Immunite humorale contre la varicelle déficiente chez les enfants infectes par le VIH: des taux d'anticorps spécifiques bas et de basse avidité

L'Huillier, Arnaud

UNIVERSITÉ DE GENÈVE

FACULTE DE MEDECINE

Section de Médecine clinique

Département de Pédiatrie

Thèse préparée sous la direction du Professeur Claire-Anne Siegrist

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IMMUNITE HUMORALE CONTRE LA VARICELLE DEFICIENTE CHEZ LES ENFANTS INFECTES PAR LE VIH: DES TAUX D'ANTICORPS SPECIFIQUES BAS ET DE BASSE AVIDITE

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Arnaud L'Huillier

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Diplômes

Ligne directe : 022 705 77 44

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Nom et prénom : L'HUILLIER Arnaud

Adresse : 29, rue du vieux-moulin, 1290 Versoix

Faculté : Médecine Département (selon structures officielles) : Pédiatrie

Directeur de thèse : Professeure Claire-Anne Siegrist

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Résumé

Résumer de manière succincte le contenu du travail de doctorat. Pour des raisons d'impression, le texte doit être limité à **150 mots au maximum**. Tout résumé dépassant ce chiffre sera retourné à son auteur.

Introduction : La varicelle induit généralement une immunité à vie. Chez les immunosupprimés, des présentations sévères ou récurrentes sont décrites. *Objectif :* Définir si les enfants HIV+ ont des anticorps anti-VZV plus bas que les adultes HIV+ et les enfants sains et le cas échéant, quelle en est la cause. *Méthode :* Dosage des anticorps anti-VZV annuellement chez 78 adultes et 97 enfants HIV+ sur 10 ans, ainsi que 97 enfants sains matchés par âge. *Résultats :* Les anticorps anti-VZV sont plus bas chez les enfants HIV+ que les adultes HIV+ et enfants sains tout au long de l'étude mais ne diminuent pas avec le temps. L'avidité des anticorps est également plus basse. *Conclusion :* Les enfants HIV+ ont une réponse humorale au VZV plus faible que les adultes HIV+ et les enfants sains. Le déclin des anticorps chez ces enfants ne résulte pas d'une perte accélérée d'anticorps ni d'une faible réponse primaire mais d'une réponse mémoire défectueuse.

Signature :

Visa du directeur de thèse :

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A Sophie, A Yves-Alain.

INTRODUCTION AU TRAVAIL

Le virus VZV (Varicella Zoster Virus) est un virus de la famille des alpha herpesvirus, organismes caractérisés par des cycles de reproduction courts, un passage rapide entre les cellules et une propension à séjourner de façon latente dans les ganglions sensitifs. Cette famille comprend, en plus du VZV (aussi appelé HHV-3), les virus herpes simplex 1 et 2 (HSV-1 ou HHV1, et HSV-2 ou HHV2, respectivement), le cytomégalovirus (CMV ou HHV-4), l'Epstein-Barr virus (EBV ou HHV-5), les HHV-6 et HHV-7 (responsables de la roséole infantile ou exanthème subit), ainsi que le HHV-8, responsable du sarcome de Kaposi.

La primo-infection par VZV se présente sous la forme de varicelle, maladie endémique très fréquente, essentiellement pédiatrique. En Suisse, où la vaccination contre la varicelle n'est recommandée qu'aux adolescents non immuns, la séroprévalence dépasse 95% entre 13 et 15 ans [1]. Cette affection, dont la transmission par voie aérienne est hautement contagieuse est caractérisée, après une période d'incubation de 10 à 21 jours, par de la fièvre, un malaise généralisé, une anorexie, des céphalées et, parfois, des douleurs abdominales modérées. Ces symptômes sont rapidement suivis par l'apparition d'un exanthème composé de macules très prurigineuses évoluant vers des vésicules liquides, devenant croûteuses. Cette maladie est le plus souvent bénigne et se résout spontanément. Elle peut néanmoins entraîner certaines complications, telles des surinfections bactériennes des lésions cutanées, une pneumonie, une hépatite, une cérébellite ou une encéphalite [2], particulièrement chez les adolescents et les adultes, mais aussi chez les patients immunosupprimés tels que les patients séropositifs pour le virus de l'immunodéficience humaine (VIH) [3-5] .

Après la primo-infection, le virus VZV reste latent dans les ganglions trigéminés et dans les ganglions spinaux de la moelle épinière. Le virus peut alors se réactiver et causer un zona (ou zoster) [6]. Le zona se présente quant à lui sous la forme d'un rash douloureux vésiculaire unilatéral limité à un dermatome.

Rarement, mais surtout chez les patients immunosupprimés, il est disséminé ou multidermatomal. La prévalence du zona augmente avec l'âge et atteint en moyenne 10-20% des adultes sains : il est 10 fois plus élevé chez les patients VIH+ [7-10].

Il est en général considéré que la primo-infection VZV entraîne une immunité humorale à vie [11, 12], de telle façon qu'une histoire clinique de varicelle est considérée comme preuve d'immunité. Cependant, la protection contre un nouvel épisode de varicelle dépend non seulement de la persistance de l'immunité humorale, mais aussi de l'immunité cellulaire [13, 14] puisque les patients ayant perdu leurs anticorps spécifiques contre VZV ne développent que rarement une varicelle. Dans les rares cas où cela arrive, la varicelle est souvent peu sévère [3, 15]. Les réactivations, symptomatiques ou non, qu'elles soient dues à un réveil du virus qui était latent dans les ganglions (réactivation endogène) ou qu'elles soient dues à un contact avec une personne infectée (activation exogène), jouent un rôle dans le maintien de l'immunité contre le VZV [16].

Le VIH est un virus à ARN (rétrovirus), qui s'attaque aux cellules du système immunitaire, particulièrement aux lymphocytes T CD4+, mais aussi aux cellules dendritiques et aux macrophages. La transmission du virus se fait principalement de manière sexuelle, par les produits sanguins ou par l'usage de drogues chez les adultes. Chez les enfants cependant, la transmission ne se fait quasiment que pendant la grossesse ou l'accouchement (transmission verticale). Le virus détruit les cellules cibles (cellules T CD4+, monocytes, macrophages, cellules dendritiques, cellules de Langerhans) par infection directe, en se liant à leur récepteur CD4 via la protéine GP120, ainsi qu'au récepteur CCR4 et/ou CXCR5 via la protéine GP41. Ceci entraîne une mort cellulaire ou apoptose, responsable des manifestations cliniques.

Il est très clairement démontré que la baisse des CD4 augmente le risque de varicelle sévère ou compliquée et aussi de zoster [17]. Les études diffèrent quant à l'influence du VIH sur l'immunité cellulaire contre le VZV: certains auteurs décrivent une réponse cellulaire altérée chez les enfants VIH+, même sous traitement antirétroviral hautement actif (HAART) [18, 19], alors que d'autres ont démontré que ces enfants produisent une réponse cellulaire adéquate [19-21]. Avant l'ère du HAART, les varicelles récurrentes étaient fréquemment décrites chez les enfants VIH+ [17, 22, 23] et le risque de zona était plus important chez les enfants et adolescents VIH+ que chez les adultes VIH+ [24]. Le taux accru de complications chez les enfants VIH+ pourrait résulter d'un défaut de réponse primaire, ce qui expliquerait le taux augmenté d'échecs vaccinaux à beaucoup de vaccinations [25].

Il a été constaté parmi les patients suivis dans notre institution que certains enfants VIH+ n'avaient pas d'anticorps contre le VZV, malgré une anamnèse positive de varicelle, confirmée par leurs médecins. Cette situation ne se retrouvait par contre pas chez les adultes VIH+. Cette situation a déjà été décrite par Bekker et collègues qui rapportaient la perte d'anticorps VZV chez 21% de leur cohorte d'enfants VIH+ traités par HAART [26].

Cette observation est surprenante: comme expliqué précédemment, la persistance de l'immunité humorale est généralement à vie [11, 12], notamment grâce aux réexpositions exogènes et aux réactivations endogènes [16]. Les observations décrites ci-dessus suggèrent des limitations dans la capacité des enfants VIH+ à générer, maintenir et réactiver leur mémoire immunologique.

Le but de ce travail est de démontrer si les enfants VIH+ ont des anticorps anti-VZV en plus faible quantité (taux d'anticorps) que les adultes VIH+, mais aussi que les enfants sains (VIH négatifs) du même

âge. De surcroît, nous souhaitons démontrer si ces enfants ont aussi des anticorps de plus « mauvaise qualité » : en effet, la force avec laquelle un anticorps spécifique se lie à son antigène s'appelle avidité. L'avidité des anticorps augmente lors de la réponse immune et en cas de réexposition à cet antigène, grâce à la maturation d'affinité des anticorps. De ce fait, l'avidité permet de différentier une primo-infection d'une réactivation[27-29].

Si nous prouvons que les enfants VIH+ ont des anticorps contre la varicelle en plus faible quantité et/ou de moins bonne qualité, nous essaierons de montrer que si est dû à une perte d'anticorps accélérée, à une réponse primaire au virus VZV trop faible ou à une réponse mémoire inadéquate. Pour étayer notre hypothèse, nous avons comparé le taux d'anticorps contre la varicelle de façon annuelle entre 1997 et 2008 chez 97 enfants et 78 adultes VIH+. De plus, nous avons également comparé notre groupe d'enfants VIH+ avec un groupe d'enfants sains, matchés par âge et par sexe. Pour étudier la mémoire B spécifique au VZV, nous avons étudié la cinétique des anticorps anti-VZV dans le temps, et mesuré leur avidité, un marqueur important de la maturation de la mémoire antigène-spécifique des cellules B [28].

INTRODUCTION

Human immunodeficiency virus (HIV)-infected children exhibit a higher susceptibility to numerous infectious diseases. As an example, complications of acute VZV infection, such as bacterial superinfections, pneumonia, cerebellitis and encephalitis [2], are more frequent in HIV-infected children [3]. This higher rate of acute complications is likely to result from impaired immune responses, as reflected by a higher rate of vaccine failure to most immunizations [25]. Before the highly-active antiretroviral therapy (HAART) era, chickenpox recurred frequently [17, 22, 23]. More recently, Bekker and colleagues reported the frequent loss of antibodies elicited by wild-type infections, or immunizations in HAART-treated children [26]. Similarly, several HIV-infected children of the Swiss Mother-Child HIV (MoCHiV) cohort had undetectable anti-VZV levels despite previously confirmed VZV infection and the use of highly sensitive assays (unpublished data). This observation is intriguing: the persistence of VZV humoral immunity is generally life-long [11] as both community re-exposure and endogenous viral reactivation concur to reactivate anti-VZV memory responses and maintain humoral immunity [16]. Taken together, above observations suggest limitations in HIV-infected children's capacity to generate, maintain and/or reactivate immune memory.

In Switzerland, where VZV immunization is recommended to non-immune adolescents, VZV is an endemic disease and seroprevalence reaches 95% between 13 to 15 years of age [1]. In addition, endogenous VZV reactivation is frequent in HIV-infected children, as reflected by higher risks of zoster [24], which should contribute to reactivate and/or maintain immune memory. To define whether the waning of anti-VZV antibodies in HIV-infected children resulted from impaired primary responses, accelerated antibody loss and/or failure to reactivate anti-VZV memory responses, we assessed anti-VZV IgG antibodies in sera prospectively collected over a 10-year period in children of the MoCHiV cohort, as compared to HIV-infected adults and to age-matched healthy children. To study VZV-specific B cell

memory, we assessed the kinetics of anti-VZV levels over time, and measured their avidity, a useful marker of the maturation of antigen-specific memory B cells [28].

MATERIAL AND METHODS

Blood samples from HIV-1 infected children were prospectively collected and frozen on a yearly basis between 1997 and 2008. All HIV-infected children of the Swiss MoCHiV cohort, which follows almost all HIV-infected children in Switzerland, were enrolled through 6 referral centers (Geneva, Lausanne, Bern, Basel, Zurich, and St-Gallen). Inclusion criteria were being HIV-positive, belonging to the MoCHiV cohort, being followed by a pediatric referral center, and having ≥2 frozen serum samples at least one year apart. Exclusion criteria included age younger than one year old to avoid misinterpretation due to maternal antibodies and serum samples drawn within 12 months of intravenous immunoglobulins administration. HIV-1 infected adults were enrolled in one center. Medical history and social demographics, including birth date, gender, date of HIV diagnosis, age at sampling, yearly CD4 counts, yearly viral load [30], treatment type and duration were obtained from the cohort registry. Age- and gender-matched children undergoing minor elective surgery and no known medical condition likely to interfere with immune competence were recruited as healthy controls in one center.

Medical history focusing on VZV-related disease and demographics was obtained, and blood was drawn once. Healthy children were distributed in 4 quartiles based on the age of the HIV-infected children (A1: <8.2yr, A2: 8.2-11.5yr, A3: 11.5-15.5yr, A4: >15.5yr).

For the 3 groups, patients (and/or their legal guardian) gave a written consent for the use of these samples and their medical data. All data were analyzed anonymously. Immunization against VZV was not recommended during the study.

To identify risk factors for the waning of VZV antibodies, we compared initially VZV-positive HIV-infected children whose VZV antibodies subsequently disappeared to age-matched randomly selected HIV-infected children whose anti-VZV levels remained above threshold in all available samples.

This study was approved by the institutional Ethics Committee in all centers, and by the scientific boards of Swiss HIV Cohort Study (SHCS), and MoCHiV.

All serum samples were obtained between January 1997 and October 2008. Determination of IgG anti-VZV antibodies was performed in the Laboratoire de Vaccinologie (University Hospitals of Geneva) using an "in-house", enzyme-linked immunosorbent assay (ELISA) which compared favorably with the Virion® commercial kit (Virion Servion, Germany) (data not shown) (Posfay-Barbe KM et al, New assays to quantify varicella immunity in pediatric liver transplant recipients, poster #G1-429, 48th ICAAC/IDSA, 2008). To maximize the assay's sensitivity, 96-wells plates (Nunc Maxisorp™, Roskilde, Denmark) were coated with a lectin affinity purified VZV glycoprotein (East Coast Bio®, North Berwick, USA). Eight serial serum dilutions were added and incubated prior to the successive addition of biotin-conjugated goat anti-human IgG antibody (Anti-Human IgG Biotin (Sigma™, St-Louis, MO)), horseradish peroxidase (HRP) streptavidin (HRP-strepatvidin Conjugate (Zymed™, San Francisco, CA)), and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Roche Diagnostics GmbH™, Rotkreuz, Switzerland)) substrate. Optical densities (OD) were read at 405nm and analyzed by comparison to a standard curve included in each plate (Soft Max Pro software, version 5). Results were compared to two reference sera: a NIBSC standard (WHO international standard, 50 IU/L) and a standard from Merck, calibrated in VZV-gp Units, previously used in vaccine efficacy studies [31]. The assay's cut-off (30 IU/L) was defined as the mean plus two standard deviations of 72 negative samples. Results below this cut-off were arbitrarily given a value of 15 IU/L. Including both standards in a large number of assays, we established that in our assay a titer of 5 VZV-gp Unit/ml (suggested as a putative protective threshold following immunization [31]) corresponded to 33.1 IU/L of the WHO international standard (not shown). We thus experimentally defined 50 IU/L as the "minimal threshold" for our assay. An anti-

VZV booster response was experimentally defined as a >4-fold increase of anti-VZV levels between 2 consecutive samples or a >2-fold increase resulting into an absolute increase ≥ 1000 IU/L (not shown).

Antibody avidity increases during the maturation process of memory B cells, such that re-exposure to endogenous or exogenous antigen results in antibodies of higher avidity. Accordingly, antibody avidity is an indirect marker for the reactivation of memory responses [29]. Avidity of anti-VZV antibodies was tested by adding various dilutions (0 to 3M) of sodium thiocyanate to serum-containing antigen-coated wells, as previously described [32-34]. Results were expressed as avidity index (AI), defined as the thiocyanate concentration at which 50% of the VZV specific antibodies were eluted. As this marker may fail to identify differences due to a small pool of high- or low-avidity antibodies, analyses were completed by calculating the percentage of antibodies dissociated at each thiocyanate concentration (AVISCAN) [35, 36]. Anti-VZV avidity was measured in serum samples of HIV-infected and healthy children. In HIV-infected children, we then compared the first available VZV-positive sample (sample 1) and the last sample (sample 2) to identify changes in AI and AVISCAN over time.

All P-values were two-tailed. P-values <0.05 were considered statistically significant. Continuous variables were assessed by parametric (Student's T) or nonparametric (Mann Whithney U) tests when appropriate, whereas categorical data were assessed with chi-squared or Fisher's exact test. Linear regression was used for uni- and multivariate analysis of potential risk factors for low anti-VZV levels and AI, whereas conditional logistic regression was used to identify potential risk factors for a complete loss of VZV antibody. All variables were examined at univariate level. After this, all the variables with a P-value <0.25 by univariate analysis were included in the multivariate model [37].

Change in anti-VZV levels over time in HIV-infected children and HIV-infected adults were analyzed using mixed linear models. This statistical model takes into account the repeated measurement of each individual across time. We included as predictors the time of measure (linear trend) and the time of

measure squared (quadratic trend) to account for a downward trend that could be faster for high level of VZV antibody and slower for lower level. We also included the group of patients (HIV-infected children, and HIV-infected adults). Finally, we adjusted for age, CD4 T-cell counts and VZV serological reactivation.

Statistical analysis were performed with SPSS (v15.0; SPSS Inc., Chicago, Illinois), except longitudinal analyses performed with the lme statistical package of the R software, v2.9.2 [38].

RESULTS

Ninety-seven vertically HIV-infected children and 78 HIV-infected adults met study inclusion criteria (Table 1). In 2008, CD4 T-cell counts and percentage (P<0.001, for both) and HIV viral load (P=0.007) were higher in HIV-infected children than adults. There were more females among HIV-infected children than adults (P=0.002). As more than 90% of the children had not been immunized against VZV, their anti-VZV levels resulted from wild-type VZV infection. The mean anti-VZV titer was 767+/-1626 IU/L (median 264, range undetectable (UD)-11245) in HIV-infected children and 2603+/-6388 IU/L (median 1535, range UD-51172, P<0.001) in adults (Figure 1). Anti-VZV levels remained lower in HIV-infected children than adults (P<0.001) after exclusion of VZV-seronegative, possibly unexposed individuals. Twenty-one percent (20/97) of HIV-infected children had undetectable VZV antibodies, compared to only 3% (2/78, P<0.001) of adults. Among patients with detectable antibodies, 8% (6/76) of HIV-infected children were below the minimal threshold of 50 IU/L, compared to 1% (1/75) of HIV-infected adults (P=NS).

Between 1997 and 2008, we tested 541 pediatric samples (mean samples/patient: 5.4, range 2-11) and 440 adult samples (mean samples/patient 5.6, range 2-6).

At baseline, differences in anti-VZV levels, viral load and CD4 counts and percentage between HIV-infected children and adults were already significant (P <0.001, <0.001, <0.001 and 0.001 respectively) (not shown). In this cross-sectional analysis, neither age, gender, ethnicity, CD4 T-cell count or percentage, viral load, age at beginning of HAART, absence, presence or duration of HAART were predictive of lower anti-VZV levels in HIV-infected children.

Changes of anti-VZV levels in HIV-infected patients

To define whether anti-VZV antibodies decline faster in HIV-infected children than adults, we assessed their change over time in all subjects that were already exposed to VZV (484 samples from 85 children and 435 samples from 77 adults). Seventeen of 85 (20%) previously VZV-positive children failed to maintain anti-VZV levels >50 IU/L, compared to 2 of 77 (2.6%, P<0.001) adults. The odds ratio for antibody waning in children, adjusted for the CD4 count, was 17.74 (P<0.001; 95%CI: 4.36-72.25). These 17 HIV-infected children were compared with 54 randomly selected age-matched HIV-infected children who maintained anti-VZV levels >50 IU/L during the entire study period. The two groups were comparable in term of gender, age, CD4 T-cell count and duration of HAART. However, antibody waning occurred more frequently in children who were not treated by HAART (P=0.048), had a higher viral load (P<0.001) or a lower CD4 percentage (P=0.011). Univariate analyses demonstrated that a higher HIV viral load (P=0.001), the absence of HAART (P=0.037) and a lower CD4 percentage (P=0.027) were significantly associated with the failure to maintain VZV antibodies. With a multivariate analysis, a higher viral load remained associated with the waning of anti-VZV antibodies (P=0.011). Longitudinal analyses showed that the trend of anti-VZV levels over time was not significant in adults (Figure 2). Anti-VZV levels were lower in children at all time points (P<0.001), but did not decline faster than in adults and even slightly increased over time (P=0.01). This remained true when adjusting for age. Thus, the failure of 20% of HIV-1 infected children to maintain anti-VZV antibodies did not reflect a general pattern of antibody loss in HIV-infected children.

Anti-VZV responses in HIV-infected and healthy children

The lower anti-VZV levels of HIV-infected children could result from weak primary anti-VZV responses. We thus compared the anti-VZV levels of the 97 HIV-infected children to those of 97 gender- and age-matched healthy children (Table 1). The mean anti-VZV titer was lower in HIV-infected than healthy

children (mean 2302+/-3889 IU/L; median 1151, range UD-29489, P<0.001) (Figure 1). Anti-VZV antibodies were undetectable in only 5% (5/97) of healthy children, compared to 21% (20/97) of HIV-infected children (P=0.001). The difference of anti-VZV levels persisted after exclusion of VZV-seronegative children (P<0.001). Among VZV-seropositive children, 8% (6/76) of HIV-infected children were below the 50 IU/L threshold, compared to 0% of healthy children (P=0.006).

An increase of anti-VZV levels was seen with age in healthy children (P=0.004) but not in HIV-infected children (Figure 3). Accordingly, anti-VZV levels were lower in HIV-infected children in all age quartiles except for A1 . This difference persisted after exclusion of VZV-seronegative patients (not shown). This suggested that weaker anti-VZV primary responses are elicited when VZV infection occurs in older HIV-infected children or that anti-VZV levels fail to increase with age in HIV-infected children.

Antibody avidity as marker of anti-VZV memory responses

To distinguish between the induction of weaker primary responses and the failure of secondary anti-VZV responses in HIV-infected children, we compared the avidity of anti-VZV antibodies in HIV-infected and healthy children. The mean avidity index (AI) of anti-VZV antibodies was lower in the 77 VZV-positive HIV-infected than in the 92 VZV-positive healthy children (mean 2.12+/-0.69 vs 2.52+/-0.67, P<0.001). This was true for all age quartiles, statistical significance being reached for A2 and A3 quartiles (A1: P=0.078, A2: P=0.025, A3: P=0.003, A4: P=0.784). The proportion of low-avidity anti-VZV antibodies (eluted <1M) was higher in HIV-infected than in healthy children (28% vs 21%, P<0.001) whereas high-avidity antibodies (eluted >3M) were fewer in HIV-infected than in healthy children (29% vs 37%, P<0.001). We identified no influence of age, gender, CD4 T-cell count or percentage, viral load, duration of HAART, or age at beginning of HAART on the avidity of anti-VZV antibodies.

A lower avidity of anti-VZV antibodies in HIV-infected than healthy children could result from limitations of the primary induction of high-affinity antibodies, as observed in HIV-infected infants [39] and/or from a less effective reactivation of VZV-specific memory B cells. We thus compared the anti-VZV levels and avidity in the first and the last available serum samples of 63 HIV-infected children with 2 VZV-positive samples separated by at least one year (median interval 4.08 years, range 1.17-9.42 years). The mean AI increased from 1.93+/-0.58 to 2.14+/-0.66 between the 2 series of samples (P=0.039). In 36/63 (57.1%) children, anti-VZV levels showed no evidence of serological booster responses: the mean AI (1.93 VS 1.95, P=0.817) remained low and even declined in 12/36 (33.3%) children. Twenty-seven children had evidence of anti-VZV booster responses. This was associated with a significant increase of the anti-VZV AI (from 1.94 (SD 0.64) to 2.39 (SD 0.82), P=0.014) and decline of low-avidity antibodies (from 31% to 24%, P=0.006). Remarkably, this avidity maturation was only observed in half (13/27, 48.1%) of these children: these were younger (5.58 vs 8.14 yr, P=0.037), had slightly higher CD4 T-cell counts (1311 vs 874, P=0.067) and their HAART treatment was started earlier (4.16 vs 7.92 yr, P=0.065) than in the 14/27 patients with no maturation of the anti-VZV avidity.

In healthy children, we observed no correlation between anti-VZV levels and AI: some children maintained low levels of high avidity antibodies, indicating successful avidity maturation. In contrast, a significant correlation between anti-VZV levels and AI was present in HIV-infected children (P=0.001): anti-VZV levels were significantly lower in children with a lower AI, i.e. no evidence of successful memory B cell maturation / reactivation. Thus, the waning of anti-VZV antibodies in a significant proportion of HIV-infected children results from the failure to maintain and/or reactivate anti-VZV memory responses.

DISCUSSION

This study shows that the waning of anti-VZV IgG antibodies in HIV-infected children compared to HIV-infected adults and healthy children is associated with lower antibody avidity, reflecting the failure to efficiently generate, maintain or reactivate memory B cell responses.

Rapid antibody decline was previously reported following the immunization of HIV-infected patients [25]. This may also affect humoral responses elicited by natural infection and results in absent or low antibody levels [20]. The lower anti-VZV levels of HIV-infected children than adults were not explained by differences in age, gender, or ethnicity. A lower exposition rate to chickenpox is unlikely, as chickenpox is an endemic disease in our country, and HIV-infected patients have normal lives with regular peer-contact.HIV-infected children had higher CD4 T-cell counts than HIV-infected adults, as expected [40]. HIV viral load in children was higher than in adults, children being less often treated with HAART (88% vs 99%) and their infection being more often suboptimally controlled [41, 42]. At a single time-point, however, higher viral load and absence of HAART were not predictive of lower anti-VZV levels. Yet, HIV-infected children were almost 18 times more likely than HIV-infected adults to lose anti-VZV antibodies. Our longitudinal analysis indicates that high viral load, absence of HAART and low CD4 percentage are indeed associated with the waning of VZV-specific antibodies.

Lower anti-VZV levels in HIV-infected children were not due to a generally accelerated antibody loss: HIV-infected children had lower levels than adults during the entire 10-year study period and their antibody levels even slightly increased over time. These lower levels could reflect impaired primary responses [20, 25]. However, anti-VZV levels were lower in VZV-positive HIV-infected than in healthy children in all age quartiles except the youngest: this suggested that primary responses to VZV exposition

were only impaired in older children, possibly as a result of HIV disease progression, and/or that some HIV-infected children failed to maintain / reactivate anti-VZV immunity.

To define whether the failure to reactivate anti-VZV memory responses may explain the lower anti-VZV levels of HIV-infected children, we compared anti-VZV levels in HIV-infected and healthy children. Anti-VZV levels increased with age in healthy children but not in HIV-infected children: this suggests that endogenous reactivation and/or exogenous exposure lead to the reactivation and differentiation of memory B cells into antibody-secreting-cells in healthy more frequently or efficiently than in HIV-infected children. As avidity increases during the immune response and after reexposition to an antigen [27, 32, 43-45], we next assessed the avidity of anti-VZV antibodies: the observation of a lower avidity of anti-VZV antibodies in HIV-infected than healthy children confirmed the impairment of their anti-VZV memory responses. This is in accordance with the recent observation that HIV-1 infection impairs the induction and avidity maturation of measles antibodies elicited by the immunization of Zambian children [46]. How HIV infection impairs the avidity maturation is not yet elucidated. Although somatic mutation of immunoglobulin genes is a T cell-dependent phenomenon, we observed no correlation between anti-VZV levels, avidity maturation and CD4 T cell counts. However, HIV has multiple direct effects on B cell responses [47] and the percentage of memory B cells was even suggested as a marker of HIV disease progression [48]. Last, HIV uptake by follicular dendritic cells affects germinal centers [49] in which affinity maturation is initiated.

Remarkably, anti-VZV levels and avidity correlated in HIV-infected children - in contrast to healthy children in whom low concentrations of high-affinity antibodies were not rare. This indicates that healthy children maintain immune memory cells over a prolonged period, producing high avidity antibodies even in the absence of boosting by antigen exposure, whereas immune memory only persists in HIV-infected

children with high anti-VZV levels. Whether these children with high anti-VZV levels of high avidity antibodies have benefited from earlier / more frequent VZV exposure, thus reactivating and maintaining their memory B cells more efficiently, is an interesting possibility. In contrast, almost a quarter of our HIV-infected children experienced a decline in anti-VZV antibody avidity over time, which was associated with a decline in their anti-VZV levels. We were unable to demonstrate in what these patients were different. They had obviously not successfully maintained functional memory cells and therefore had to generate a "new primary response" of low magnitude and avidity at time of repeat exposure.

This study has some limitations. Precise information about chickenpox history is lacking: some VZV-seronegative children may have been considered as "unexposed" even if they were already exposed and subsequently lost their antibodies, and we could not assess possible correlations between age at VZV infection and immune responses. Specific risk factors for the loss of anti-VZV immunity could have been missed, although we examined many factors commonly used as markers of HIV disease and management. Finally, we obtained a single sample from healthy children and could therefore not compare the kinetics of their anti-VZV levels over time with that of HIV-infected patients.

This study has several clinical implications. Physicians taking care of HIV-infected children should be aware that a history of chickenpox or VZV immunization does not provide lifelong humoral immunity [18, 20], unlike in healthy children [11, 12, 18, 20]. Cell-mediated immunity (CMI) may contribute to the persistence of protection and/or reduce disease severity even in absence of antibodies [3, 15]. However, CMI may remain appropriate [19-21] or be altered even in HAART-treated children [18, 19], such that its contribution to protection may not be predicted for a given patient. As a consequence, it may be useful to obtain VZV serology at time of exposure, especially in children with delayed and/or partly effective treatment and persistent HIV viral load – identified here as a determinant of antibody loss. Due to our

study design, we couldn’t evaluate the risk of VZV disease recurrence in patients who lost anti-VZV humoral immunity nor check if booster VZV immunization reactivates immune memory cells. Finally, although VZV immunization is effective in HIV-infected children [50], its long-term efficacy should be repeatedly assessed through serologies as vaccine-induced responses are significantly weaker than those elicited by natural infection.

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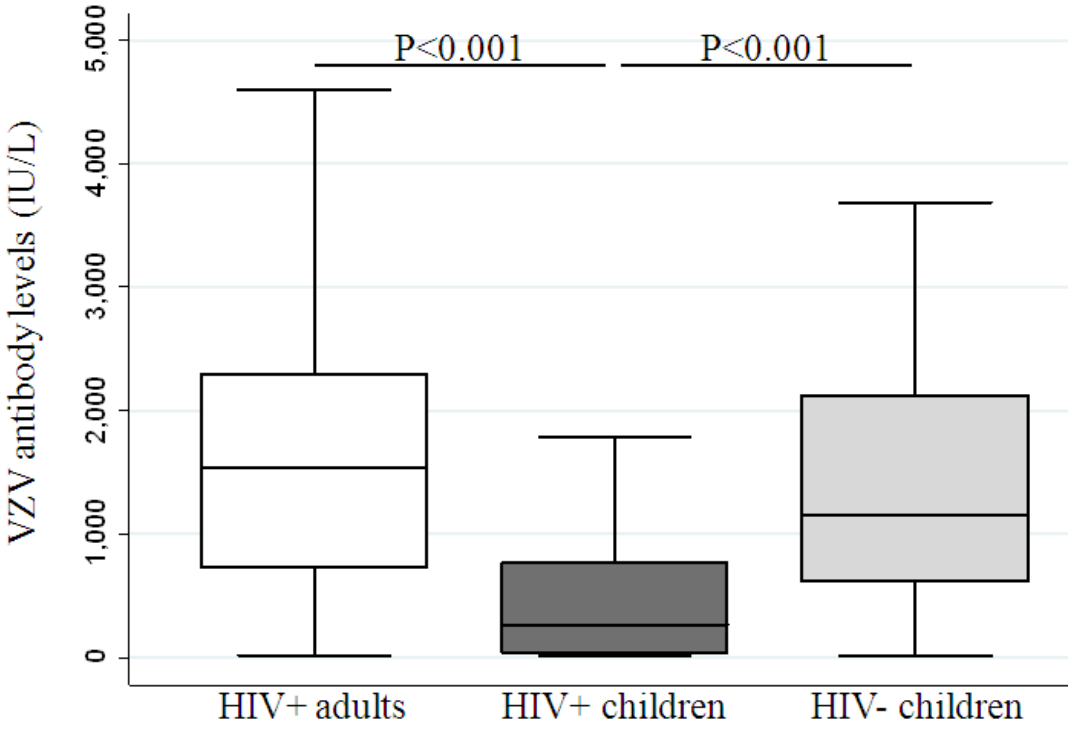
Table 1: Demographics in HIV-infected adults and children, and HIV negative children (2008).

		HIV+ adults (n=78)	**HIV+ children (n=97)**	**HIV- children (n=97)**
Female gender, % (n)		30 (23)	53 (51)	53 (51)
Mean age, years (SD)		49.3 (7.7)	12 (4.6)	13.2 (5.9)
Ethnic origin, % (n)				
Caucasian		76 (59)	34 (33)	unknown
african		15 (12)	33 (32)	
hispano-american		4 (3)	2 (2)	
asian		4 (3)	5 (5)	
missing information		1 (1)	26 (25)	
Mean viremia, copies/ml (SD)		6912 (36335)	18041 (75507)	-
Patients with undetectable viremia, % (n)		74 (58)	53 (51)	-
Mean CD4 T-cell percentage (SD)		22.5 (10.3)	30.1 (10)	-
Mean CD4 T-cell, abs. number/mm3 (SD)		467 (337)	804 (448)	-
Patients with CD4 T-cell count, % (n)				
<200/mm3		17 (13)	5 (5)	-
200-350/mm3		23 (18)	5 (5)	-
>350/mm3		58 (45)	85 (82)	-
missing information		2 (2)	5 (5)	-
CDC stage, % (n)				
A		27 (21)	29 (28)	-
B		40 (31)	31 (30)	-
C		33 (26)	37 (36)	-
missing information		0 (0)	3 (3)	-
Patients, % (n)				
Treated with HAART		99 (77)	88 (85)	-
Treated with ART only		1 (1)	3 (3)	-
without treatment		0 (0)	8 (8)	-
missing information		0 (0)	1 (1)	-
Mean age at beginning of	ART, years (SD)	37.1 (7.1)	4 (3.8)	-
	HAART, years (SD)	39.1 (7.6)	5.5 (4.3)	-

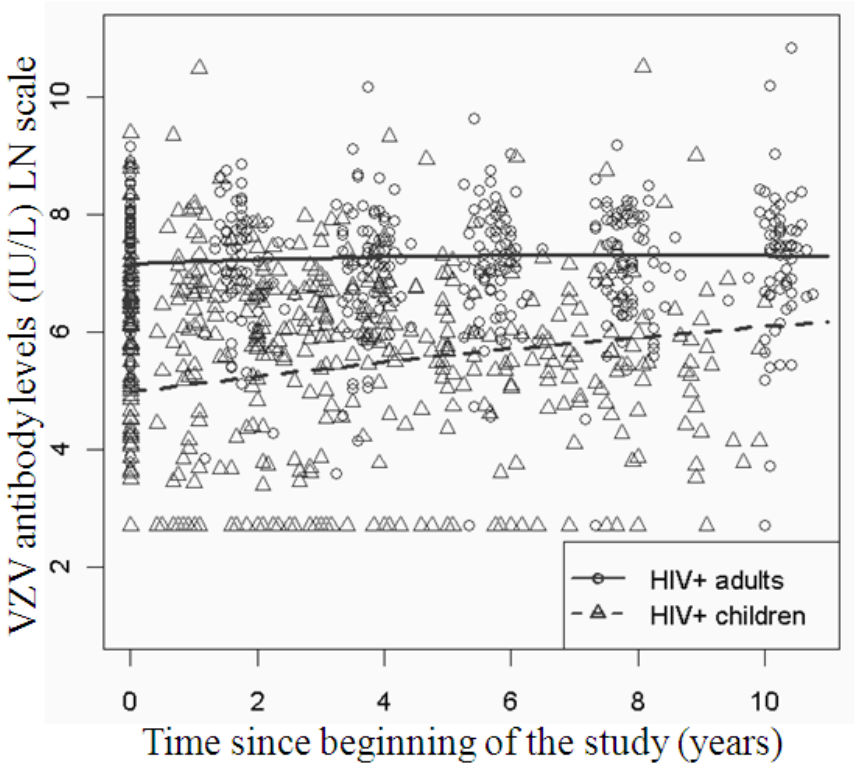
SD: Standard Deviation; CDC: Centers for Disease Control and Prevention; HAART: Highly Active Anti Retroviral Therapy; ART: Anti Retroviral Therapy

Figure 1: Comparison of anti-VZV levels between HIV-infected adults and children, and HIV negative children (2008).



VZV: Varicella-Zoster Virus, IU/L: International Units per liter

Figure 2: Predicted evolution in anti-VZV levels with time in HIV-infected adults and children.



VZV: Varicella-Zoster Virus, IU/L: International Units per liter, LN: Natural Logarithm

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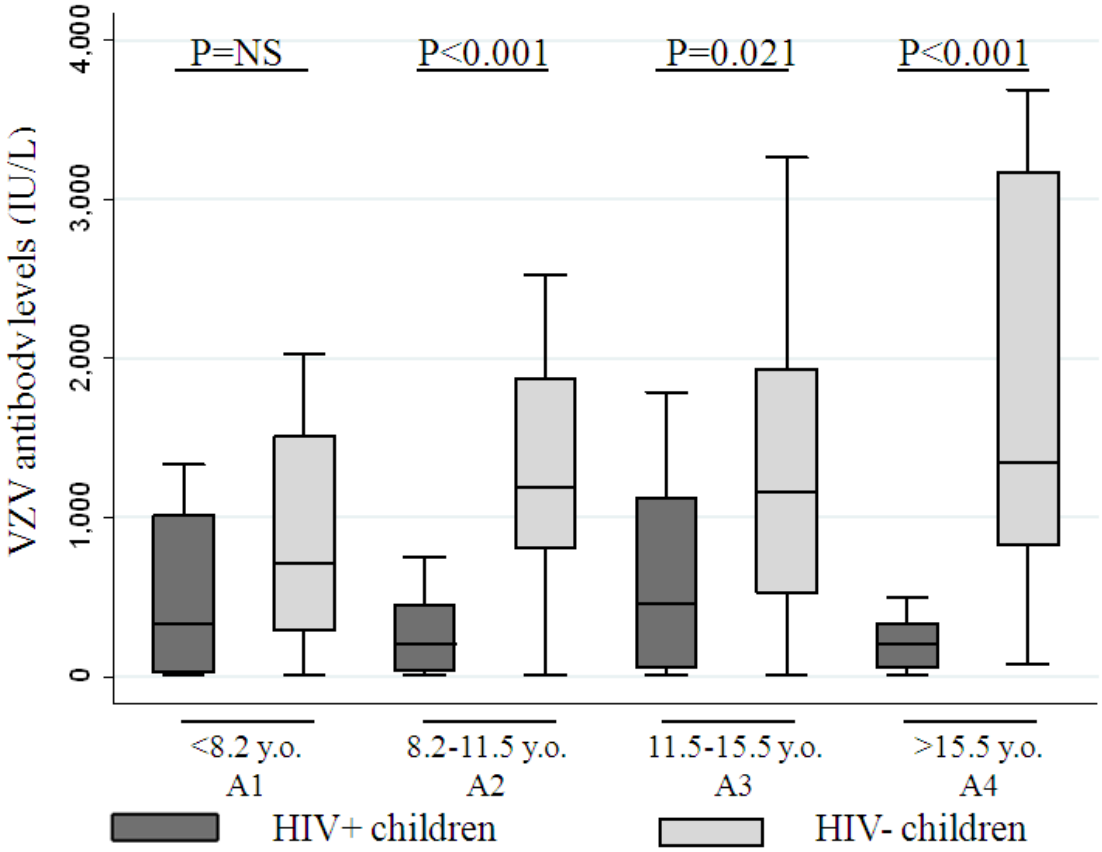
Figure 3: Anti-VZV levels in HIV-infected and healthy children by age quartiles (2008).



VZV: Varicella-Zoster Virus, IU/L: International Units per liter