Immune response to measles vaccine in Peruvian children

Norma L. Bautista-López,[1] Abraham Vaisberg,[2] Rosa Kanashiro,[3] Herminio Hernández,[4] & Brian J. Ward[5]

Objective To evaluate the immune response in Peruvian children following measles vaccination.
Methods Fifty-five Peruvian children received Schwarz measles vaccine (about 10^3 plaque forming units) at about 9 months of age. Blood samples were taken before vaccination, then twice after vaccination: one sample at between 1 and 4 weeks after vaccination and the final sample 3 months post vaccination for evaluation of immune cell phenotype and lymphoproliferative responses to measles and non-measles antigens. Measles-specific antibodies were measured by plaque reduction neutralization.
Findings The humoral response developed rapidly after vaccination; only 4 of the 55 children (7%) had plaque reduction neutralization titres <200 mIU/ml 3 months after vaccination. However, only 8 out of 35 children tested (23%) had lymphoproliferative responses to measles antigens 3–4 weeks after vaccination. Children with poor lymphoproliferative responses to measles antigens had readily detectable lymphoproliferative responses to other antigens. Flow cytometric analysis of peripheral blood mononuclear cells revealed diffuse immune system activation at the time of vaccination in most children. The capacity to mount a lymphoproliferative response to measles antigens was associated with expression of CD45RO on $CD4^+$ T-cells.
Conclusion The 55 Peruvian children had excellent antibody responses after measles vaccination, but only 23% (8 out of 35) generated detectable lymphoproliferative responses to measles antigens (compared with 55–67% in children in the industrialized world). This difference may contribute to the less than uniform success of measles vaccination programmes in the developing world.

Keywords Measles vaccine/immunology; Immunity, Cellular; Antibody-producing cells; Infant; Seroepidemiologic studies; Peru (*source: MeSH*).

Mots clés Vaccin antimorbilleux/immunologie; Immunité cellulaire; Cellule productrice anticorps; Nourrisson; Etude séro-épidémiologique; Pérou (*source: INSERM*).

Palabras clave Vacuna antisarampión/inmunología; Inmunidad celular; Células productoras de anticuerpos; Lactante; Estudios seroepidemiológicos; Perú (*fuente: BIREME*).

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Voir page 1044 le résumé en français. En la página 1044 figura un resumen en español.

Introduction

The correlates of immunity for natural measles and measles vaccination are not fully understood. Although high titres of neutralizing antibodies can protect against infection, evidence from case reports (*1, 2*), investigations of outbreaks in humans (*3*), and animal studies (*4*) suggests that some individuals who fail to develop or sustain high antibody titres after measles vaccination can nonetheless be protected against natural infection. Most people with limited or transient production of antibodies after revaccination have strong measles-specific lymphoproliferative responses (*5*) and patients who have had bone marrow transplants and have pre-existing lymphoproliferative memory of measles antigens make few or no antibodies in response to revaccination (*6*). When both humoral and lymphoproliferative responses were measured after measles vaccination, similar patterns were seen in both human and non-human primates (*4–9*). In the absence of maternal antibodies, virtually all subjects mount a strong humoral response (>95%), and more than half (56–66%) have detectable lymphoproliferative responses to measles antigens (*4–9*).

With few exceptions (*10*), detailed studies of the immune response to standard-titre measles vaccina-

[1] Graduate student, McGill University Department of Parasitology, Montreal, Quebec. Currently a postdoctoral fellow at the University of Alberta, Edmonton, Canada.

[2] Professor of Biology and Dean of the Faculty of Science, Universidad Peruana Cayetano Heredia, Lima, Peru.

[3] Research Associate, Department of Biology, Universidad Peruana Cayetano Heredia, Lima, Peru.

[4] Professor and Chief of Paediatrics, Department of Pediatrics, Universidad Peruana Cayetano Heredia, Lima, Peru.

[5] Director of McGill University Division of Infectious Diseases, and Associate Professor of Medicine and Microbiology, McGill University, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada (email: brian.ward@mcgill.ca). Correspondence should be addressed to this author.

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tion have been conducted in the industrialized world, where these vaccines have been successful. Although measles vaccination is highly effective in children in the developing world when performed in carefully monitored settings (*11–13*), vaccination programmes in the developing world have not been uniformly successful (*14*). Among the many factors that may contribute to this discrepancy are the strain of the vaccine, the handling and quality of the vaccine, and host factors, such as age at vaccination, maternal antibody status, nutritional status, and whether there are intercurrent infections (*15–17*).

In this study, the phenotype and activation state of peripheral blood mononuclear cells (PBMCs) were examined in Peruvian children at the time of vaccination, and immune responses to measles antigens were monitored at 1–4 weeks (cellular and humoral responses) and at 3 months (humoral only) after vaccination.

Methods

Participants

Children were recruited during routine vaccination clinics associated with the Hôpital del Rimac (affiliated to Universidad Peruana Cayetano Heredia) in San Martin de Porras, Lima, Peru. Socioeconomic conditions in this sector of Lima vary widely; it is home to middle-class professionals and newly arrived migrants. The study was approved by the ethics committees of both the Montreal General Hospital Research Institute (McGill University, Montreal, Quebec) and Universidad Peruana Cayetano Heredia (Lima, Peru).

Fifty-five children (25 boys and 30 girls) undergoing routine measles immunization with Schwarz vaccine ($10^{3.0}$–$10^{3.7}$ plaque forming units (pfu)) at 9 months of age were studied. After informed written consent was obtained from the parent or parents, heparinized blood samples (1–5 ml) were obtained by venepuncture immediately before vaccination (n = 55) and at a single, randomly selected time between 1 and 4 weeks after vaccination: at week 1, n = 5; week 2, n = 15; week 3, n = 17; and week 4, n = 18. A sample was collected from all children 3 months after vaccination.

Vaccination booklets were not inspected, but vaccine coverage in this community exceeds 90% for BCG (bacillus Calmette–Guérin) and at least two DPT (diphtheria–pertussis–tetanus) immunizations at 9 months of age (*18*).

For comparison we have included unpublished flow cytometric data for PBMCs isolated from 57 Canadian children (30 boys and 27 girls) immediately before vaccination at about 13 months of age (416 $\pm$ 2.9 days). The recruitment of these children and their pattern of humoral and cellular immune responses following vaccination with MMR (measles–mumps–rubella) II (Merck, West Point, PA) have been reported (*9*).

Samples

Plasma samples and PBMCs were aliquoted and stored until used in assays as previously described (*5*). Briefly, the PBMCs were isolated by differential density centrifugation, enumerated by trypan blue exclusion, aliquoted, and cryopreserved in liquid nitrogen at –180 °C. The cells were subsequently batched and sent to McGill University, Montreal, Quebec, in nitrogen vapour for further analysis. Samples taken at 3 months after vaccination were left to clot overnight at 4 °C before centrifugation (600 *g* for 10 minutes); the supernatant was then aliquoted and frozen at –70 °C until use.

Measurement of neutralizing antibodies

Measles-specific neutralizing antibodies were measured in the Virology Laboratory of the Universidad Peruana Cayetano Heredia (Lima, Peru) by plaque reduction neutralization as described elsewhere (*12, 19*). The antibody titres are reported in milli-International Units (mIU)/ml, based on extrapolation from a standard curve generated using the WHO standard antiserum (5 IU of #66/202; WHO International Laboratory for Biological Standards, South Mimms, England) that was included in each assay run. The minimum antibody concentration detectable was 33 mIU/ml. Based on data suggesting that concentrations >200 mIU/ml protect against natural infection (*20, 21*), children with titres $\geqslant$200 mIU/ml were considered to be seropositive.

Antigen preparations

Measles antigen was prepared from a wild-type virus (CHI-1, gift of W. Bellini, Centers for Disease Control and Prevention, Atlanta, GA) as previously described (*5*). A clarified and filtered Vero cell lysate served as the control antigen. The other antigens used were: tetanus toxoid (gift of B. Latham, Boston, MA), diphtheria toxoid, whole pertussis antigen (gifts of R. Wittes, Connaught Laboratories, Willowdale, ON), and whole, inactivated BCG (gift of D. Radziok, Montreal, Quebec). The protein content of the antigen preparations was estimated using a modified Bradford assay (BioRad Laboratories, Hercules, CA).

Lymphoproliferation assay

Antigen-specific cell-mediated immunity was measured using a solid-phase lymphoproliferation assay as previously described (*5, 9*). Briefly, flat-bottomed, 96-well plates (Nunc, Roskilde, Denmark) were coated with antigens (50 µl per well at 10 µg/ml); control and test antigens were also coated on the same plates. PBMCs resuspended in RPMI 1640 media containing 5% (v/v) heat-inactivated autologous plasma were distributed (2×10^5 cells per well) and cultured for 6 days before the addition of 1 µCi of [^{3}H]thymidine (ICN, Costa Mesa, CA). DNA was harvested on glass-fibre filters 24 hours later and counts per minute (cpm) were used to calculate stimulation indices (stimulation index = cpm for antigen-stimulated wells/cpm for the

Table 1. **Molecular markers used in flow cytometric analysis and their principal functions**

Cell type	CD designation	Function
$CD4^+$ T-cells	CD11 (LFA-1)	Integrin adhesion molecules, co-stimulatory
	CD25	IL[a]-2 receptor, activated T-cells
	CD28	Co-stimulatory molecule, binds to CD80 and 86
	CD30	Associated with a T-helper 2 cytokine production
	CD45RA	Naive T-cell marker
	CD45RO	Memory T-cell marker
	CD122	IL-2Rβ, constitutive expression
	HLA-DR	MHC[b] II upregulated during T-cell activation
$CD8^+$ T-cells	CD11, CD25, and CD30	(as indicated for $CD4^+$ T-cells)
	CD122 and HLA-DR	Activation marker
	CD38	Early activation marker
	CD69	Activation marker
B-cells (CD19)	CD23	Activation marker
	CD25	(as indicated for $CD4^+$ T-cells)
	CD80	Ligand for CD28, co-stimulatory molecule
	CD86	Ligand for CD28, co-stimulatory molecule
Natural killer cells (CD16)	CD69	(as indicated for $CD8^+$ T-cells)
Macrophages (CD14)	CD38, CD80, and CD86	(as indicated for $CD4^+$ and $CD8^+$ T-cells)
	CD54 (ICAM-1)	Activation marker, binds to CD11

[a] IL = interleukin.

[b] MHC = major histocompatability complex.

Table 2. **Development of the immune response to measles antigens in 55 Peruvian children after administration of standard titre measles (Schwarz) vaccine at 9 months of age**

Time sample taken	Humoral response	Cellular response
	PRN[a, b] (mIU/ml)	Stimulation index[a, c]
Before vaccination	36.0 ± 8.7	1.08 ± 0.11
1–2 weeks after vaccination	296.5 ± 105.5	1.99 ± 0.40
3–4 weeks after vaccination	1266.3 ± 156.3	3.60 ± 0.80
3 months after vaccination	1212.0 ± 144.7	NT[d]

[a] Results are expressed as mean values ± standard error of the mean.

[b] PRN = plaque reduction neutralization. PRN titres ⩾200 mIU/ml are considered to be protective.

[c] Indices ⩾3 are considered to be significant.

[d] NT = not tested.

controls). A score of ⩾3 on the stimulation index was considered to indicate an important lymphoproliferative response. In North American children lymphoproliferative responses to measles antigen are first detectable 1–2 weeks after vaccination and are well developed by 3–4 weeks post vaccination (*9*).

Direct immunofluorescence staining

Cryopreserved cells were thawed at 37 °C, washed twice in HBSS (Hank's balanced salt solution (300 *g* for 10 minutes)), and resuspended in 200 µl PBS (phosphate buffered saline) containing 5% (w/v) bovine serum albumin (Boehringer Mannheim, Indianapolis, IN). Identical protocols for isolation of PBMCs, cryopreservation, and flow cytometric analyses were used for both the Peruvian and Canadian samples. Aliquots were triple stained, according to the manufacturers' instructions (Becton Dickinson, San Jose, CA; Dako, Carpinteria, CA; or Pharmingen, San Diego, CA), using combinations of monoclonal mouse antibodies conjugated with FITC (fluorescein isothiocyanate), PE (phycoerythrin), or perCP (peridinin chlorophyll A); the specificities of these antibodies are listed in Table 1. After incubation for 30 minutes at 4 °C, the cells were washed in cold PBS, resuspended in 150 µl PBS with 1% paraformaldehyde (v/v), and kept at 4 °C until use. Three-colour fluorescence analysis was performed with a FACScan flow cytometer (Becton Dickinson). For each sample, 15 000 events were acquired using log-amplified fluorescence and linearly amplified side-scatter and forward-scatter signals. All samples were analysed by setting appropriate side-scatter and forward-scatter gates around the lymphocyte and monocyte populations.

Statistical analysis

The data were first reviewed for distribution patterns, and skewed data were log transformed to approximate a normal distribution. Comparisons between groups were performed using Student's *t* test. When data remained skewed after log transformation, the Mann–Whitney U test was used. Correlations were calculated using the Spearman Rank correlation coefficient. All analyses were performed using Statview 512 software (SAS, Cary, NJ).

Results

Results are expressed as mean values ± standard error of the mean.

Humoral response: neutralizing antibody titres

Low concentrations of maternal neutralizing antibodies were detectable in 14 of the 55 samples (25%) taken before vaccination (plaque reduction neutralization (PRN) titre 36 ± 8 mIU/ml, range 8–235). Protective concentrations of measles-specific neutralizing antibodies developed rapidly after vaccination (Table 2). For example, 11 of 15 samples (73%)

obtained 2 weeks after vaccination had titres > 200 mIU (564.2 ± 196 mIU/ml). Three months after vaccination, 51 of 55 children (93%) had titres ⩾200 mIU/ml (1214 ± 145 mIU/ml, $P < 0.0001$ compared with pre-vaccination). Thus only 4 of 55 samples (7%) failed to achieve protective titres (having titres of 104.3 ± 45.4 mIU/ml). Children with detectable maternal antibodies had lower titres at 3 months than those without such antibodies (985.4 ± 287 mIU/ml vs 1292 ± 168 mIU/ml), but this difference did not reach statistical significance. Neither the rate of development nor the magnitude of the antibody response to vaccination differed significantly between boys and girls.

Cellular response: measles-specific lymphoproliferation

As expected, lymphoproliferative responses to measles antigens were undetectable before vaccination (stimulation index 1.1 ± 0.1) (Table 2). Significant lymphoproliferative responses to measles antigens first became apparent in the second week after vaccination (4 of 15 samples had stimulation indices ⩾3). By 3–4 weeks after vaccination 8 out of 35 children (23%) had a stimulation index ⩾3 (3.6 ± 0.8 vs 1.0 ± 0.1 in the remaining children). Children who had maternal antibodies present at the time of vaccination had slightly lower lymphoproliferative responses at 3–4 weeks than those without maternal antibodies (2.4 ± 0.7 vs 3.8 ± 1.0), but this difference did not reach statistical significance. There was no difference between boys and girls in lymphoproliferative responses to measles antigen.

Cellular responses to other antigens

Despite the weak cellular response to measles antigens, the Peruvian children had vigorous lymphoproliferative responses to other antigens. Roughly half of the children had significant responses (stimulation index ⩾3) to BCG (56% (31/55), index 9.8 ± 2) and tetanus toxoid (49% (27/55), index 11.7 ± 1.8). Fewer children had a stimulation index ⩾3 in response to whole pertussis antigen (29% (14/49, index 4.5 ± 1.1) and diphtheria toxoid (18% (10/55), index 2.4 ± 0.5). Although children with a measles-specific index ⩾3 generally had stronger lymphoproliferative responses to other antigens (Table 3), many of the children with little or no cellular response to measles antigens after vaccination had readily detectable lymphoproliferative responses to other antigens. Correlations between the cellular responses to measles and other antigens were barely significant or of borderline significance (e.g. for BCG, $r = 0.24$, $P<0.05$; for tetanus toxoid, $r = 0.16$, $P = 0.1$).

Relationship between humoral and cellular responses

At 3–4 weeks after vaccination, high antibody titres and strong cell-mediated immune responses ($Ab^H CMI^H$) were detected in only 8 of 35 children (23%) (Table 4). Almost three-quarters of these children (25/35, 71%) had strong humoral responses without evidence of measles-specific lymphoproliferation ($Ab^H CMI^L$); and 2 of the 35 (6%) had neither significant humoral nor lymphoproliferative responses to measles antigens ($Ab^L CMI^L$). These two children remained Ab^L at 3 months after vaccination (PRN titres 125.5 ± 67.5 mIU/ml). Antibody titres tended to be lower in $Ab^H CMI^H$ children, but this difference did not reach statistical significance ($Ab^H CMI^H$: 1287 ± 176 vs $Ab^H CMI^L$:1483 ± 355).

PBMCs and co-stimulatory molecules

With the exception of basic subset measures of PBMCs (for example, enumeration of T-cells and $CD4^+/CD8^+$ ratios), little is known about activation and co-stimulatory molecule expression on PBMCs in healthy children in the developing world. As a result, much of the data is descriptive. Basic subset proportions (40% $CD4^+$ T-cells, 27% $CD8^+$ T-cells, 20% B-cells, and 6% monocytes) as well as $CD4^+/CD8^+$ ratios (1.7 ± 0.1) were similar to those reported for children in the developing world at 9–12 months of age (*22–26*). Measles vaccination had no impact on the subset distribution of PBMCs as has been reported (*27*) and it had little overall effect on the expression of activation and co-stimulatory molecules (data not shown).

The most striking aspect of the cytometric analysis was the marked state of activation of PBMCs at the time of vaccination. Compared with 13-month-old Canadian children at the time of vaccination, the Peruvian children had diffuse activation of $CD4^+$ T-cells, $CD8^+$ T-cells, B-cells, and monocytes (Fig. 1). None of the Peruvian children was seriously ill at the time of vaccination.

Compared with the Canadian children, the Peruvian children also had markedly higher expression of CD45RO on $CD4^+$ T-cells (18 ± 4% vs 31 ± 2%, $P <0.0001$), suggesting that there is a much more rapid acquisition of T-cell antigens in the developing world. There were also marked differences between the Peruvian and Canadian children in terms of co-stimulatory molecule expression at the time of vaccination. Overall, Peruvian children had lower CD28 expression on $CD4^+$ T-cells (94.8 ± 0.9% vs 96.0 ± 99%, $P = 0.09$) and on $CD8^+$ T-cells (59.4 ± 2.3% vs 85.8 ± 1.2%, $P <0.0001$); Peruvian children had higher expression of CD80 ($P <0.0001$) and CD86 ($P<0.0001$) on B-cells and CD86 ($P = 0.06$) on monocytes. Unfortunately, antibodies for CTLA-4 (CD152), the other T-cell ligand for CD80/86, were not commercially available at the time this study was performed.

Maternal antibodies and lymphocyte activation state

Compared with children who had no maternal antibodies at the time of vaccination, children with detectable maternal antibodies had significantly higher expression of CD45RA on $CD4^+$ T-cells

Table 3. **Cellular responses to non-measles antigens in Peruvian children grouped according to their cellular response to measles antigens**

Antigen[a]	Stimulation index[b, c]		*P*-value
	< 3 for measles antigens	⩾3 for measles antigens	
Tetanus toxoid	9.2 ± 1.8[b]	27.2 ± 15.5	<0.03
BCG[d]	8.2 ± 1.9	34.5 ± 14.0	<0.001
Diphtheria toxoid	1.7 ± 0.2	3.2 ± 1.2	<0.04
Whole pertussis	1.6 ± 0.2	17.9 ± 13.2	<0.008

[a] Lymphoproliferative responses to non-measles antigens were determined before measles vaccination at 9 months of age.

[b] See footnote a, Table 2.

[c] See footnote c, Table 2.

[d] BCG = Bacillus Calmette–Guérin.

Table 4. **Numbers of Peruvian children (*n* = 35) with humoral and cellular responses to measles antigens 3–4 weeks after vaccination**

Cellular response	Humoral response	
	PRN[a] <200 mIU/ml	PRN ⩾200 mIU/ml
Stimulation index[b] <3	0 (0)[c]	8 (23)
Stimulation index ⩾3	2 (6)	25 (71)

[a] See footnote b, Table 2.

[b] See footnote c, Table 2.

[c] Figures in parentheses are percentages.

(91.6 ± 1.3 % vs 88.4 ± 1.3 %, $P<0.05$) and CD23 on B-cells (71.6 ± 4.5% vs 60.6 ± 2.9%, $P<0.01$), and lower expression of CD25 on $CD4^+$ T-cells (9.4 ± 0.9% vs 12.6 ± 1.2%, $P<0.04$).

PBMC markers and immune responses

The presence of a detectable lymphoproliferative response to measles antigens among the Peruvian children was strongly correlated with the expression of CD45RO on $CD4^+$ T-cells ($r = 0.46$, $P<0.007$). Compared with Ab^HCMI^L children, those who developed both humoral and cell-mediated immune responses (Ab^HCMI^H) had significantly greater expression of CD45RO on $CD4^+$ T-cells (33.9 ± 1.8% vs 28.8 ± 2.1%, $P<0.05$). Expression of CD45RO on $CD4^+$ T-cells was in turn associated with increased expression of a constellation of activation markers on other PBMC subsets, including CD38 ($r = 0.487$, $P<0.0002$) and HLA-DR ($r = 0.24$, $P = 0.07$) on $CD8^+$ T-cells, CD69 on natural killer cells ($r = 0.258$, $P = 0.06$), CD80 ($r = 0.226$, $P = 0.09$) and CD23 ($r = 0.504$, $P<0.0001$) on B-cells, and CD38 on monocytes ($r = 0.351$, $P<0.008$).

Discussion

Vaccine-induced immunity in the developing world

Although a substantial number of immunological investigations after natural measles infection have been conducted in the developing world (*27, 28–32*), few detailed studies of vaccine-induced immunity have been performed (*11, 12, 26*). One of the most striking observations in this study was the relative weakness of the lymphoproliferative responses induced by vaccination in Peruvian children (23% scored ⩾3 on the stimulation index) compared with the reported values for North American children vaccinated at either 13 months of age (61% reached stimulation index ⩾3) (*9*) or 6 months of age (63% reached ⩾3) (*8*). However, the Peruvian children mounted excellent humoral responses to vaccination with antibody titres equal to or superior to those reported for North American children (*8, 9*). Although lymphoproliferation was measured at only one point after vaccination, our recent description of the kinetics of this response in Canadian children suggests that measles-antigen-specific lymphoproliferation is fully developed by 3–4 weeks after vaccination and persists at detectable concentrations in many children for at least 5–10 years (*9*).

This combination of strong antibody and weak lymphoproliferative responses to measles antigens has also been described 4–5 years after vaccination in children originally enrolled in trials of high-titre measles vaccines in Haiti, Senegal, and Sudan (*33–35*). The vaccines administered in these studies varied by strain (Moraten, Schwarz, Edmonston-Zagreb, and Biken-CAM), viral titre ($10^{3.7}$ to $10^{6.2}$ pfu), and age at vaccination (5–24 months); this suggests that factors other than strain, dose, and age at vaccination contribute to the relatively poor cellular response to measles antigens among children in the developing world. However, other aspects of cellular responsiveness (such as antigen-specific cytokine production and cytotoxic T-lymphocyte activity) were not measured in this or most of the previous studies (*8, 9, 33–35*). Measles-specific cytotoxic T-lymphocyte (*11*) and cytokine responses (*36*) are certainly measurable parameters after vaccination in young children in both the developing and industrialized world. The limited lymphoproliferative responses in this study were not caused by international transport of cells or a general inability of Peruvian children to mount such responses, since the stimulation index for non-measles antigens (such as tetanus toxoid and pertussis) were comparable to those seen in North American children (*9, 37*).

It is likely that many factors contribute to the discrepancy between the general success of measles control efforts in the industrialized world and the ongoing struggle with the virus in the developing world. These include straightforward issues of resources and infrastructure (for example, the maintenance of the cold chain (*38*) and the purchase of suboptimal products (*15*)) but may also include

more subtle limitations related to the target populations themselves, such as maternal antibody status, nutritional status, and whether there are intercurrent infections. Certainly, maternal antibody status has long been recognized as a major confounding factor in measles vaccination campaigns (*39–41*).

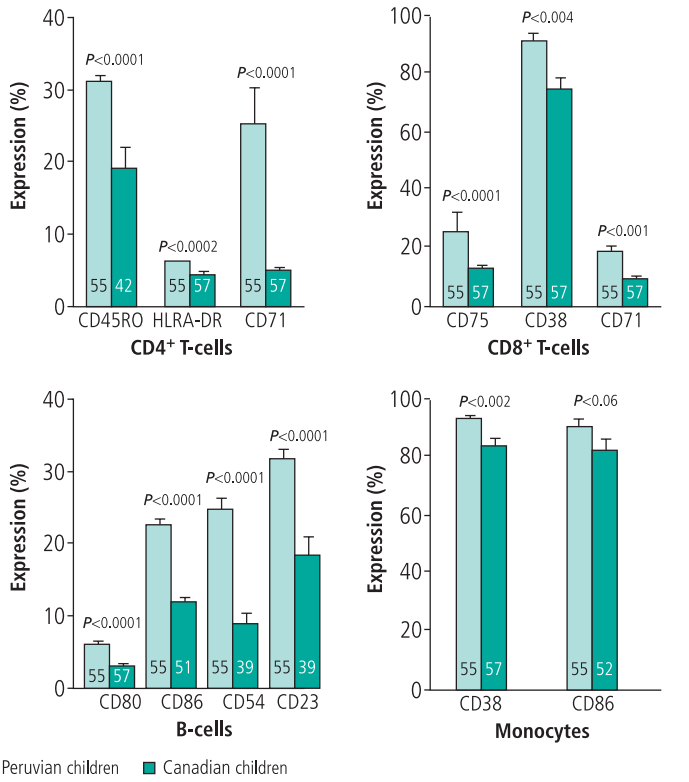
Age at immunization

The choice of 9 months of age for routine measles immunization in the developing world reflects a compromise forced by the opposing pressures of high attack rates and high mortality in children younger than 1 year of age and the risk of failure of measles vaccine in children who have high maternal antibody titres (*41*). In this study low titres of maternal antibodies were detected in only about 25% of the children at the time of vaccination and these titres had little impact on antibody production or lymphoproliferative responses (with the probable exception of the two children who had maternal antibodies and who had neither a cellular nor humoral response after vaccination).

There were, however, striking associations between maternal antibody status and the expression of CD45RO on $CD4^+$ T-cells and, in turn, between this marker of prior antigen exposure and a broad range of activation markers on other PBMCs. Although all of the 9-month-old Peruvian children had evidence of diffuse PBMC activation compared with 13-month-old Canadian children, the Peruvian children with the highest expression of CD45RO (and the most activated PBMCs) were significantly more likely to respond to measles vaccination with a "balanced" humoral and lymphoproliferative response.

T-cell expression of the mutually exclusive isoforms of CD45R (RA, RO) is thought to reflect cumulative antigen exposure, such that 90–95% of cord blood $CD4^+$ T-cells are $CD45RA^+$ while 40–60% of adult $CD4^+$ T-cells are typically $CD45RO^+$ (*42, 43*). It is tempting to speculate how the progressive loss of maternal antibodies during the first year of life could permit a more controlled acquisition of antigen exposure (such as through infectious agents). Children with low maternal antibody titres at birth or who lose maternal antibodies rapidly might reasonably be expected to have a precocious transition to an adult pattern of CD45RO expression on $CD4^+$ T-cells. It is less clear how such an early transition could influence the pattern of immune response to a live attenuated viral vaccine. Furthermore, the correlation between maternal antibody titres at the time of vaccination and the serological response is far from perfect (*44–46*). It is certainly possible that CD45RO expression is simply a marker for the development of a capacity to respond to any antigen with a proliferative response. In this study, children with strong lymphoproliferative responses to measles antigens had higher lymphoproliferative responses to non-measles antigens, but there was no clear relationship between CD45RO expression on $CD4^+$ T cells and cellular responses to non-measles antigens.

Fig. 1. **Expression of selected activation markers and co-stimulatory molecules on peripheral blood mononuclear cells isolated before measles vaccination from 9-month-old Peruvian children (*n* = 55) and 13-month-old Canadian children (*n* = 57)**



Figures inside the bars represent numbers of children.

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Unfortunately, in this study a detailed clinical evaluation was not performed at the time of vaccination. Vaccination was deferred in children with serious illnesses in accordance with local practice, but infectious episodes in the recent past, mild current illnesses, and prodromal states were not identified: any of these might have been associated with evidence of activation of PBMCs. Although it is not possible to distinguish between these states, our data support the current recommendation to administer measles vaccine despite mild intercurrent infection (*47*); the Peruvian children with evidence of ongoing immune activation generated excellent antibody titres as well as mounting detectable lymphoproliferative responses to measles antigens.

Importance of cell-mediated immunity

The precise implications of a limited lymphoproliferative response after vaccination are not known, but evidence is accumulating that cellular immunity needs to be considered in evaluating vaccine-induced protection (*1–5, 9*). "Experiments of nature" (*1*), case reports (*2*), seroepidemiology, outbreak investigations (*3*), and animal studies (*4*) all suggest that the cellular response is sufficient to protect against

measles and may be necessary for such protection in many circumstances. These observations and our findings suggest that investigations directed towards identifying factors that limit the capacity of children in the developing world to mount lymphoproliferative responses to vaccination may shed light on the suboptimal efficacy of measles vaccine in this setting (*48*). Such factors might include nutritional status, specific micronutrient status (for example, of zinc, selenium, or vitamin A), and chronic infections with immunomodulating organisms such as Epstein-Barr virus, human herpes virus 8, and malaria parasites (*49–52*). A more detailed understanding of the immune response to measles vaccination in children in the developing world and the factors that limit or modify this response may be critical to the success of the drive towards eradicating measles. ■

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Conflicts of interest: none declared.

Résumé

Réponse immunitaire au vaccin antirougeoleux chez des enfants péruviens

Objectif Evaluer la réponse immunitaire chez des enfants péruviens après vaccination antirougeoleuse.

Méthodes Cinquante-cinq enfants péruviens ont été vaccinés contre la rougeole par le vaccin de souche Schwarz (environ 10^3 unités formant plage) vers l'âge de 9 mois. Des prélèvements de sang ont été réalisés une fois avant la vaccination puis deux fois après la vaccination, la première au bout de 1 à 4 semaines et la deuxième au bout de 3 mois, pour l'évaluation du phénotype des cellules de l'immunité et de la réponse lymphoproliférative aux antigènes rougeoleux et non rougeoleux. Les anticorps spécifiques de la rougeole ont été mesurés selon la méthode de neutralisation par réduction des plages.

Résultats La réponse humorale se développait rapidement après la vaccination, puisque seuls 4 enfants sur 55 (7 %) avaient un titre en anticorps <200 mUI/ml au bout de 3 mois. Néanmoins, seuls 8 enfants sur 35 (23 %) présentaient une réponse lymphoproliférative aux antigènes rougeoleux 3 à 4 semaines après la vaccination. Les enfants ayant une faible réponse lymphoproliférative aux antigènes rougeoleux avaient des réponses lymphoprolifératives facilement détectables vis-à-vis d'autres antigènes. L'analyse des mononucléaires du sang périphérique par cytométrie de flux a mis en évidence chez la plupart des enfants une activation diffuse du système immunitaire au moment de la vaccination. La capacité à opposer une réponse lymphoproliférative aux antigènes rougeoleux était associée à l'expression de CD45RO à la surface des cellules T $CD4^+$.

Conclusion Les 55 enfants péruviens étudiés avaient une excellente réponse en anticorps à la suite de la vaccination antirougeoleuse, mais seuls 23 % (8 sur 35) avaient une réponse lymphoproliférative détectable vis-à-vis des antigènes rougeoleux (contre 55-67 % chez les enfants des pays industrialisés). Cette différence peut expliquer la réussite inégale des programmes de vaccination antirougeoleuse dans les pays en développement.

Resumen

Respuesta inmunitaria a la vacuna antisarampionosa en niños del Perú

Objetivo Evaluar en niños peruanos la respuesta inmunitaria consecutiva a la vacunación contra el sarampión

Métodos Se administró la vacuna antisarampionosa Schwarz (unas 10^3 unidades formadoras de placas) a 55 niños peruanos de unos 9 meses de edad. Se tomaron muestras de sangre antes de la vacunación, y otras dos veces después de la vacunación: una muestra entre 1 y 4 semanas después de la vacunación y una última muestra a los 3 meses de la vacunación, para evaluar el fenotipo de las células inmunitarias y las respuestas linfoproliferativas a los antígenos sarampionosos y no sarampionosos. Se midieron los anticuerpos específicos para el sarampión mediante neutralización por reducción del número de placas.

Resultados La respuesta humoral se produjo rápidamente después de la vacunación; sólo 4 de los 55 niños (7%) presentaron títulos <200 mUI/ml 3 meses después de la vacunación. Sin embargo, únicamente 8 de los 35 niños analizados (23%) presentaban respuestas linfoproliferativas a los antígenos sarampionosos 3-4 semanas después de la vacunación. Los niños con respuestas linfoproliferativas bajas a los antígenos sarampionosos presentaban respuestas linfoproliferativas fácilmente detectables frente a otros antígenos. El análisis por citometría de flujo de la células mononucleares de la sangre periférica reveló una activación difusa del sistema inmunitario en el momento de la vacunación en la mayoría de los niños. La capacidad de organizar una respuesta linfoproliferativa frente a los

antígenos sarampionosos se asoció a la expresión de CD45RO en los linfocitos T $CD4^+$.

Conclusión La producción de anticuerpos en respuesta a la vacunación antisarampionosa fue excelente en los 55 niños peruanos, pero sólo el 23% (8 de 35) presentaron respuestas linfoproliferativas detectables a los antígenos sarampionosos (en comparación con 55-67% en los niños del mundo industrializado). Es posible que esa diferencia contribuya a explicar el irregular éxito de los programas de vacunación en el mundo en desarrollo.

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