

## Persistence of measles neutralizing antibody related to vaccine and natural infection acquired before HIV infection

M. B. ISA<sup>1,2†</sup>, J. V. PAVAN<sup>1\*†</sup>, P. SICILIA DON<sup>1</sup>, S. GRUTADAURIA<sup>3</sup>,  
L. C. MARTINEZ<sup>1</sup>, M. O. GIORDANO<sup>1</sup>, G. MASACHESSI<sup>1,4</sup>, P. A. BARRIL<sup>1,4</sup>  
AND S. V. NATES<sup>1</sup>

<sup>1</sup> Virology Institute 'Dr J. M. Vanella', School of Medical Sciences, National University of Córdoba, Córdoba, Argentina

<sup>2</sup> University Clinic 'Reina Fabiola', Córdoba, Argentina

<sup>3</sup> Institute 'Sanatorio Allende', Córdoba, Argentina

<sup>4</sup> National Council of Science of Technology, Argentina (CONICET)

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### SUMMARY

Little is known about long-lasting measles protective immunity when exposure to wild-type or vaccine measles virus precedes HIV infection. The results obtained suggest that measles immunity wanes and the lowest measles geometric mean titres (GMT) were significantly associated with measles vaccine-induced immunity in individuals that later developed HIV infection (86% prevalence, GMT 164 mIU/ml) compared to naturally induced immunity in HIV-infected adults (100% prevalence, GMT 340 mIU/ml,  $P=0.0082$ ) or non-HIV infected adults (100%, GMT 724 mIU/ml,  $P=0.0001$ ), and vaccine-induced immunity in non-HIV-infected adults (100%, GMT 347 mIU/ml,  $P=0.017$ ). The study was conducted in an area without wild-type virus circulation since 2000. The absence of virus circulating may alter the paradigm of lifelong immunity to measles virus after vaccination. As the proportion of HIV-infected individuals possessing only vaccine-induced immunity continues to grow, checking the status of measles immunity in this group is strongly recommended.

**Key words:** HIV-infected adults, measles, measles immunity.

### INTRODUCTION

Currently, in many countries of Africa, America, Asia and Europe measles virus continues to spread [1]. Therefore, the WHO has announced a new plan to eradicate measles and rubella throughout the world [2]. The Measles & Rubella Initiative is a five-part

plan to reduce measles deaths by 95% globally by 2015 and to eliminate both measles and rubella in the six WHO regions by 2020. Argentina achieved measles elimination in 2000, defined as the interruption of year-round endemic measles transmission. However, importation of measles into Argentina continues to occur [3]. There was one imported measles case in 2009, two imported cases and 15 import-related cases in 2010 in Buenos Aires province (after the World Cup in South Africa), and in 2011 there were two import-related measles cases in Rio Negro province and one imported case in Santa Fe province without confirmed secondary cases. In this context,

\* Author for correspondence: J. V. Pavan, MD, PhD, Virology Institute 'Dr J. M. Vanella', School of Medical Sciences, National University of Córdoba, Córdoba, Argentina.

(Email: jorgepavan@gmail.com or jpavan@mater.fcm.unc.edu.ar)

† These authors contributed equally to this work.

the maintenance of high level population measles immunity is essential to prevent measles outbreaks and to sustain measles elimination. Thus, measles immunity research is important in evaluating potential measles susceptibility groups; HIV-infected individuals are such a group. Little is known about the long-term persistence of protective measles antibody several years after natural infection or vaccination in HIV-infected adults receiving highly active antiretroviral therapy (HAART) and in the absence of re-exposure to the virus, i.e. in the American epidemiological scenario [4]. In order to better understand the interaction between HIV chronic infection and measles immunity, a study was undertaken to evaluate long-lasting natural and vaccine measles protective immunity in HIV-infected adults. The results obtained may provide data to facilitate a revision of measles immunization recommendations when the measles elimination goal has reached.

## METHODS

### Background

The present study was conducted in Córdoba City, Argentina. Measles vaccine was introduced in Córdoba city in 1971 for children aged 1–4 years (National Law No. 19968) as a discontinuous programme of immunization, reaching low overall vaccination coverage (between 50% and 70%). As consequence, measles remained endemic in Córdoba and outbreaks were registered. The current vaccination programme (one dose of measles vaccine at age 12 months) started in 1978 and it was only in 1993 that mass immunization became available for subjects aged 1–15 years.

### Patient population

During January–December 2010, a total of 97 serum samples, 55 obtained from HIV-infected individuals and 42 obtained from non-HIV-infected individuals were used to measure measles antibody. The patients were selected by a random process, using a random number table, including about four or five individuals per month from each studied group.

Sera of HIV-infected patients were from individuals who received HAART and underwent periodic controls at the University Clinic 'Reina Fabiola' and at the Institute 'Sanatorio Allende' (Córdoba city). Sera from non-HIV-infected individuals were obtained

from subjects donating to the Blood Bank of the National University of Córdoba, Argentina. A voluntary informed consent for participation in the study was obtained from each individual in accordance with the ethical principles of the Declaration of Helsinki and the additional requirements of local and national authorities.

Only one serum sample from each individual was obtained and sera were stored at  $-20^{\circ}\text{C}$  until they were tested for measles antibody.

The birth date of the subjects enrolled in this study was used as a proxy for individual source of measles immunity (natural measles infection or vaccination) according to the following criteria: (1) Individuals born before 1967 (aged  $>43$  years at the time of sampling) were presumed to have naturally acquired measles infection taking into account that most people in Argentina aged  $\geq 5$  years in 1971 were infected by wild measles virus and were never included in a vaccination programme. (2) Individuals born after 1978 (aged  $<33$  years at the time of sampling) were presumed to have acquired immunity after measles immunization. This population was re-immunized during the mass immunization campaign in 1993, given that the cohort born after 1978 was aged 1–15 years in 1993. The criterion of considering patients aged  $<33$  years old as vaccinated is supported by the vaccine coverage rates that the measles programme achieved since 1978 in Córdoba City, Argentina (between 81% and 93% in 1-year-old children) and up to 97% in the mass immunization campaign in 1993 in the population aged 1–15 years. According to the two criteria cited above, the total serum samples ( $n=97$ ) were assigned into four groups according to the presumed source of measles infection (natural or vaccination) and the HIV infection status (HIV-infected or non-HIV-infected individuals). The serum samples were categorized as follows:

*Group A1.* This group comprised of 17 samples obtained from HIV-infected adults, aged  $>43$  years (median 57 years, range 44–76 years) presumed to be naturally infected with measles.

*Group A2.* This group comprised of 38 samples obtained from HIV-infected adults, aged  $<33$  years (median 27 years, range 21–32 years) presumed to be vaccinated against measles.

*Group B1.* This group comprised of 24 samples obtained from non-HIV-infected adults, aged  $>43$  years (median 54 years, range 44–60 years) presumed to be naturally infected with measles.

*Group B2.* This group comprised of 18 samples obtained from non-HIV-infected adults, aged <33 years (median 25 years, range 18–32 years) presumed to be vaccinated against measles.

In addition, mean measles antibody titres were analysed against CD4 T-cell counts in the HIV-infected adults, according to the CDC 2012 classification system for HIV-infected adults [5]. Of the total HIV serum samples analysed ( $n=50$ ), the CD4 T-cell count record was available, distributed in the following categories: category I ( $>500$  cells/mm<sup>3</sup> of blood,  $n=31$ ); category II (between 200 and 499 cells/mm<sup>3</sup> of blood,  $n=15$ ) and category III ( $<200$  cells/mm<sup>3</sup> of blood,  $n=4$ ).

### Serological assay

A seroneutralization assay was performed as described by Nates *et al.* [6]. The highest dilution of serum that completely inhibited the cytopathic effect was regarded as the end point of antibody titration. Titres  $\geq 1:2$  were considered positive. Measles virus control and an internal reference preparation of measles antibody were used in every 96-well microculture plate and diluted in the same manner as the test samples. Results were converted to milli-International Units per millilitre (mIU/ml) of serum, based on parallel assay results. Titres  $\geq 32$  mIU/ml were defined as seropositive based on the test sensitivity at the first serum dilution of 1:2.

### Statistical analyses

Geometric mean titres (GMTs) were calculated only for individuals with detectable antibodies. The reciprocal of measles neutralization titres were transformed into base 2 logarithms and expressed as antilogarithms and GMTs were calculated. The normal distribution of data was checked. Proportional differences in measles prevalence between study groups were evaluated by Fisher's exact test. Comparisons of GMTs between groups were conducted using Student's *t* test, analysis of variance and Tukey's *post-hoc* test. *P* values  $<0.05$  were considered statistically significant.

## RESULTS

The frequency of measles antibody by study groups is summarized in Table 1. The global measles

Table 1. Frequency of measles neutralizing antibody by study group

Study group*	<i>N</i>	Measles seroprevalence†	95% CI
Group A1	17	100	90–100
Group A2	38	86.8‡	75.3–98.3
Group B1	24	100	91.5–100
Group B2	18	100	90–100
Total	97	94.8	

CI, Confidence interval.

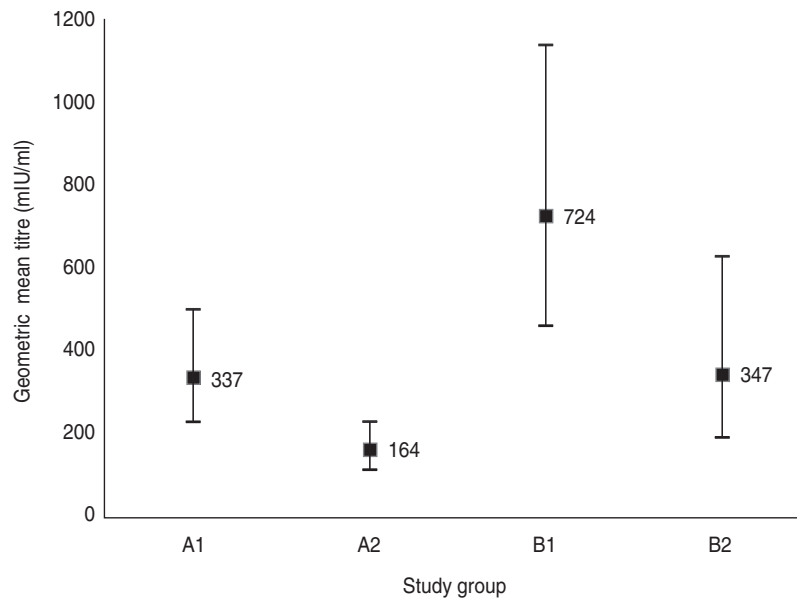
\* Group A1: HIV-infected individuals presumed to have naturally acquired measles infection. Group A2: HIV-infected individuals presumed to have acquired immunity after measles immunization. Group B1: Non-HIV-infected individuals presumed to have naturally acquired measles infection. Group B2: Non-HIV-infected individuals presumed to have acquired immunity after measles immunization.

† Neutralization antibody titre  $\geq 32$  mIU/ml was considered as protective antibody level.

‡  $P=0.008$

neutralizing antibody rate was 94.8% (range 86.8–100%). Measles neutralizing antibody was detected in 90.9% of HIV-infected adults, showing similar antibody prevalence compared to non-infected adults ( $P=0.07$ ). Nevertheless, within measles-vaccinated HIV-infected individuals, a small group of subjects susceptible to measles was detected. This was reflected in a statistically significant difference in measles seropositivity in HIV-infected individuals presumed to have derived their immunity from measles vaccination compared to the other study groups ( $P=0.008$ ). In contrast, similar measles antibody prevalence was detected in HIV-infected individuals, presumed to have measles natural immunity (group A1) and non-HIV-infected individuals (groups B1 + B2) ( $P>0.05$ ).

The antibody titre was measured in the 92 measles-seropositive individuals. The results are depicted in Figure 1. By comparison, the GMT in non-HIV-infected individuals [groups B1+B2: 530 mIU/ml, 95% confidence interval (CI) 260–760] was significantly larger than the GMT revealed in the HIV-infected individuals (groups A1+A2: 211 mIU/ml, 95% CI 161–276; 530 vs. 211,  $P=0.0001$ ). In addition, a significantly larger GMT level was detected in both HIV-infected and non-HIV-infected individuals who were presumed to have natural measles infection compared to measles-vaccinated individuals [groups A1 vs. A2 ( $P=0.008$ ), groups B1 vs. B2 ( $P=0.04$ )].



**Fig. 1.** Distribution pattern of measles antibody titre in the study groups. This shows the shape of the distribution, its central value (media) and 95% confidence intervals (vertical bars). Group A1: HIV-infected individuals presumed to have naturally acquired measles infection. Group A2: HIV-infected individuals presumed to have acquired immunity after measles immunization. Group B1: Non-HIV-infected individuals presumed to have naturally acquired measles infection. Group B2: Non-HIV-infected individuals presumed to have acquired immunity after measles immunization. Groups A1 vs. A2 ( $P=0.0082$ ), A1 vs. B1 ( $P=0.015$ ), A2 vs. B1 ( $P<0.0001$ ), A2 vs. B2 ( $P=0.017$ ), B1 vs. B2 ( $P=0.04$ ).

Finally, a slight difference, although not statistically significant, was revealed in measles GMT levels for HIV-infected individuals included in CD4 T-cell count category III (GMT=152) compared to those included in category II (GMT=164,  $P>0.05$ ). Similar results were seen in measles GMT levels for category II (GMT=164) compared to category I (GMT=232,  $P>0.05$ ) and category III compared to category I ( $P>0.05$ ).

## DISCUSSION

Seroprevalence studies of measles in HIV-infected adults provide different information than studies of measles in children, because measles vaccination or natural infection in most adults could have occurred before they became infected with HIV. A severe dysfunction of long-term serological memory has been suggested by vaccination studies in chronically HIV-infected individuals [7]. Recent published studies have reported that the loss of memory B cells and the decrease in memory B-cell function in chronically HIV-infected individuals cannot be reversed by HAART [8]. Under this new scenario, our study is the first to document the waning of measles neutralization antibody in relation to vaccine-acquired immunity prior to HIV infection.

Waning of measles antibody does not necessarily equate to waning immunity because cell-mediated responses are known to play an important role in protection. However, specific antibody detection is the only way to measure the protection against measles. Measurement of antibodies to measles virus by a neutralization assay is best correlated with protection from infection and remains the gold standard for measuring protective antibody levels [9]. Therefore, the results reported in our study mirror the level of protective immunity against measles virus.

Our results indicate that care should be taken when prevalence of measles antibody in HIV-infected populations is analysed because the source of measles immunity would set up the long-lasting immunity leading to lower measles antibody rates in HIV-infected individuals presumed to be vaccinated against measles. This could be explained by taking into account the fact that natural immunity to measles is more robust than immunity following immunization. Previously reported data [10, 11], does not discriminate measles seroprevalence according to the source of measles infection.

In the present study, significant lower neutralizing measles GMTs were detected in both groups of HIV-infected individuals (groups A1 and A2) compared to the similar non-infected study groups (groups

B1 and B2), respectively. Moreover, higher significant neutralizing measles GMTs were detected in the naturally infected group compared to the vaccinated group, when analysing the HIV-infected groups. The long-lasting neutralizing measles GMTs in HIV-infected individuals has not been previously reported for naturally infected or vaccinated sources of immunity; therefore, there is no data available to compare our results with. Lower measles GMTs in HIV-infected individuals could be explained by the fact that during HIV infection, a massive depletion of activated memory CD4 T-cells occurs throughout all compartments of the body [8, 12, 13]. Titanji *et al.* [14] found that memory B-cell loss is a progressive event occurring in the course of HIV infection, not readily apparent during the early stages of HIV infection, but very significant at the chronic stage of infection.

Finally, in agreement with other studies, there was no correlation between changes in measles antibody titres and CD4 T-lymphocyte count [10].

These results suggest that measles immunity following natural measles infection may persist in HIV-infected adults in geographical areas without measles virus circulation. By contrast, many adults infected with HIV, presumed to be vaccinated against measles many years before becoming infected with HIV, appear to be insufficiently protected against measles. In this way, the importance of knowing the measles immune status of HIV-infected individuals is likely to be critical at the individual level to prevent measles infections if such patients are exposed.

In addition, concern may exist because the proportion of the population possessing only vaccine-induced immunity continues to grow. Similarly, opportunities for boosting caused by wild-type measles exposure are becoming increasingly rare, and waning antibody titres could, over time, result in an accumulation of measles-susceptible individuals in the population.

In order to achieve a high level of measles population immunity, a control programme should be maintained at 93–95%. Consequently, there would be little margin for even small increases in the number of susceptible people. Therefore, checking the status of measles immunity in HIV-infected individuals is strongly recommended. The importance of waning humoral immunity as an impediment to measles elimination remains an open question.

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## DECLARATION OF INTEREST

None.

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