed twice with phosphate buffered saline. Data were acquired until 10.000 events in the CD3 gate (Epics XL-MCL flow cytometer; Beckman-Coulter, USA) and analyzed (Expo software; Beckman Coulter, USA). Isotype controls were used to discriminate specific antibody staining.

**Isolation of mononuclear cells**

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by Ficoll-Hypaque (Amershan Biosciences, USA) density gradient sedimentation according to the manufactorer´s instruction and diluted to $5x10^6$ cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2mM L-glutamine, 100U/ml penicilin and 100µg/ml streptomycin (complete medium, CM)

**Target cells staining**

K562, human chronic myelogenous leukemia cells, were cultured in CM at 37°C in a 5% $CO_2$ atmosphere. Target cells were harvested in the log phase of growth, washed in RPMI 1640, diluted to $5x10^6$ cells/ml in 500µL of diluent A and labeled with 4,4µL $PKH_2$ (PKH2 Green Fluorescent Cell Linker Kit for General Cell Membrane Labeling, Sigma, USA). The cells were washed and diluted in CM ($1x10^5$ cells/ml).

**Cytotoxicicity activity assay**

The assay was performed as previously described [20-22]. Cells were co-incubated at effector to target (*E:T*) ratios of 12.5:1 , 25:1 and 50:1 in a total volume of 200µl in polystyrene tubes for 2h at 37°C in a 5% $CO_2$ atmosphere. Propidium iodide (PI) (20µl/ml) (Sigma, USA) was added to every tube and the samples were acquired (Epics XL-MCL flow cytometer; Beckman-Coulter) until 10.000 events in the PKH2 positive region (fig.1). Target cells were incubated alone in CM to measure spontaneous cell death.

The cytotoxicicity activity was calculated by the equation:

% specific cell death =

(% $PI^+$ (dead) targets - % spontaneous $PI^+$ (dead) targets)/ (100-% spontaneous $PI^+$ (dead) targets) x100

**ARV group division**

The study had access to the ARV prophylaxis status during prenatal from 29 of the HEU infants included in the cytotoxic assay. Two mothers received no ARV drugs during pregnancy and five ones received ARV combination without protease inhibitors. The HEU infants were sorted in groups according with the duration of mother's ARV therapy and tested for cytotoxicity activity.

**Statistical analysis**

Statistical analysis was performed using a SPSS software version 7.5.1 (SPSS Inc., Chicago, IL, USA). Data were expressed as medians and extremes and significance level was considered for $p<0.05$. Comparison of variables between groups was performed by Mann-Whitney test and within groups by Friedman test. Correlations were evaluated by Spearman test. The $33^{th}$ and $66^{th}$ percentiles of mother's ARV therapy duration were used to separate the HEU infants into groups and the statistical analysis was performed with Kruskal Wallis test. Prism software (version 4.0; GraphPad Software) was employed for the figures. The z-score was calculated for the anthropometric profiles in the time of the blood collection with an anthropometric calculator (WHO Anthro v 3.0.1).

## RESULTS

### Study population

The HEU group was compound of 18 males and 15 females, with ages varying from 6.6 to 10.8 months (median 7.6), birth weight from 1300 to 3430g (median 2740g) and gestational age from 31.4 to 40.3 weeks (median 37.4). The median gestational age for the 26 full term infants of the group was 37.6 weeks (37.0-40.3) and for the seven preterm (gestational period < 37 weeks) it was 35.5 weeks (31.4-36.3).

In the time of the assay, among the HEU preterm infants, one had low height-for-age and low weight-for-age, one had low height-for-age and was overweight for the height and one had only low height-for-age. Among the HEU term infants, two had only low height-for-age, two were overweight for the height and had high mass index body- for-age and one had low height-for-age and was overweight for the height.

Six (18.18%) of the HEU infants were delivered vaginally. All the newborns received azidothymidine (AZT) for 40 days and Trimethoprim (TMP) –Sulfamethoxazole (SMX) for *Pneumocystis jiroveci* prophylaxis. In the time of the assay, 14 (42.42%) infants were still taking TMP-SMX.

In the NE group there were 22 male and 25 female infants with ages varying from 7.1 to 10.8 months (median 7.7). The median birth weight was 3230g (2170-4495) and the gestational age was 39.0 weeks (37.0-41.1). All of them were full term infants.

The median birth weight and gestational age were different between NE and HEU infants (Mann-Whitney test, p=0<0.001) even when the preterm infants were not included (Mann-Whitney test, p=0.037).

**Peripheral blood counts and immunophenotyping**

The hematological and immunological aspects from the HEU and from NE infants are contained in Table I and II, respectively. The groups showed differences in the numbers (relative and absolute) of T lymphocytes and in the subsets TCD4+ and TCD8+, but not in the CD3-CD16+56+ cells (fig.2). The groups did not differ in the hematological features, but in the HEU group, five infants had previous history of anemia (hemoglobin level below 9.0g/dl) treated with ferrous sulfate and one infant had anemia in the time of the study.

**Cytotoxicity activity assay**

The percentages of specific target cells death for the HEU and NE infants are presented in Table III. The cytotoxicity activity for the HEU preterm infants did not differ from that one for the full term infants (Mann-Whitney test, $p>0.05$ for all *E:T* ratio), so they were grouped together to compare with the NE infants. There was no difference in the cytotoxicity activity between the HEU and the NE (Mann-Whitney test, $p>0.05$) (fig. 3).

The cytotoxicity activity of the HEU infants was not correlated to CD8+ T cell numbers neither to NK absolute number for the 12.5:1 and 50:1 (*E:T*) cell ratio (Spearman´s test, $p>0.05$). There was correlation between relative number of NK cell and cytotoxicity activity for the 25:1 (*E:T*) cell ratio (Spearman´s test, $p=0.032$). The duration of mother's ARV therapy did not affect the cytotoxicity activity (fig 4) (Kruskal wallis test, $p>0.05$).

## DISCUSSION

We observed lower birth weight in the HEU group when compared to the NE. Low birth weight in newborns from HIV-positive mothers has been extensively reported and it seems to be associated with clinical, behavioral, psychosocial and demographic factors, such as ethnicity, no ARV treatment in the past or smoking or illicit substances during pregnancy [22-24]. In the HEU neonates, lower birth weight and lower gestational age can also be attributed to the recommendation of elective cesarean for this group [18].

The placental transfer of HIV proteins and the ARV drugs used in vertical transmission prevention have been considered potential factors in causing alterations in haematopoietic parameters [10-12]. The mother´s ARV prophylaxis is related to a more profound anemia in the newborn [12], but not with intrauterine growth retardation [25]. At the time of the study we did not find alterations in the hematological aspects of the HEU infants, probably because the HEU infants that previously had anemia were treated with ferrous sulfate.

Our data show that, at $7^{th}$ month of age, the absolute and relative numbers of TCD3+, TCD4+ and TCD8+ lymphocytes are diminished in HEU infants compared to NE infants. Diminished TCD4+ was also found by Clerici et al [14] in the HEU newborn and by Gesner et al [13] in 6-month-old HEU infants, but Clerici et al [14] reported increased TCD8+ in the newborn and Gesner et al [13] did not observe difference in TCD8+. Ono et al [18] reported a trend to higher numbers of TCD3+ and TCD4+ in 12-month-old HEU infants, although without a significant difference compared to the controls. A European Collaborative Study [16] showed that TCD4+ counts below the cut-off to moderate immunodeficiency and to severe immunodeficiency, would have

occurred in an estimated 18% and 8% of at 1-year-old HEU infants, respectively. An estimated cumulative 14% of infants would have had an event below the TCD8+ cut-off.

In normal children, the absolute numbers of total lymphocytes, TCD3+ and TCD4+ increase immediately after birth, remain relatively stable until 2 years of age and gradually decrease to adult levels. However, the number of TCD8+ lymphocytes remains stable from birth up to 2 years of age, followed by a gradual decrease toward adult levels. The percentage of TCD3+, TCD4+ and TCD8+ lymphocytes shows a slight fluctuation, while the CD4/CD8 ratio has lower median values (1.0 to 2.0) at birth and higher values (2.0 to 3.0) during the first 2 years of life [26, 27]. We observed a median CD4/CD8 ratio of 3.1 for the HEU infants.

The reduction in T subsets for the HEU infants and no alteration in total lymphocytes can be explained by the possible presence of immature lymphocytes [14]. Because of the limited amount of blood collected from the infants, the B cell immunophenotyping could not be included.

We observed no alterations in the NK values and cytotoxicity activity of the 7-month-old HEU infants. A similar result was found by Clerici et al [14] for the newborn from HIV-infected mothers. However, Ono et al [18] observed higher absolute and relative numbers of these cells in 12- month-old HEU infants.

In healthy children, the absolute number of NK cells dramatically decreases during the first 2 months of life and thereafter remains stable. The percentage of NK cells declines immediately after birth, followed by a slow increase at adult age [2, 26, 27].

Around 90% of peripheral blood and spleen NK cells are $CD3^{-}CD16^{+}CD56^{dim}$ and are cytotoxic. In contrast, most NK cells in lymph nodes and tonsils are CD3-

$CD16^{+}CD56^{bright}$ and lack cytotoxic function [3]. The cytolytic capacity results from the release of granzymes and perforin in response to a net of signals. The acquisition of this function is closely related to the expression of inhibitory receptors that recognize self-MHC, providing a mechanism of tolerance. However, the precise transition of non-cytolytic cells to cytolytic cells is not yet completely established [28]. The cytotoxicity function becomes competent between 1 and 5 months of age and reaches complete maturity by the age of four [2].

HIV infection is associated with a decrease of peripheral $CD3\text{-}CD16^{+}CD56^{bright}$ [29] and emergence of $CD3^{-}CD16^{+}CD56^{-}$ [30]. In perinatally HIV-infected children, the NK cells have lower levels of degranulation marker CD107 and increased expression of receptors correlated with CD4+ T lymphocyte depletion [31].

Exposure to HIV does not inevitably lead to infection [32] and there is growing evidence that NK function is one of the multiple factors involved in this resistance [6, 7, 8]. The NK cells from uninfected intravascular drug users, compared to controls not exposed to HIV or with drug users that became HIV-infected, show a higher constitutive degranulation potential in the absence of exogenous stimulation [8], increased *in vitro* NK activity against tumoral cells and production of the chemokines CCL3, CCL4 e CCL5 [6]. Among HIV-exposed individuals by sexual intercourse, the uninfected ones have a higher percentage of NK cells in comparison to the infected ones and increased stimulated production of the IFN-γ than the non exposed [7]. This resistance is attributed to genetic variants of HLA molecules with a potential lower affinity for their inhibitory NK cell receptors [38].

In the intrauterine exposure to HIV, NK cytokines production seems to have a relevant role in the prediction of the infants that will become infected. Tiemessen et al [39]

reported detectable production of IFN-γ and IL-2 in HEU infants and their mothers. However, this response was not detected in uninfected mothers and in their infants, as well as HIV-infected infants and mothers, suggesting that IFN-γ and IL-2 are a protective cytokine response.

Unlike what is reported for adults, we observed no alterations in NK and cytotoxicity activity for the HEU infants. Despite the alterations in T lymphocytes, the exposure to HIV and the ARV therapy seems not to disrupt this important mechanism of antiviral defenses in the infants.

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**FIGURES**

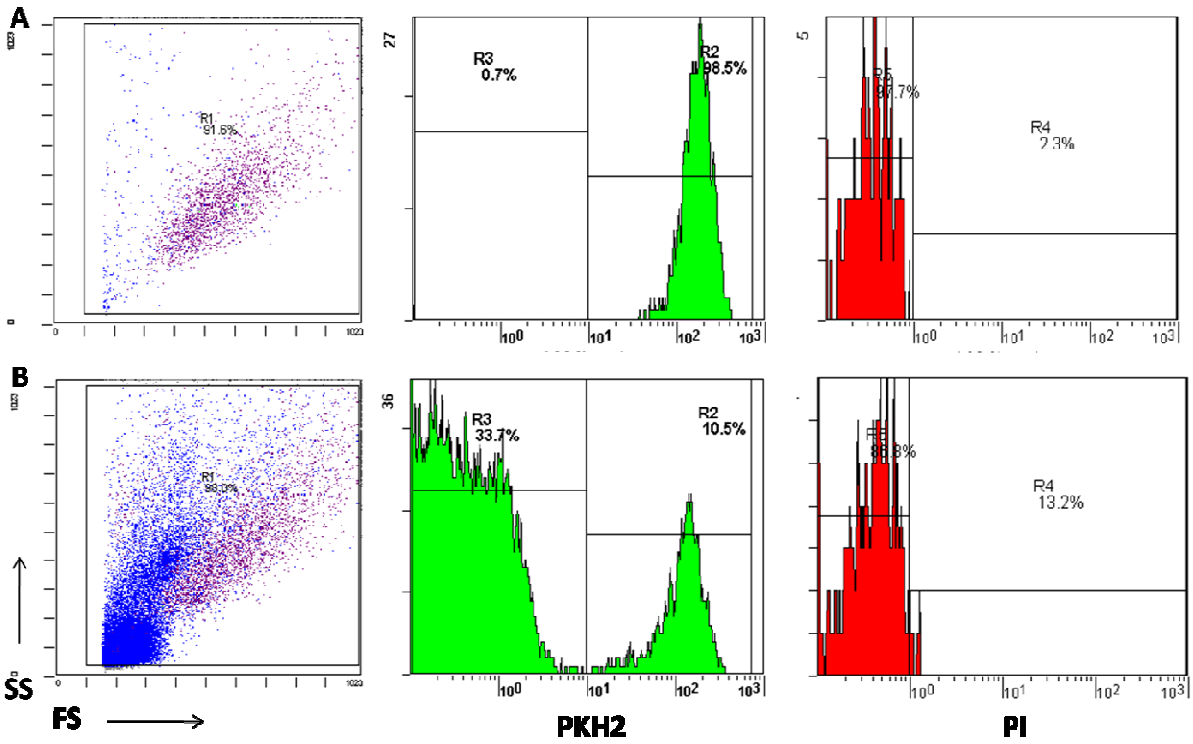


**Fig 1.** Aquisition of cytotoxicity activity: target cells incubated alone (A) and with effector cells (B). Forward and Side Scatters (FS and SS) were used to gate on target and effector cells, from which PKH2-target cells were separated from effector cells. The dead cells were identified (cells labeled with propidium iodide) from the gate of PKH2 positive cells.

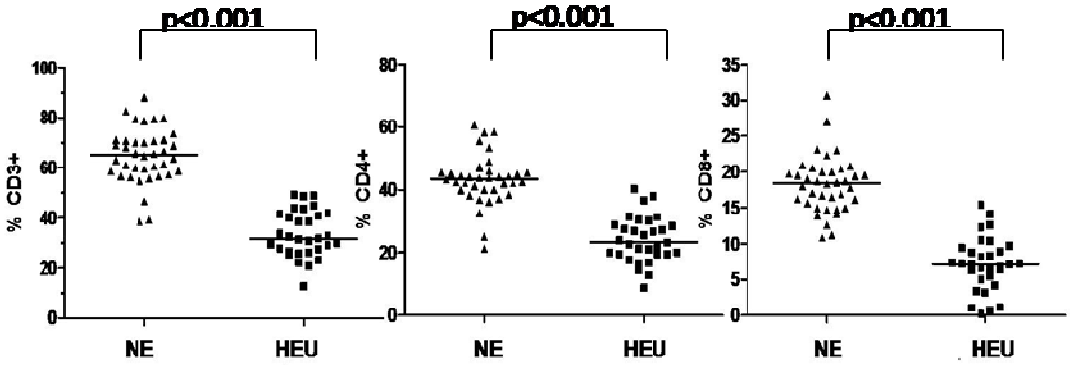


**Fig 2.** Distribution of the relative numbers of TCD3+ cells and the subsets CD4+ and CD8+ T cells for the HIV-exposed uninfected (HEU) infants and for the control infants not exposed to HIV.

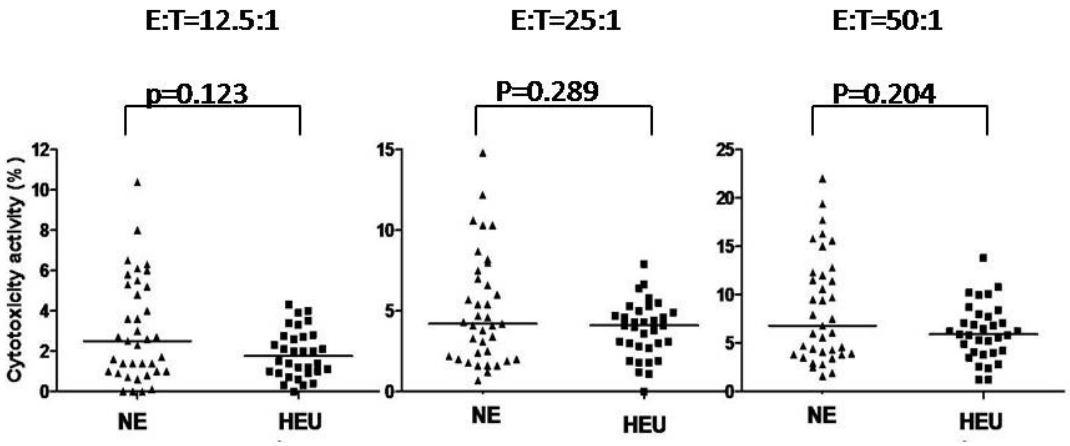


**Fig 3.** Distribution of the percentage of specific target cell death for the HIV-exposed uninfected (HEU) infants and for the control infants not exposed to HIV, with three effector:target cells (E:T) ratios.

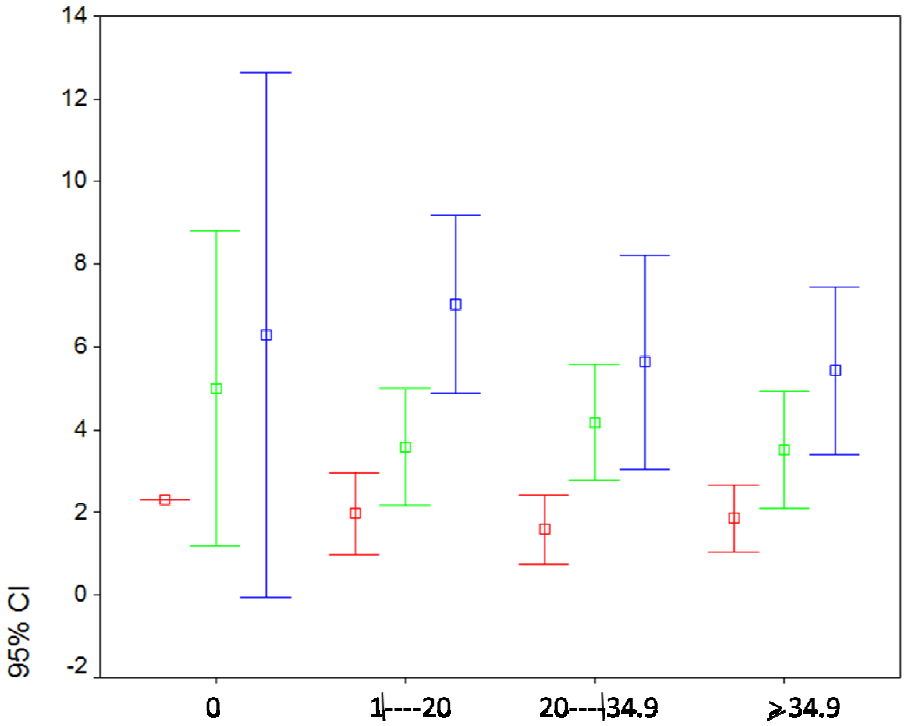


**Fig 4.** Error bar of the cytotoxicity activity (%) for the groups of HIV exposed uninfected infants, sorted according to the duration in weeks of the mother's ARV therapy in the prenatal period: 0 weeks (n=2), 1 to 20 weeks (n=11), 20 to 34.91 weeks (n=9) and more than 34.91 weeks (n=7). Values for the effector:target cells (E:T) ratios: 12.5:1 (red), 25:1 (green) and 50:1 (blue).

**Table I.** Hematological features of control infants not exposed (NE) to HIV and of HIV-exposed uninfected infants (HEU).

| | WBC ($x10^3/mm^3$) | Lymphocytes ($x10^3/mm^3$) | RBC ($x10^6/mm^3$) | Hct (%) | Mcv (fl) | Hemoglobin (g/dl) |
|---|---|---|---|---|---|---|
| NE | 11.05 | 7.4 | 4.65 | 34.8 | 74.55 | 11.35 |
| (n=38) | (5.86-20.40) | (5.12-13.02) | (4.10-6.05) | (29.3-41.7) | (59.20-82.30) | (9.6-14.2) |
| HEU | 11.45 | 7.56 | 4.64 | 35.9 | 75.9 | 11.8 |
| (n=28) | (6.70-20.20) | (4.28-14.5) | (4.08-5.99) | (27.0-39.1) | (49.90-88.00) | (8.5-13.4) |
| P(*) | 0.554 | 0.943 | 0.844 | 0.247 | 0.180 | 0.488 |

Values are presented as medians (minimum and maximum). WBC= white blood cells; RBC= red blood cells; HCT=hematocrit; MCV= mean corpuscular volume.
(*) Mann-Whitney test.

**Table II.** NK cell, T cell, CD3+ and CD 4+ T cell subset relative and absolute numbers of peripheral blood lymphocytes and CD4/CD8 ratios for control infants not exposed (NE) to HIV and for HIV-exposed uninfected infants (HEU).

| | CD3-CD16+CD56+ | | CD3+ | | CD3+CD4+ | | CD3+CD8+ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Relative[1] | Absolute[2] | Relative[1] | Absolute[2] | Relative[1] | Absolute[2] | Relative[1] | Absolute[2] | Ratio 4/8 |
| NE | N=9 | N=9 | N=38 | N=29 | N=38 | N=29 | N=38 | N=29 | N=38 |
| | 9.0 | 0.61 | 65.2 | 4.74 | 43.4 | 3.24 | 18.4 | 1.30 | 2.4 |
| | (5.5-16.3) | (0.34-1.3) | (38.5-88.3) | (2.10-9.14) | (21.3-60.7) | (1.25-5.53) | (10.8-30.6) | (0.65-2.60) | (1.4-4.3) |
| HEU | N=33 | N=27 | N=30 | N=25 | N=30 | N=25 | N=30 | N=25 | N=30 |
| | 7.2 | 0.46 | 32.0 | 2.41 | 22.1 | 1.65 | 7.1 | 0.55 | 3.1 |
| | (2.7-15.7) | (0.21-2.28) | (12.8-49.2) | (1.11-4.62) | (8.7-40.2) | (0.63-3.82) | (1.0-15.4) | (0.05-1.47) | (1.4-19.3) |
| p(*) | 0.198 | 0.139 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.003 |

Values are presented as medians (minimum and maximum).
(1)Percentages of the cells expressing the indicated markers for the lymphocyte population presented.
(2) Subset counts expressed as number of cells per microliter x $10^3$.
(*) Mann-Whitney test.

**Table III.** Cytotoxicity activity for the HIV-exposed uninfected (HEU) infants and for the control infants not exposed (NE) to the virus, with three effector:target cells (E:T) ratios

| Groups | E:T | | | p(1) |
|---|---|---|---|---|
| | 12.5:1 | 25:1 | 50:1 | |
| HEU N=32 | 1.75 [0 – 4.3] | 4.05 [0 – 7.9] | 5.85 [1.2 – 13.8] | < 0.001 |
| NE N=35 | 2.50 [0 – 10.4] | 4.2 [0.7 – 14.8] | 6.8 [1.6 – 22.0] | < 0.001 |
| p(2) | 0.123 | 0.289 | 0.204 | |

Values are percentage of specific target cell death presented as medians (minimum and maximum).

(1) Friedman test; (2) Mann-Whitney test

# 4- CAPÍTULO II

**IMPAIRED VACCINE HUMORAL RESPONSE AMONG HIV-EXPOSED UNINFECTED INFANTS**

**Revista a ser submetido:** Clinical and Vaccine Immunology

# IMPAIRED VACCINE HUMORAL RESPONSE AMONG HIV-EXPOSED UNINFECTED INFANTS

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**SUMMARY**

The World Health Organization recommends vaccination for the newborns from HIV-infected mothers. Most of the vaccines are known to be safe to the HIV-exposed infants, but they are not always effective, especially to the infants that become infected. Little is known about its effectiveness for the HIV-exposed infants that do not become infected. We evaluate the antibody response to hepatitis B, tetanus and diphtheria vaccine in vertically HIV-exposed uninfected infants and compared with control infants not exposed to the virus. Quantitative determination of anti-HBs (mIU/ml) was performed blindly on serum samples using microparticle enzyme immunoassay (MEIA) from Axsym Ausab®. Reliability of the measurements was assessed by the intra-class correlation coefficient (ICC) stratified by sample origin and include lower and upper limits. Specific neutralizing antibodies against diphtheria and tetanus were evaluated in sera by a modified toxin binding inhibition (ToBI) test. 6.7% of the HIV-exposed uninfected individuals were non-responders (anti-HBs < 10 mIU/mL) and 64.4% were very good responders (anti-HBs ≥ 1000 mIU/mL), whereas only 3.6% of the not exposed infants were non-responders ($\chi^2$ = 10.93, fd= 1). The HIV-exposed uninfected infants presented protective titres for diphtheria and tetanus although lower geometric mean anti-tetanus titres was observed. Our data point to the necessity to evaluate vaccine immune response in these children and reinforced that the alterations in lymphocytes reported for the newborns from HIV-infected mothers interfere with the effectiveness of vaccination.

**Keywords:** HIV, infants, hepatitis B vaccine, DTP vaccine

## INTRODUCTION

The World Health Organization (WHO) recommends vaccination for the newborns from HIV-infected mothers [1], especially to prevent opportunistic infections. HIV-infected women co-infected with opportunistic pathogens might be more likely than uninfected women to transmit these infections to their infants [2]. The infants born from HIV-infected mothers have an increased morbidity and mortality than the infants born from healthy mothers [3].

The current WHO guidelines for immunizing children known to have HIV infection and infants born to HIV-infected women differ only slightly from the general guidelines for other infants. Serious complications resulting from immunization with live vaccines in patients infected by HIV have been described with Bacillus Calmette-Guérin (BCG), oral polio and measles vaccines [4].

In general, routine vaccination is safe and efficient when applied to children without immunosuppression [5], but not always effective [1]. The vaccination program for the HIV-exposed infants should consider that the development of their immune system was under unusual conditions generated by the maternal infection. Proteins from HIV are able to cross the placental barrier and cause a state of immune activation in the offspring [5]. The ARV drugs used in the prophylaxis of HIV-infected pregnant women have been suggested to act as a potential cytokine expression modulation, causing changes in the normal placental environment [6].

The intra-uterine exposure to HIV and ARV interfere with the thymic maturation pathway and selection of T lymphocytes, resulting in defects in CD4+ and CD8+ lymphocytes and a peripheral increase in immature T lymphocytes that can persist until

seven years old in the HIV-exposed uninfected (HEU) children [7,8]. The newborns from HIV-infected mothers also present enhanced expression of CD40L on activated T lymphocytes [9] and alterations in dendritic cells [10] that may interfere with the antigen presentation and immune response to T-dependent antigens.

The studies concerning the efficacy of the vaccines in the HIV-exposed infants have been focusing on children who become infected [11-15]. The aim of this study was to evaluate the effectiveness of the hepatitis B, diphtheria and tetanus vaccines in HIV-exposed uninfected infants.

## METHODS

### Study population

The HIV-1-exposed infants (HEU) were recruited in Pediatrics Immunodeficiency Out-patients Unit at the University of Campinas Clinical Hospital (UNICAMP, Campinas, Brazil) and the healthy infants not exposed (NE) to HIV were recruited in Campinas Public Health Centers. Exposed infants with two HIV-1 undetectable viral loads in RNA polymerase chain reaction assays (with a lower limit of quantification at 50 copies of RNA/mL) were categorized as uninfected infants.

For the quantitative determination of anti-HBs were included 45 HEU infants with median age of 7.5 months (6.9-11.2) and of diphtheria and tetanus antitoxins were included 32 HEU infants with median age of 7.5 months (6.7-10.7). All serological analysis was performed with 112 NE infants with median age of 7.7 (6.0-11.4).

The study was approved by Ethical Committee from UNICAMP. Written informed consent was obtained from each infant´s parent or legal guardian.

### Vaccination

The infants received the vaccines following the Brazilian national schedule. The vaccines were manufactured by the Butantan Institute, São Paulo, Brazil. The antigens were adsorbed onto aluminum hydroxide and thimerosal was used as a preservative. Hepatitis B vaccines are composed of highly purified preparations of hepatitis B surface antigen (HBsAg) obtained from yeast cells transfected with viral DNA (recombinant vaccine) (Butang®, 10µg HBsAg with 0.625mg aluminum hydroxide and 0.05mg thimerosal). The

vaccine is usually given as three intramuscular doses over a 6-month period, with the first dose given at birth and the second dose at the first month.

DTP (diphtheria-tetanus-pertussis) vaccine contained four protective units of *Bordetella pertussis* toxin and two protective units of diphtheria and tetanus toxoids (1.25 mg of aluminum hydroxide, and 0.2 mg of thimerosal). Since 2002, DTP and *Haemophilus influenzae* type b (Bio-Manguinhos, Rio de Janeiro, Brazil) vaccines have been administered as a single injection (DTP/Hib). Hib is composed of 10mg *H. influenzae* type b capsular polysaccharide and conjugated to 20-40µg tetanus toxoid as protein carrier [16]. The national health schedule recommends three intramuscular doses of the tetravalent DTP/Hib at 2, 4 and 6 month of life and DTP booster with 15 months and 4-6 years.

**Quantitative determination of anti-HBs**

Quantitative determination of anti-HBs (mIU/ml) was performed blindly on serum samples using microparticle enzyme immunoassay (MEIA) from Axsym Ausab® (ABBOTT Laboratories, ABBOTT Park IL, USA). Reliability of the measurements was assessed by the intra-class correlation coefficient (ICC) stratified by sample origin and include lower and upper limit. The seroprotection was defined as anti-HBs titer $\geq$ 10 IU/ml.

**Quantitative determination of tetanus and diphtheria antitoxin** - ***ToBI test***

This test was performed as described by Hendriksen et al [17] but employing diphtheria toxoid instead of toxin [18, 19].

Specific neutralizing antibodies (Abs) against diphtheria and tetanus were evaluated in sera by a modified Toxin Binding Inhibition (ToBI) test, performed in two steps: (1) seroneutralization and (2) ELISA.

**(1) Seroneutralization**

Round bottomed plates (96 wells) were blocked with 0.1% BSA. After washing, two fold serial dilutions of sera samples (1:4 to 1:512) and reference anti-tetanus toxoid (TTxd) or anti-diphtheria toxoid (DTxd) antitoxin standards were applied. Toxoid solution at 0.1 Lf/mL was added, except in negative control, and incubated for 1h at 37°C. This mixture was used in the next step.

**(2) ELISA**

Flat bottomed plates (96 wells, NUNC Maxisorp) were coated with anti-TTxd or anti-DTxd equine serum at 0.1 IU/well (50 mM carbonate buffer, overnight). Plates were washed and blocked as above. After washing, the mixtures from the seroneutralization step were transferred to the ELISA plates, incubated (90 min, 37°C) and washed. An appropriate dilution of a horseradish peroxidase-labelled equine anti-TTxd or anti-DTxd (prepared from purified F(ab')2 fragments supplied from Fundação BioManguinhos, Rio de Janeiro, as described elsewhere [18]) was added and the plates were incubated (2h, room temperature). Peroxidase substrate was added to the wells. The reaction was stopped and the absorbance (450 nm) recorded in a microplate reader. The average of two independent assays was expressed in IU/mL relative to the reference antiserum.

**Immunophenotyping**

Samples of whole blood from the HEU infants were incubated with the anti-human CD3/CD4 fluorescent conjugated monoclonal Abs (Beckman Coulter, USA) for 20 minutes at room temperature. The red blood cells were lysed with ammonium chloride solution ($NH_4Cl$ 0.15M, $KHCO_3$ 10mM, EDTA 4Na 37 mg/L) and washed twice with phosphate buffered saline. Data were acquired until 10.000 events in the CD3 gate (Epics XL-MCL

flow cytometer; Beckman-Coulter, USA) and analyzed (Expo software; Beckman Coulter, USA). Isotype controls were used to discriminate specific antibody staining.

**Blood collection**

One milliliter of peripheral blood was collected in EDTA-tubes to immunophenotyping and three milliliters in serum-separating tubes to evaluate immune responses to hepatitis B, diphtheria and tetanus. The collection was done for about one month after the third dose from each vaccine.

**Statistical considerations**

Comparison of vaccine response between groups was performed by Chi-Square ($\chi^2$) test with critic $\chi^2$ value of 5.99 (fd, freedom degree = 1) for hepatitis B and by Fisher´s test for diphtheria and tetanus, with significance level for $p < 0.05$. To evaluate the anti-diphtheria and anti-tetanus titers, calculation of the geometric mean titers (GMTs) of Abs was performed on $\log_{10}$-transformed data, and we report the antilogarithms. For each group, GMTs and 95% CIs were calculated. For comparison of the logarithm of the titers, Student's *t* test was applied for independent samples. Comparisons manifesting a two-tailed *P* value of <0.05 were considered statistically significant [20]. Correlation between CD4 counts and diphtheria and tetanus titres was performed by Spearman test.

## RESULTS

### Anti-HBs titres

Table 1 shows anti-HBs titres in serum from both groups after administration of the third dose of hepatitis B vaccine. 6.7% of the HEU infants were non-responders (anti-HBs < 10 mIU/mL) and 64.4% were very good responders (anti-HBs ≥ 1000 mIU/mL), whereas only 3.6% of the NE infants were non-responders. There was a significant difference in the number of non-responder individuals between the HEU and the NE group ($\chi^2 = 10.93$, fd = 1). There was no difference in the CD4 T-lymphocytes percentages between the groups of responders and non-responders from HEU group (Mann-Whitney, p=0.766).

**Table 1:** Distribution of anti-HBs (mUI/mL) for the HIV-exposed uninfected (HEU) infants and for the not exposed (NE) infants, after the third dose of hepatitis B.

| Number and percentages of vaccines | | Anti-HBs (mUI/ml) | | |
|---|---|---|---|---|
| | | < 10 | 10 \|--- 1000 | ≥ 1000 |
| **HEU** | 45 | 3 | 13 | 29 |
| | % | 6.7 | 28.9 | 64.4 |
| **NE** | 112 | 4 | 65 | 43 |
| | % | 3.6 | 58.0 | 38.4 |

### Anti-diphtheria and Anti-tetanus titres

The titres of anti-diphtheria and anti-tetanus, the mean geometric titres and 95% CI serum anti-diphtheria and anti-tetanus IgG levels (IU/mL) are shown in table 2. The non-responsiveness for diphtheria (< 0.1 IU/mL) was 5.3% and 3.6% for the HEU and for the NE infants, respectively (Fisher´s exact test p=0.4). All HEU infants and 98.2% of the NE presented protective response to tetanus (Fisher´s exact test p=0.5).

The difference in the GMTs between groups was not significant for anti-diphtheria but it was significant for anti-tetanus (t-test, p = 0.810 and 0.013, respectively). There were no correlation between CD4 T-lymphocytes percentage and diphtheria titers or tetanus titers from HEU infants (Sperman´s test, p=0.064 and 0.085, respectively).

**Table 2:** Protection against tetanus toxin and diphtheria toxin, geometric mean titres and 95% CI for serum anti-diphtheria and anti-tetanus IgG levels (IU/mL) for the HIV-exposed uninfected (HEU) infants and for the not exposed (NE) infants.

| Antibodie and group | No. of subjects considered protected*/total | Titer (IU/ml) | |
|---|---|---|---|
| | | Geometric Mean | 95% CI |
| **Anti-tetanus toxin IgG** | | | |
| HEU | 19/19 | 1.520 | 1.247-1.851 |
| NE | 108/112 | 2.712 | 2.258-3.258 |
| **Anti-diphtheria toxin IgG** | | | |
| HEU | 18/19 | 0.526 | 0.286-0.967 |
| NE | 110/112 | 0.559 | 0.465-0.672 |

*Titre >0.01 IU/ml for anti-tetanus and titre>0.1 IU/ml for anti-diphtheria

## DISCUSSION

We observed for the HEU infants a poor response to hepatitis B vaccine and lower geometric mean antitoxin titres to tetanus than NE infants.

Since 1998 the Brazilian National Immunization Program (NIP) has incorporated neonatal hepatitis B vaccination into the routine infant immunization schedule as recommended by World Health Organization in 1991 [21]. This strategy was proved economically feasible with the recombinant yeast-derived hepatitis B vaccine Butang® manufactured by Butantan Institute, São Paulo, Brazil [22,23].

The hepatitis B vaccine induces helper T lymphocytes responses and antibody production [24]. In a previous study from our team, we showed that 99.6% of healthy infants mounted seroprotection one month after completion of the 3-dose schedule [25]. Reports on the titres kinetics show that anti-HBs concentrations decline rapidly within the first year after primary immunization and more slowly thereafter. But even if anti-HBs concentrations decline to below 10 mIU/mL, immune memory persists for long time and the children are capable of develop an anti-HBs response upon exposure to hepatitis B virus later in life [26].

About 5-10% of healthy adults do not present anti-HBs protective concentrations and it has been suggested that non-response to hepatitis B vaccine can be due to prior exposure to occult hepatitis B virus or due to immunological and immunogenetic mechanisms [27]. Polymorphism in the major histocompatibility complex (MHC) genes leading to varied response to HBsAg due to differential presentation of antigen by different MHC molecules seems to play an important role in the non-response to hepatitis B antigens [28]. Host factors, such as increasing age, obesity and smoking cigarettes, are known to

cause a decrease in the response to vaccine [29]. HIV infection is also largely associated with a fail to respond to hepatitis B vaccine, with only 25-50% of HIV-infected children developing protective antibody. In some studies the response rates correlate with CD4 T-lymphocytes counts [1].

Our result shows that the intra uterine exposition to HIV is also a condition that leads to a poor response to hepatitis B vaccine, even in those cases that the newborns do not become infected. Similar percentages of non-responsiveness to hepatitis B vaccine among HEU infants were reported by Thaithomyanon et al [30] and Rutstein et al [31] (8.1 % and 8.0% of non-responders, respectively). As both studies focused on the HIV-infected infants, their limitation is that the HEU group was used only as a control group to compare with the infected infants, without a NE group.

The neonates and infants from HIV-infected mothers present several alterations in the immune system that may interfere with the antigen presentation and response to T-dependent antigens. They present reduction in CD4 T cells and an increase in CD8 T and in immature T lymphocytes [7] and enhanced expression of CD40L on activated T lymphocytes [8]. In the first year of life, Bunders et al [27] and Gesner et al [32] report for the HEU infants a reduction in the counts of CD4 and CD8 T subsets. Velilla et al [9] observed for the HIV-exposed newborns a higher frequency of myeloid dendritic cell, higher up-regulation of both CD80 and CD86 and distinct pattern of up-regulation of the inhibitory molecule B7-H1 upon stimulation.

Unlike what we observed for the anti-HBs response, the percentages of non-responders to tetanus and diphtheria toxins seen in this study did not differ between the HEU and the NE infants.

Widespread childhood immunization with DTP, introduced more than 65 years ago, has largely eradicated diphtheria and tetanus from many countries. In Brazil, the DTP vaccine manufactured by Butantan Institute has been available since 1992 and has been administered free of charge to almost 100% of newborn children [33].

The data about vaccine failure rates to diphtheria and tetanus in HIV-infected child are variable. Following primary immunization in infancy, 40–100% of symptomatic and asymptomatic HIV-infected children respond to diphtheria and tetanus toxoids by developing protective levels of diphtheria and tetanus antitoxins. However, they develop lower GMTs and are more likely than uninfected persons to lose antibody within a few years after vaccination [1].

Similar to what is seen for the HIV-infected children, our data indicate that the HEU infants also respond with protective tetanus and diphtheria antitoxins titers, but present lower GMT of anti-tetanus toxin IgG.

We did not observe for the HEU infants differences in the percentage of CD4 T-lymphocytes between the groups of responders and non-responders for Anti-HbsAg titres. There was also no correlation between CD4 percentage and anti-tetanus or anti-diphtheria titres. Gesner et al [32] report normal cellular proliferative response to tetanus and diphtheria antigens for a group of HEU infants with persistent low CD4 percentages. Taken together these data indicate that the poor response for anti-HBs and the lower GMTs to tetanus toxoid may be more related to qualitative changes in T lymphocytes and in antigen presentation than to TCD4 counts.

Interestingly, the only HEU infant that did not respond with protective titres to diphtheria was a very good responder to anti-HBs. These differences may due in part to the

vaccines composition. While the hepatitis B vaccine is a recombinant vaccine, the DTP vaccine contains diphtheria and tetanus toxoids. Although the vaccines are T-dependent, their presentation to dendritic cells must be different. Furthermore, this same infant presented anti-tetanus titres of 2.01 IU/mL. Together with DTP vaccine, the infants receive the Hib vaccine with tetanus toxoid as protein carrier, reinforcing the tetanus vaccine. It is necessary to investigate the kinetics from these titres after the booster doses of DTP.

The poor response to hepatitis B vaccination and the lower geometric mean antitoxin titres to tetanus found for the HEU infants in this study corroborates that intrauterine exposure to HIV interferes with the effectiveness of early vaccination. It is necessary to include the anti-HBs serology one month after the third dose of hepatitis B vaccine as a medical routine to the infants born from HIV-infected mothers.

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# 5- DISCUSSÃO

Nossos dados mostraram que os lactentes com exposição vertical ao HIV e não infectados (ENI) apresentam baixo peso ao nascimento e reduzida contagem de linfócitos TCD3, TCD4+ e TCD8+ em relação aos lactentes não expostos (NE) ao vírus. Em relação à resposta às vacinas, encontramos uma reduzida resposta protetora à vacina da hepatite B, menores títulos geométricos de anticorpos IgG para o toxóide tetânico e normais para o toxóide diftérico para os lactentes ENI. Além disso, encontramos para esse grupo uma contagem normal de células natural killer e preservada atividade citotóxica de células mononucleares do sangue periférico.

Diversas recomendações têm sido empregadas com sucesso para reduzir a transmissão vertical do HIV. Entretanto, algumas dessas medidas, associadas ao ambiente intra-uterino alterado pela infecção materna, podem interferir no desenvolvimento do feto/embrião.

Semelhante ao nosso resultado, diversos estudos têm mostrado baixo peso de nascimento nos recém-nascidos de mães infectadas pelo HIV e isso está relacionado à etnia, à ausência de tratamento durante a gravidez, ao uso de cigarro e drogas ilícitas na gestação (ICKOVICS *et al*. 2000, FLORIDIA *et al*. 2008, IBIETA *et al*. 2009). A submissão das gestantes infectadas pelo HIV à cesárea eletiva na 38ª semana de gestação pode ser fator contribuinte para o menor peso de nascimento (ONO *et al*. 2008).

A passagem placentária de proteínas do HIV, associada à exposição às drogas ARV, causa, nos recém–nascidos, disfunção mitocondrial (BARRET 2003), alterações hematológicas (CONNOR *et al*. 1994, LE CHENADEC *et al*. 2003, FEITERNA-SPERLING *et al*. 2007) e um estado de ativação imune (RICH *et al*. 1997). A exposição intrauterina a gp120 pode fazer com que essa proteína se ligue ao CD4, impedindo os timócitos de interagirem com moléculas MHC II e, consequentemente, impossibilitando o

desenvolvimento normal de linfócitos T. Os recém nascidos de mães infectadas pelo HIV apresentam uma redução em linfócitos T CD4+ e aumento de TCD8+ e de linfócitos imaturos, que persiste em crianças de 7 anos de idade. (CLERICI *et al*. 2000).

Nossos resultados sobre contagem de linfócitos TCD3, TCD4+ e TCD8+ para esses lactentes aos 7 meses de idade estão parcialmente de acordo com o observado por GESNER *et al*. (1994), em um estudo com lactentes ENI do nascimento até 27 meses de idade. O grupo observou porcentagem reduzida de linfócitos TCD4+ em todo o período, porém de forma significativa apenas entre 4 e 6 meses de idade, mas em nenhum período obteve diferença significativa para a porcentagem de TCD8+.

Os estudos sobre células NK e atividade citotóxica em indivíduos ENI são focados em adultos e mostram aumento do número de células NK, produção de citocinas por essas células e atividade citotóxica nos indivíduos ENI em relação aos que se tornam infectados (SCOTT-ALGARA *et al*. 2003; MONTOYA *et al*. 2006, RAVET *et al*. 2007). Ao contrário dos resultados para adultos ENI, nossos dados mostraram número normal de células NK e atividade citotóxica semelhante ao dos lactentes não expostos ao HIV. CLERICI *et al*. (2000) observaram contagem normal de NK nos recém-nascidos de mães infectadas pelo HIV, enquanto ONO *et al*. (2008) relataram um número aumentado dessas células em lactentes ENI aos 12 meses de idade. Nossos dados sugerem que as alterações relatadas por ONO *et al*. (2008) podem ocorrer após os sete meses de idade.

Além das alterações de células T, VELILLA *et al*. (2008) observaram que os recém-nascidos expostos ao HIV possuem uma maior freqüência de células dendríticas mielóides em relação aos recém-nascidos de mães não infectadas e que essas células possuem um padrão distinto de expressão de moléculas após estimulação. Essas alterações, em conjunto, podem interferir com a resposta imune a antígenos T dependentes.

Os resultados obtidos mostram que os lactentes ENI apresentam uma reduzida resposta protetora à vacina da hepatite B, menores títulos geométricos de anticorpos IgG para o toxóide tetânico e normais para o toxóide diftérico. Esses dados são semelhantes aos encontrados para crianças infectadas pelo HIV. Embora essas crianças tenham uma menor resposta protetora para anti-HBs, os dados sobre soroproteção para difteria e tétano são variados, sendo bem estabelecido apenas que elas possuem menores títulos de anticorpos para tais antígenos (MOSS *et al*. 2003).

A menor resposta protetora para vacina da hepatite B e a menor média geométrica de títulos antitetânicos observada para os lactentes ENI reforçam as observações de que as alterações em T e em células dendríticas encontradas nesses indivíduos interferem com a eficiência da vacinação. A sorologia para anti-HBs um mês após a terceira dose da vacina deve ser incluída como exame de rotina para os lactentes ENI e, em caso de ausência de resposta protetora, novas doses da vacina devem ser aplicadas. Mais estudos sobre a cinética do título de anticorpos antitétano e antidifteria após as doses de reforço da vacina DTP são necessários.

# 6- CONCLUSÕES

Em relação aos lactentes não expostos ao HIV, os lactentes com exposição vertical ao HIV e não infectados apresentaram:

1. Menor peso de nascimento.
2. Menores contagens de linfócitos T e das subpopulações TCD4 e TCD8.
3. Contagens semelhantes de células natural killer.
4. Atividade citotóxica de células mononucleares do sangue periférico preservada.
5. Baixa resposta protetora à vacina de hepatite B.
6. Número semelhante de indivíduos respondedores para difteria e tétano.
7. Média Geométrica semelhante para título antidiftérico e menor para título antitetânico.

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# 8- ANEXOS

## 8.1 Carta de Consentimento Livre e Esclarecido

Carta de Consentimento Livre e Esclarecido (resolução 196/96-251/97)

Pesquisa Clínica:

**TÍTULO**: "Caracterização e Atividade de Células Natural Killer e Maturação de Células B em Lactentes com Exposição Vertical ao Vírus da Imunodeficiência Humana"

**Pesquisadores:** Professores Doutores: Marcos Tadeu Nolasco da Silva e Maria Marluce dos Santos Vilela.

**LOCAL**: Centro de Investigação Pediátrica (CIPED) – FCM-UNICAMP.

**1. INTRODUÇÃO**: As informações a seguir descreverão esta pesquisa e o papel que você terá como participante. Os pesquisadores responsáveis pelo estudo responderão a quaisquer perguntas que você possa ter sobre este termo e sobre o estudo. Por favor, leia-o cuidadosamente, e não hesite em perguntar qualquer coisa sobre as informações abaixo.

**2. PROPÓSITO**: Você está sendo convidado a participar de uma pesquisa clínica cujo objetivo é caracterizar células natural killer e avaliar a atividade dessas células e a maturação de células B de lactentes com e sem exposição vertical ao HIV. Serão coletados aproximadamente 10mL de sangue periférico para realização dos ensaios de atividade de células natural killer, fenotipagem, produção de citocina intracelular, quantificação viral e hemograma. Para você decidir se deseja ou não participar deste estudo de pesquisa, você deve entender o bastante para fazer uma decisão consciente. Este processo é conhecido como consentimento informado.

**3. RETROSPECTIVA**: A Síndrome da Imunodeficiência Adquirida (AIDS) é causada por um retrovírus da família Lentiviridae, Vírus da Imunodeficiência Humana (HIV). Exposição do feto a partículas solúveis do HIV afeta a maturação do sistema imune, sendo isso amplamente registrado para células T. Por outro lado, existem poucos estudos sobre a maturação de células B e o sistema imune inato de lactentes filhos de mães infectadas pelo HIV-1. Células natural killer (NK) desempenham papéis predominantes na resposta imune inata celular e sua função na proteção e controle da infecção por HIV-1 não

é totalmente conhecida. O presente trabalho tem como objetivo avaliar a maturação de células B e atividade citolítica e produção de citocinas pelas células NK em lactentes com exposição vertical ao HIV, mas não infectados, utilizando métodos de citometria de fluxo. O estudo contribuirá para o entendimento dos possíveis efeitos desta exposição ao HIV-1 sobre a ontogenia do sistema imune inato e adaptativo do lactente.

**4. DESCRIÇÃO DO ESTUDO**: Os responsáveis de todos os lactentes de mães soropositivas em tratamento no Hospital de Clínicas (HC) da Unicamp nos sete primeiros trimestres de execução do projeto serão convidados a participar do estudo. Será estudada ainda a resposta imune de trinta crianças sem exposição vertical ao HIV. Os responsáveis pelos lactentes que concordarem em participar da pesquisa serão entrevistados, terão todas as dúvidas esclarecidas e o termo de consentimento a ser assinado. A coleta de sangue e exames não interferirão nos atendimentos médicos. A coleta de sangue durará cerca de 5 minutos e o método de coleta utilizado não acarretará qualquer risco, custo ou dano imediato ou tardio ao paciente. Não serão realizadas intervenções terapêuticas durante o período de estudo, exceto as realizadas pela equipe médica.

**5. DESCONFORTO, RISCOS E BENEFÍCIOS ESPERADOS**: O método de coleta utilizado, ao qual os participantes da pesquisa serão submetidos, não acarreta qualquer risco, custo ou dano imediato ou potencial ao participante. Por outro lado, oferece a possibilidade de gerar conhecimento para compreensão dos efeitos no sistema imune que a exposição vertical a partículas do vírus do HIV causa.

**6. EXCLUSÕES**: critérios de exclusão no grupo desta pesquisa:

- Lactente portador de qualquer doença neurológica, convulsão ou que esteja em uso de anticonvulsivante.
- Lactente portador de malformação congênita, de doença genética ou de condição clínica grave.
- Lactente submetido à transfusão sanguínea.

**7. COMPENSAÇÃO**: Não existem danos imediatos ou futuros previsíveis decorrentes da pesquisa e, portanto, a mesma não inclui a possibilidade de indenização.

**8. CONFIDENCIALIDADE DOS REGISTROS**: Você tem direito a privacidade e toda informação que for obtida em relação a este estudo permanecerá confidencial nos

âmbitos possíveis da lei, assegurando proteção de sua imagem, sigilo e respeitando valores culturais, sociais, morais, religiosos e éticos. Como condição de sua participação nesta pesquisa, você permite acesso aos dados obtidos durante o estudo, aos pesquisadores envolvidos neste estudo, aos membros da Comissão de Ética responsáveis pela análise do projeto e a agência financiadora. Os resultados deste projeto de pesquisa poderão ser apresentados em congressos ou em publicações, porém sua identidade não será divulgada nessas apresentações.

**9. DIREITO em PARTICIPAR, RECUSAR ou SAIR:** Ao participar, você concorda em cooperar com os procedimentos que foram executados e que foram descritos acima, não abrindo mão de seus direitos legais ao assinar o termo de consentimento informado. Sua participação neste estudo é voluntária e você poderá recusar-se a participar ou poderá interromper sua participação a qualquer momento sem penalidades ou perda dos benefícios aos quais de outra forma tenha direito.

O pesquisador tem o direito de desligá-lo do estudo a qualquer momento que julgar necessário. Se você sair voluntariamente ou for retirado pelo pesquisador, poderá ser solicitado que volte para eventual coleta de sangue.

**10. CONTATOS**: Se ainda houver qualquer dúvida sobre o estudo você poderá receber mais esclarecimentos falando com os médicos: Marcos Tadeu Nolasco da Silva ou Maria Marluce dos Santos Vilela.

Endereço: Centro de Investigação em Pediatria (CIPED)- Faculdade de Ciências Médicas - UNICAMP, Campinas, SP, CEP: 13083-970, Fone: (XX-19) 3521- 8989 ou 3521-8963.

## 8.2 Termo de Consentimento Livre e Esclarecido

Declaro, por livre e espontânea vontade, que permito a participação de _____, sob registro de nascimento _____, que se encontra sob responsabilidade de _____, com idade de ____ anos, com o RG de no _____, residente em _____, cujo grau de parentesco é _____, na pesquisa intitulada "Caracterização e atividade de Células Natural Killer e Maturação de Células B em Lactentes com Exposição Vertical ao Vírus da Imunodeficiência Humana (HIV)".

Esta pesquisa tem como objetivo caracterizar células natural killer, avaliar a atividade dessas células e a maturação de células B de lactentes com e sem exposição vertical ao HIV Atesto que recebi esclarecimentos quanto aos propósitos e procedimentos a serem utilizados durante o estudo, tais como:

a) entrevista com profissional da área da saúde;

b) coleta de sangue periférico para a realização de exames.

Comprometo-me a seguir as orientações recebidas, tendo a garantia de receber resposta a qualquer pergunta e esclarecimento a qualquer dúvida acerca dos assuntos relacionados à pesquisa.

Estou ciente que não receberei remuneração em troca da participação, que os dados obtidos serão mantidos em sigilo, que posso deixar de participar da pesquisa no momento em que desejar e que a desistência não influenciará no atendimento que venho recebendo.

De acordo,

Responsável pelo participante: _____.

Profa. Dra. Maria Marluce dos S. Vilela (Tel: (19) 3521-8963): _____

Campinas, ___ de _____ de 2007.

Secretaria do Comitê de Ética em Pesquisa do Hospital das Clínicas – UNICAMP: (19) 3521-8936.Faculdade de Ciências Médicas, Rua: Tessália Vieira de Camargo, 126 Cidade Universitária "Zeferino Vaz", Campinas - SP - Brasil - CEP: 13083-970, Cx. Postal: 6111. E-mail: cep@fcm.unicamp.br.

## 8.3 Carta de Aprovação do Comitê de Ética da Faculdade de Ciências Médicas da Unicamp

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 23/10/07.
(**PARECER CEP:** N° 135/2002)

# PARECER

**I-IDENTIFICAÇÃO:**

PROJETO: **"AVALIAÇÃO DA IMUNOGENECIDADE E SEGURANÇA DA VACINA COMBINADA BCG E HEPATITE B DO INSTITUTO BUTANTAN, NA CRIANÇA".**

PESQUISADOR RESPONSÁVEL: Maria Marluce dos Santos Vilela.

**II - PARECER DO CEP**

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP tomou ciência e aprovou o Adendo que inclui o projeto de pesquisa intitulado "**CARACTERIZAÇÃO E ATIVIDADE DE CÉLULAS NATURAL KILLER E MATURAÇÃO DE CÉLULAS B EM LACTENTES COM EXPOSIÇÃO VERTICAL AO VÍRUS DA IMUNODEFICIÊNCIA HUMANA**", referente ao protocolo de pesquisa supracitado.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

Homologado na X Reunião Ordinária do CEP/FCM, em 23 de outubro de 2007.

**Profa. Dra. Carmen Silvia Bertuzzo**
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

Comitê de Ética em Pesquisa - UNICAMP
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## 8.4 Trabalhos apresentados em Congresso

**Abramczuk BM**, da Silva MTN, Lima SCBS, Zorzeto TQ, Vilela MMS. *Vertical exposure to HIV-1 does not affect the NK cell activity maturation,* apresentado sob a forma de pôster no 32º Congresso da Sociedade Brasileira de Imunologia, 2008, Ribeirão Preto.

**Abramczuk BM**, Mazzola TN, Moreno YMF, Blotta MH, da Silva MTN, Vilela MMS. *Hepatitis b vaccination among HIV-exposed uninfected infants*, apresentado sob a forma de pôster no 2º Congresso Europeu de Imunologia, 2009, Berlim, Alemanha.

## 8.5 Pôsteres apresentados em Congressos

# VERTICAL EXPOSURE TO HIV-1 DOES NOT AFFECT THE NK CELL ACTIVITY MATURATION

*Abramczuk,B.M.; da Silva, M.T.N.;*
*Lima, S.C.B.S.; Zorzeto,T.Q.; Vilela,M.M.S.*

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Faculty of Medical Sciences, PO Box 6111,University of Campinas - UNICAMP, Zip Code 13083-887, Campinas, SP, Brazil.

### INTRODUCTION

The immune system undergoes a dramatic transition at birth, from a sheltered intra-uterine environment to a distinct environment of the outside world. This transition is then followed by a gradual, age-dependent maturation. Natural killer (NK) cells play an integral role in the innate immune response. NK mediates a cell contact-dependent cytolysis of virally infected and transformed cells and provides help to adaptive responses through cytokine secretion. Early in life functional NK is completely mature. The aim of this study was to evaluate the NK activity in vertically HIV-exposed infants.

### METHODS

We investigated the NK cell activity in 33 HIV exposed uninfected children followed at the Pediatrics Immunodeficiency outpatients unit State University of Campinas Medical School, with ages varying from 6.6 to 10.83 months (median, 7.57) and 38 controls not exposed to HIV, with ages varying from 7.1 to 10.77 months (median, 7.63). Peripheral blood mononuclear cells (PBMC), in three different concentrations, were incubated at 37°C for 2 hours in a 5% $CO_2$ atmosphere with target cell K562 ($1x10^5$cel/mL) labeled with $PKH_2$ (Sigma, EUA). Propidium iodide (Sigma, EUA) was added and the cell lysis percentage was acquired in an Epics XL-MCL flow cytometer (Beckman-Coulter, USA) (fig. 1) . The relative NK cell ($CD3^-CD16^+CD56^+$) population was also determined for the HIV exposed children and for nine controls.

### RESULTS

The NK activity medians for the HIV exposed children were 1.75% (0.0 - 4.3), 4.1 (0.0 -7.9) and 5.9% (1.2 -13.8) for $0.625x10^6$cel/mL, $1.25x10^6$cel/mL and $2.5x10^6$cel/mL PBMC concentrations, respectively. In the control group, these medians were 2.5% ± 2.57, 4.2 ± 3.47 and 6.8 ± 5.57 (fig.2). No differences in NK activity were found between the HIV exposed children and the control group (Mann-Whitney test, p = 0.123, p = 0.289 and p = 0.204).

The median of the NK cell percentage for the HIV exposed group was 7.2 (2.7 16.7) and for the controls was 9.0 (5.5-16.3). There were no differences between the HIV exposed children and the controls (Mann-Whitney test, p = 0.206). There was no correlation between the percentage of NK cells and the cell activity from the HIV exposed children (Spearman's test, p= 0.307 , 0.103 and 0.918).

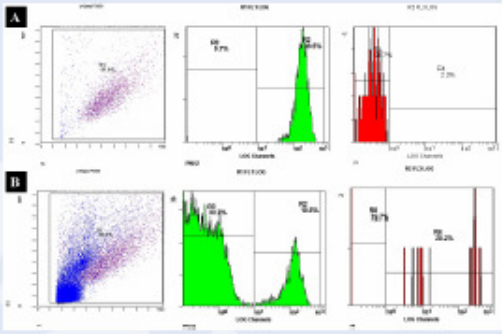



***Fig.1.*** Data acquisition
A.Negative control; K562 cells labeled with PKH2.
B.NK assay with PBMC 1.25x106cel/mL (target:effector ratio 1:25)

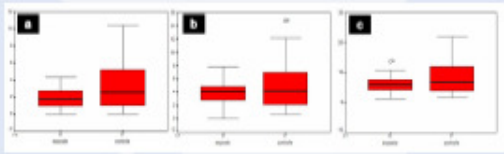


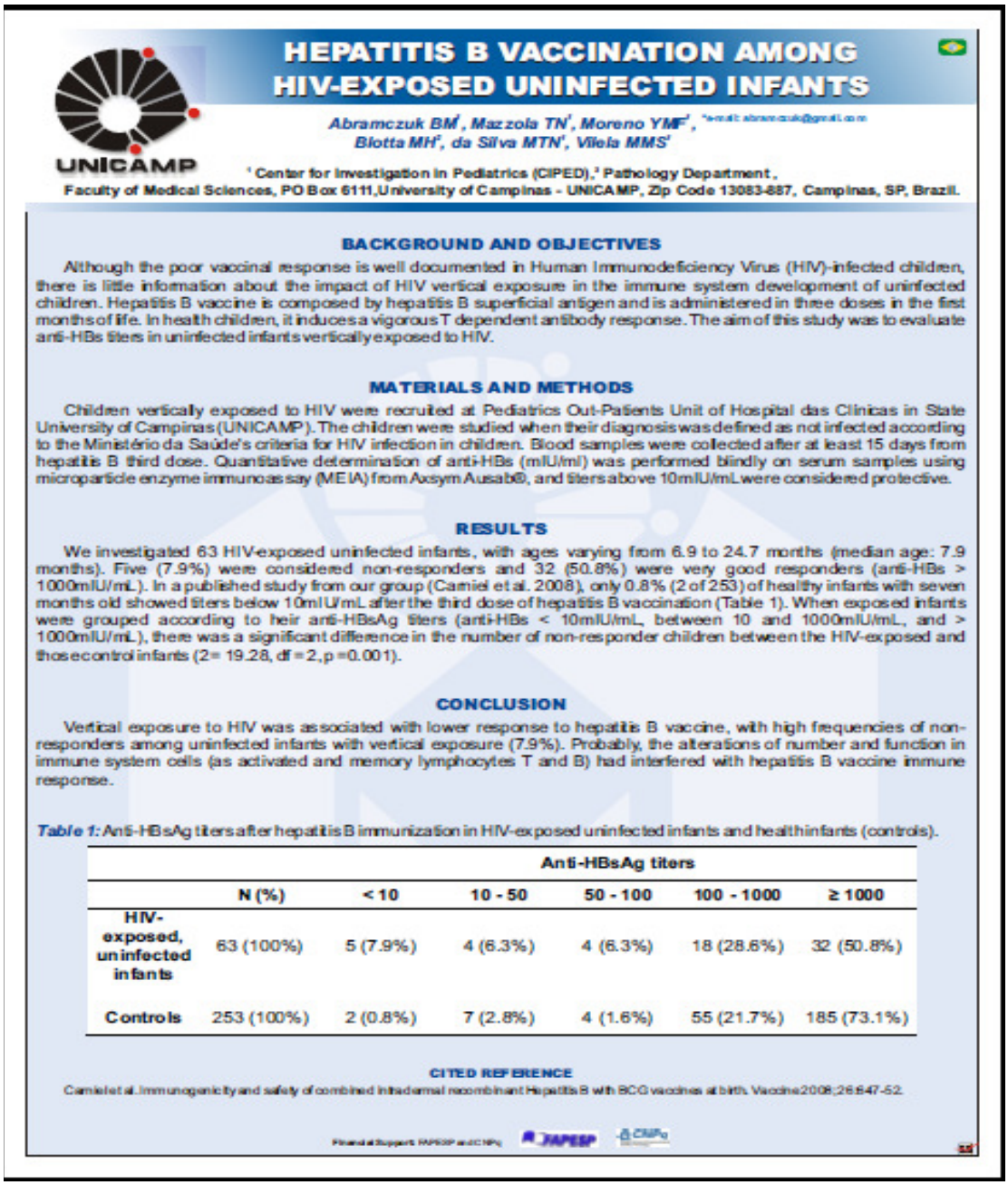

***Fig.2.*** Box plot of the NK activity for the HIV exposed uninfected children and for the controls, for target/effector cells ratio 1/12.5 (a), 1/25 (b) and 1/50 (c)

### CONCLUSION

These data suggest that the maturation of NK cell activity is not affected by the in utero exposure to HIV.

Financial Support: FAPESP and CNPq

Pôster apresentado no 32º Congresso da Sociedade Brasileira de Imunologia, 2008, Ribeirão Preto.



# HEPATITIS B VACCINATION AMONG HIV-EXPOSED UNINFECTED INFANTS

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## BACKGROUND AND OBJECTIVES

Although the poor vaccinal response is well documented in Human Immunodeficiency Virus (HIV)-infected children, there is little information about the impact of HIV vertical exposure in the immune system development of uninfected children. Hepatitis B vaccine is composed by hepatitis B superficial antigen and is administered in three doses in the first months of life. In health children, it induces a vigorous T dependent antibody response. The aim of this study was to evaluate anti-HBs titers in uninfected infants vertically exposed to HIV.

## MATERIALS AND METHODS

Children vertically exposed to HIV were recruited at Pediatrics Out-Patients Unit of Hospital das Clínicas in State University of Campinas (UNICAMP). The children were studied when their diagnosis was defined as not infected according to the Ministério da Saúde's criteria for HIV infection in children. Blood samples were collected after at least 15 days from hepatitis B third dose. Quantitative determination of anti-HBs (mIU/ml) was performed blindly on serum samples using microparticle enzyme immunoassay (MEIA) from Axsym Ausab®, and titers above 10mIU/mL were considered protective.

## RESULTS

We investigated 63 HIV-exposed uninfected infants, with ages varying from 6.9 to 24.7 months (median age: 7.9 months). Five (7.9%) were considered non-responders and 32 (50.8%) were very good responders (anti-HBs > 1000mIU/mL). In a published study from our group (Camiel et al. 2008), only 0.8% (2 of 253) of healthy infants with seven months old showed titers below 10mIU/mL after the third dose of hepatitis B vaccination (Table 1). When exposed infants were grouped according to heir anti-HBsAg titers (anti-HBs < 10mIU/mL, between 10 and 1000mIU/mL, and > 1000mIU/mL), there was a significant difference in the number of non-responder children between the HIV-exposed and those control infants ($2 = 19.28$, $df = 2$, $p = 0.001$).

## CONCLUSION

Vertical exposure to HIV was associated with lower response to hepatitis B vaccine, with high frequencies of non-responders among uninfected infants with vertical exposure (7.9%). Probably, the alterations of number and function in immune system cells (as activated and memory lymphocytes T and B) had interfered with hepatitis B vaccine immune response.

*Table 1:* Anti-HBsAg titers after hepatitis B immunization in HIV-exposed uninfected infants and health infants (controls).

| | | Anti-HBsAg titers | | | | |
|---|---|---|---|---|---|---|
| | N (%) | < 10 | 10 - 50 | 50 - 100 | 100 - 1000 | ≥ 1000 |
| HIV-exposed, uninfected infants | 63 (100%) | 5 (7.9%) | 4 (6.3%) | 4 (6.3%) | 18 (28.6%) | 32 (50.8%) |
| Controls | 253 (100%) | 2 (0.8%) | 7 (2.8%) | 4 (1.6%) | 55 (21.7%) | 185 (73.1%) |

CITED REFERENCE

Camiel et al. Immunogenicity and safety of combined intradermal recombinant Hepatitis B with BCG vaccines at birth. Vaccine 2008; 26:647-52.

Financial Support: FAPESP and CNPq

Pôster apresentado no 2º Congresso Europeu de Imunologia, 2009, Berlim, Alemanha.