

## The Effect of Edmonston-Zagreb and Schwarz Measles Vaccines on Immune Responses in Infants

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The effects of measles immunization on immune responses in infants and the roles of vaccine strain and age of immunization are not known. Eighty-eight children were immunized at 6 or 9 months of age with the Edmonston-Zagreb (EZ) or Schwarz (SW6, SW9) strain of measles vaccine. Children were studied before and 2 weeks and 3 months after immunization. Seroconversion was similar, but geometric mean neutralizing titers at 3 months differed by vaccine group: SW9, 1367 mIU/mL; SW6, 982; and EZ, 303 ( $P = .003$ ). Mitogen-induced lymphoproliferation was decreased at 2 weeks in the SW9 group and at 3 months in all groups and was negatively correlated with measles antibody level at 3 months ( $r = -.387$ ,  $P = .003$ ). CD8 T cells, soluble CD8, neopterin, and  $\beta_2$ -microglobulin were increased at 2 weeks in the SW9 group, and soluble CD8 and  $\beta_2$ -microglobulin remained elevated at 3 months. Therefore, measles immunization resulted in suppression of lymphoproliferation, which was most evident in infants with the highest antibody responses and most immune activation.

Measles remains a major public health problem in children aged <1 year in developing countries [1]. One of the reasons for this continued problem is the inability to successfully immunize young infants while passively derived maternal antibody is still present [2, 3]. Various strategies based on altering the strain, dose, and route of immunization have been devised to attempt to lower the age of measles immunization [4–8] but have been complicated by observations of higher mortality over a 3-year follow-up period among children given high-titer vaccines at 4–6 months of age [9–12].

Immunosuppression leading to increased susceptibility to secondary infection is a well-recognized complication of natural measles [13–17], but there is no evidence that routine immunization with live attenuated measles virus vaccines leads to clinically important immune suppression. However, the ef-

fect of measles immunization on immune responses in infants has not been systematically studied, and it is possible that subtle abnormalities of potential importance are induced.

Studies with the high-titered vaccines have suggested that dose of virus is most clearly associated with higher mortality, but virus strain and age of immunization remain potential determinants of immune suppression. The Edmonston-Zagreb (EZ) and Schwarz strains of measles vaccine have different passage histories and several identified amino acid differences in structural proteins that could influence *in vivo* viral replication and immune responses [18]. The effect of age of immunization on immune responses other than induction of antibody has not been assessed. Therefore, we examined the effects of virus strain and age at vaccination on immune responses after measles immunization. This study examined whether the EZ and Schwarz measles vaccines had any immediate or delayed effects on immune responses and whether any differences observed were associated with the age or sex of the child.

### Materials and Methods

**Study subjects.** Children attending two immunization clinics in the greater Cape Town area were enrolled into the study, one clinic in a high-risk area for measles and the other clinic in a low-risk area. The immunization policy at these clinics was to use either high-titered EZ or Schwarz at 6 months (with a repeat dose at 9 months) and Schwarz at 9 months, respectively. However, when assayed independently, both vaccines were actually of similar titer (see below). At the former clinic (high-risk area), 64 children were enrolled into the study; the first 38 received EZ vaccine

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Informed consent was obtained from the parents or guardians of all patients. The study was approved by the Ethics and Research Committee of the University of Cape Town, the Joint Committee for Clinical Investigation of the Johns Hopkins University School of Medicine, and the Institutional Review Board of the Centers for Disease Control and Prevention.

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**Table 1.** Characteristics of the infants and mothers in a study of the effects on children of measles vaccines.

	Measles vaccine group			P*
	Edmonston-Zagreb (6 months)	Schwarz		
		6 months	9 months	
<b>Infants</b>				
No.	30	24	21	
Age (months)	6.2 (5.5–7.5)	6.4 (5.5–8)	9 (8–12)	
Boys:girls	16:14	15:9	10:11	
% of expected weight	103 (93–110)	104 (96–113)	109 (100–115)	.06
Serum retinol (µg/dL)	21.7 (16.8–28.3)	25.2 (19.3–29.9)	20.4 (15.9–33.1)	.48
<b>Mothers</b>				
No.	24	19	17	
Age (years)	25 (21–30)	23 (22–29)	23 (18–25)	.18
Education (highest standard passed)	6 (5–8)	6 (4–8)	8 (5–10)	.38
Rooms in house	2 (1–3)	1 (1–1)	2 (1–2)	.02
Persons/room	3 (2–4.5)	3 (2–5)	2 (2–5)	.46

NOTE. Data are median (25th–75th centiles) except age, which is given as mean (range).

\* Kruskal-Wallis test for comparisons between 3 groups.

and the subsequent 26 received Schwarz vaccine at 6 months of age (SW6). At the other clinic (low-risk area), 24 children received Schwarz vaccine at 9 months of age (SW9). Only well-nourished infants (>10th percentile weight/age) with no history of a significant illness (requiring a visit to a health worker in the prior month) were included in the study. At enrollment a full medical history was taken and the children were examined.

Venous blood (5–10 mL) was obtained for measles serology, complete blood cell count, serum retinol level, and immunologic investigations at baseline and 2 weeks and 3 months after vaccination. Human immunodeficiency virus screening was done at baseline. Measles vaccine was administered immediately after the first blood samples were taken. A second Schwarz vaccine was given at age 9 months to the SW6 group after collection of the 3-month blood sample.

The study commenced before the recommendation of the World Health Organization to stop the use of the high-titered vaccine, and no EZ vaccine was administered after the World Health Organization decision to withdraw this vaccine.

**Measles vaccine.** EZ vaccine (lot 318/6) was obtained from the Institute of Immunology (Zagreb, Croatia), and Schwarz vaccine (Rimevax, lot M151C41A) was obtained from SB Biologicals (Rixensart, Belgium). Potency testing was done by the US Food and Drug Administration (Bethesda, MD). The titer for the EZ vaccine was  $10^{4.26}$  and for the Schwarz vaccine was  $10^{4.29}$  pfu/dose. International reference 82-68 was assayed simultaneously, and the titer was  $10^{4.08}$  pfu/dose (reference titer =  $10^{3.75}$  pfu/dose). No corrections in reported titer were made. Therefore, both vaccines were of medium titer.

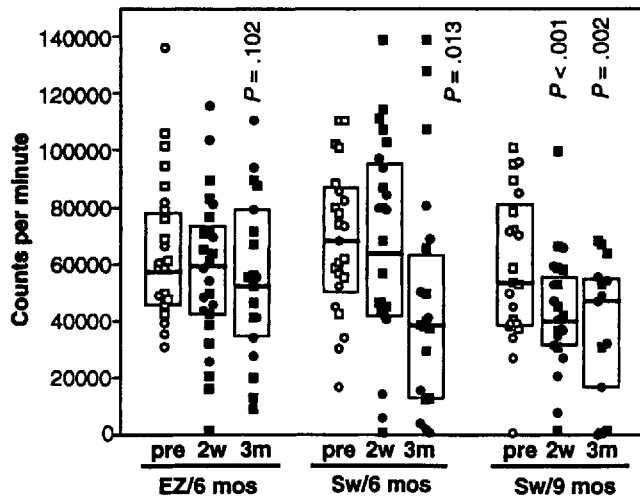
**Measles antibody.** Measles antibody was assayed by plaque reduction neutralization [19]. The lowest plasma dilution tested was 1:8. Criteria for seroconversion were a 4-fold rise in antibody concentration at 2 weeks, a 2-fold rise at 3 months, or a concentration >200 mIU/mL at either time, provided the antibody level before immunization was <200. Data from children who seroconverted were used to compare amounts of antibody induced in the different groups.

**Table 2.** Antibody responses to measles immunization: seroconversion and concentration of measles virus neutralizing antibody (mIU/mL) in seroconverters after immunization.

	Measles vaccine group						
	Edmonston-Zagreb (6 months)		Schwarz (6 months)		Schwarz (9 months)		P*
	No.	GMT	No.	GMT	N	GMT	
Seroconverters	26/30 (87%)		22/24 (92%)		20/21 (95%)		
Pre	26	19 (4–36)	22	8 (4–31)	20	4 (4–4)	.013
2 weeks	22	88 (28–181)	21	419 (165–853)	20	743 (215–1507)	<.001
3 months	20	303 (188–549)	20	982 (239–1403)	14	1367 (577–1745)	.003

NOTE. GMT, geometric mean titer measured by plaque reduction; data are median (25th–75th centiles). NS, not significant.

\* Kruskal-Wallis test for comparisons between 3 groups.



**Figure 1.** Proliferation of peripheral blood mononuclear cells in response to phytohemagglutinin before (pre; open symbols) and 2 weeks (2w) and 3 months (3m) after (solid symbols) measles immunization with Edmonston-Zagreb (EZ) or Schwarz (Sw) vaccine. Proliferation was assessed by incorporation of tritiated thymidine. Bars indicate medians; boxes, 25th and 75th percentiles.  $\square$ ,  $\blacksquare$ , boys;  $\circ$ ,  $\bullet$ , girls. *P* values (Wilcoxon signed rank test) compare postimmunization with preimmunization values.

**Immunologic investigations.** Lymphocyte subsets (CD3, CD4, and CD8 T cells, B cells, and NK cells) were analyzed on whole blood by flow cytometry using the Coulter Profile 2 system and directly conjugated monoclonal antibodies (Coulter, Miami, FL). The remainder of the blood was centrifuged and the plasma removed and frozen in aliquots at  $-20^{\circ}\text{C}$ . The cells were diluted and the mononuclear cells recovered by density gradient separation on Ficoll-Paque (Pharmacia, Piscataway, NJ). Cells were frozen and stored in liquid nitrogen. At the time of the study, samples from all time points for a single patient were analyzed simultaneously. Viability was determined by trypan blue dye exclusion, and only samples with  $>80\%$  viability were used. Cells were cultured in triplicate ( $10^6$  viable cells/mL) and stimulated with phytohemagglutinin (PHA-P; Sigma, St. Louis;  $2.5\ \mu\text{g}/\text{mL}$ ) using standard methods [20].

Plasma samples were analyzed by EIA for the presence of soluble interleukin (IL)-2 receptor, soluble CD4, and soluble CD8 (T Cell Sciences, Cambridge, MA) and by RIA for neopterin (Henning-Berlin; DRG International, Mountainside, NJ) and  $\beta_2$ -microglobulin (Pharmacia). Standards were provided by the manufacturers and assays were done according to the manufacturers' instructions.

**Data analysis.** Data were analyzed using Epi Info version 5 (USD, Stone Mountain, GA), SAS (SAS Institute, Cary, NC), and Statview 4.01 (Abacus Concepts, Berkeley, CA) programs. Between groups, categorical data were compared using the  $\chi^2$  test, and continuous data were compared using the nonparametric Kruskal-Wallis test. The Wilcoxon signed rank test was used to assess differences between values before and after vaccination. Statistical significance was defined as  $P < .05$ .

## Results

**Baseline characteristics.** Of the 88 children who were enrolled in the study, 13 were excluded because of failure of

follow-up ( $n = 11$ ) or serologic evidence of measles before immunization ( $n = 2$ ). The children in each group were from a similar racial and socioeconomic background. Baseline characteristics of the groups were comparable (table 1). All children were negative for antibody to human immunodeficiency virus.

**Antibody responses to measles vaccines.** Residual maternal antibody levels were generally low at 6 months and absent at 9 months in this population (table 2). There were no differences in the percentage of children who seroconverted after administration of the two vaccines (table 2). Of the seroconverters who returned for the 3-month follow-up, the percentages with 2-fold rises were as follows: EZ, 95%; SW6, 95%; and SW9, 100%. The percentages with plaque reduction neutralization titers  $>200$  mIU/mL were 70% for EZ, 80% for SW6, and 100% for SW9. The median measles antibody titers in the SW6 and SW9 groups were significantly greater than in the EZ group at 2 weeks ( $P < .001$ ) and at 3 months ( $P = .003$ ) after immunization. Although the antibody titers in the SW9 group were greater than in the SW6 group, the differences were not significant at either 2 weeks ( $P = .25$ ) or 3 months ( $P = .2$ ).

**Mitogen-induced lymphoproliferation.** To determine the effect of successful measles immunization on general immune responses, the ability of lymphocytes from seroconverting infants to respond to PHA was measured (figure 1, table 3). There were no differences between groups in proliferation before immunization. At 2 weeks after immunization, proliferation was lower in the SW9 group compared with the other vaccine groups and compared with preimmunization proliferation in this group. At 3 months after immunization, proliferation was significantly decreased compared with preimmunization values in both Schwarz groups, and there were no differences between the groups.

Regression analysis of proliferative responses to PHA and levels of antibody to measles for all immunized infants showed that lower responses to PHA at 2 weeks were associated with higher antibody responses at 3 months (figure 2) ( $r = -.387$ ,  $P = .003$ ). This was true for all vaccine groups and for both boys and girls.

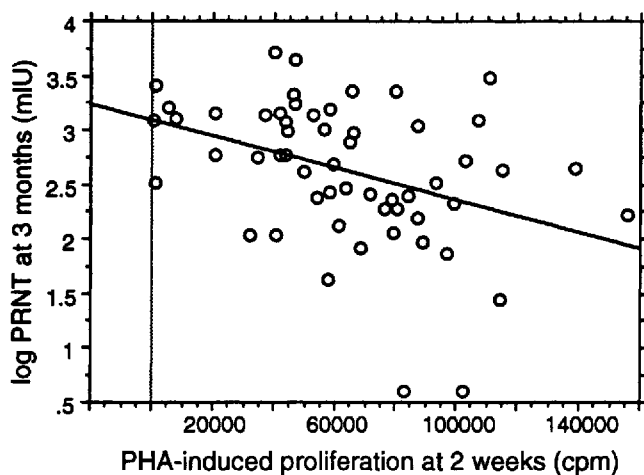
**Lymphocyte subsets.** To determine whether the changes in lymphoproliferation were associated with altered lymphocyte subsets, the numbers of CD3-, CD4-, and CD8-positive lymphocytes were measured (table 4). The median values for lymphocyte subsets were generally lower in the SW6 group. Significant differences between pre- and postimmunization values were seen for an increased absolute CD8 cell count and a decreased CD4:CD8 ratio at 2 weeks in the SW9 group. There were no substantial differences in B lymphocytes or NK cells (data not shown).

**Plasma indicators of immune activation.** Activated T lymphocytes shed soluble forms of cell surface molecules that provide a measure of immune activation (table 3). The median values of soluble IL-2 receptor and soluble CD4 were higher in the SW6 group, but this was true before as well as after immunization. No significant changes in plasma levels of soluble IL-2 receptor or soluble CD4 were detected after immuniza-

**Table 3.** Phytohemagglutinin-induced lymphocyte proliferation and plasma levels of soluble indicators of immune activation in 3 measles vaccine groups.

Variable	Measles vaccine group						P2
	Edmonston-Zagreb (6 months)		Schwarz (6 months)		Schwarz (9 months)		
	Median	P1	Median	P1	Median	P1	
<b>Lymphoproliferation (cpm)</b>							
Pre	58,646		64,830		55,420		.60
2 weeks	59,721	.07	62,443	.86	41,133	<.001	.04
3 months	53,665	.10	38,678	.013	48,033	.002	.28
<b>Soluble IL-2 receptor (U/mL)</b>							
Pre	2600		3473		2316		.002
2 weeks	2624	.63	3647	.53	2767	.09	.002
3 months	2139	.26	3288	.88	2925	.06	<.001
<b>Soluble CD4 (U/mL)</b>							
Pre	22		27.5		20		.11
2 weeks	20	.89	29	.17	20.5	.98	.002
3 months	22	.59	27	.65	21.5	.10	.015
<b>Soluble CD8 (U/mL)</b>							
Pre	1200		1040		958		.004
2 weeks	1235	.66	1040	.29	1238	<.001	.55
3 months	1175	.18	980	.65	1115	.02	.53
<b><math>\beta_2</math>-microglobulin (mg/L)</b>							
Pre	2.70		2.52		2.24		.037
2 weeks	2.90	.48	2.75	.36	2.65	<.001	.58
3 months	2.80	.84	2.75	.24	2.38	.04	.36
<b>Neopterin (nmol/L)</b>							
Pre	8.9		10.2		10.1		.49
2 weeks	7.9	.33	12.4	.60	15.2	.015	.005
3 months	8.6	.35	11.1	.74	9.7	.80	.18

NOTE. P1, Wilcoxon signed rank test for intragroup comparisons before and after immunization; P2, Kruskal-Wallis test for comparisons between 3 groups.



**Figure 2.** Lymphoproliferation and antibody responses. Regression analysis of proliferation of mononuclear cells from all immunized children in response to phytohemagglutinin (PHA) at 2 weeks and plaque reduction neutralizing (PRNT) antibody response to measles virus at 3 months after immunization.  $r = -.387$ ,  $P = .003$ .

tion. However, soluble CD8 was increased 2 weeks and 3 months after immunization in the SW9 group.

Two other sensitive indicators of immune activation during viral infection are production of neopterin by activated macrophages and increased release of  $\beta_2$ -microglobulin, the constant chain of the major histocompatibility class I molecule. Plasma levels of both neopterin and  $\beta_2$ -microglobulin were elevated at 2 weeks after immunization in the SW9 group, and  $\beta_2$ -microglobulin was still elevated at 3 months.

*Differential responses between boys and girls.* Data stratified by sex were also analyzed. No substantial differences were revealed that were not present when the groups were analyzed as a whole, and changes were not confined to either boys or girls. For instance, depressed proliferation to PHA was present in both boy and girl infants in the SW9 group at 2 weeks ( $P = .008$ , boys;  $.021$ , girls) and at 3 months ( $P = .047$ , boys;  $.025$ , girls) after immunization.

**Discussion**

Immune suppression associated with immune activation is well described during natural measles [21–24] but has not

**Table 4.** Distribution of leukocyte parameters in 3 measles vaccine groups.

Variable	Measles vaccine group						P2
	Edmonston-Zagreb (6 months)		Schwarz (6 months)		Schwarz (9 months)		
	Median	P1	Median	P1	Median	P1	
White blood cell count ( $10^9/L$ )							
Pre	11.6		10.4		9.7		.27
2 weeks	11.5	.89	10.7	.33	11.8	.02	.90
3 months	11.3	.97	10.4	.86	9.6	.12	.19
Absolute CD3 cell count ( $10^9/L$ )							
Pre	3.8		3.4		3.5		.20
2 weeks	4.2	.43	3.0	.41	4.1	.05	.06
3 months	4.1	.80	2.9	.47	4.1	.10	.07
Absolute CD4 cell count ( $10^9/L$ )							
Pre	2.5		2.0		2.1		.19
2 weeks	2.5	.33	1.7	.18	2.2	.11	.029
3 months	2.3	.69	1.9	.02	2.0	.88	.30
Absolute CD8 cell count ( $10^9/L$ )							
Pre	1.3		0.9		1.1		.19
2 weeks	1.4	.13	0.9	.49	1.4	.01	.041
3 months	1.3	.79	0.9	.20	1.2	.06	.008
CD4:CD8 ratio							
Pre	1.8		2.0		1.7		.11
2 weeks	1.9	.84	1.8	.13	1.5	.01	.17
3 months	1.6	.23	2.4	.93	1.3	.55	.07

NOTE. P1, Wilcoxon signed rank test for intragroup comparisons before and after immunization; P2, Kruskal-Wallis test for intergroup comparisons between 3 groups.

been studied after primary immunization with live attenuated measles vaccine given in infancy [25]. The reports of higher mortality after immunization of 4- to 6-month-old girls with high-titered measles vaccines [9–12] has increased the need to understand the generalized effects of immunization on immune responses. Our studies have shown that decreases in mitogen-induced lymphoproliferation are common and that these abnormalities are present 3 months after measles immunization of infants. Immune suppression was most profound in infants with the highest antibody responses and was associated with increased numbers of circulating CD8 T cells and with increased plasma levels of soluble surface molecules and cellular products associated with immune activation. These data suggest that both immune suppression and activation may be necessary correlates of a vigorous immune response to live attenuated measles virus vaccine.

Seroconversion rates in the 3 groups of vaccinees were similar (>80%). However, median antibody concentration in the EZ group was significantly lower than in the other groups. These results are similar to those of almost all of the studies comparing EZ with Schwarz vaccine. While the EZ vaccine often produces higher seroconversion rates, the magnitude of the antibody response is lower [4, 5]. The reason for this is not known.

Immune responses during natural measles are characterized by vigorous and sustained antibody responses, activation of CD8 and CD4 T cells [24, 26], suppression of delayed-type hypersensitivity skin test responses [13, 27, 28], and decreased mitogen-induced proliferation of lymphocytes [29]. Depressed skin test responses and lymphoproliferation can often be detected months after recovery from measles [28, 29]. A number of studies have documented similar changes in immune responses after measles immunization [30]. The only study to focus on altered immune responses in very young children reported decreased lymphoproliferative responses to concanavalin A, tetanus toxoid, or purified protein derivative 8–21 days after measles-mumps-rubella immunization in 18-month-old Swedish children and after Schwarz vaccine administration in 9- to 44-month-old children from Guinea Bissau [25]. We have shown that mitogen-induced lymphoproliferation was suppressed after immunization of both 6- and 9-month-old infants with the Schwarz strain of measles virus. This abnormality was more apparent at 3 months than at 2 weeks. Lymphoproliferation was also lower at 3 months after immunization of 6-month-old infants with the EZ strain, but this was not statistically significant. Lower PHA responses at 2 weeks were associated with higher antibody responses at 3 months.

The most marked immunologic changes were found in the group receiving Schwarz vaccine at 9 months. Two weeks after immunization, these infants had a significant increase in the absolute CD8 cell count, a decrease in CD4:CD8 ratio, suppression of lymphoproliferative responses, and an increase in plasma soluble CD8, neopterin, and  $\beta_2$ -microglobulin. The abnormalities in lymphocyte proliferation, soluble CD8, and  $\beta_2$ -microglobulin persisted for at least 3 months after immunization and suggest a role for CD8 T cells in this suppression.

T cell function is mediated primarily through secretion of cytokines that act on macrophages, B cells, and T cells. Both CD4 and CD8 T cells can be grouped generally into type 1 and type 2 T cells on the basis of cytokines produced. Type 1 T cells produce IL-2 and interferon- $\gamma$ , which promote delayed-type hypersensitivity responses and lymphoproliferation and suppress the activity of type 2 T cells [31]. Type 2 T cells produce IL-4, IL-5, and IL-10, which promote antibody responses and suppress the activity of type 1 T cells [32–34]. Clones of antigen-specific CD8 T cells have been described that help B cells and suppress T cell proliferation and cytotoxicity through production of IL-4, IL-10, and transforming growth factor- $\beta$  [35–37]. Such suppressor CD8 T cells are functionally active in lepromatous leprosy [38] and murine lymphomas [35]. We hypothesize that activation of CD8 and possibly CD4 suppressor T cells occurs in response to measles vaccine. These cells produce cytokines that provide B cell help and inhibit T cell proliferation, accounting for both the excellent antibody responses in vivo and suppression of lymphoproliferation in vitro [39].

The extent of the immune activation and immune suppression may be linked to the degree of measles virus replication in vaccine recipients. Nine-month-old infants are likely to have higher levels of vaccine virus replication, since residual maternal antibody is lower than at 6 months. We did not evaluate children who received high-titer vaccine. Therefore, we can only speculate that the younger age or larger amount of virus may have resulted in a more sustained pattern of suppression or activation leading to the differences in mortality.

Immunologic studies done on cells from children 2–3 years after they received high-titer vaccine have shown a lower percentage of CD4 cells, lower CD4:CD8 cell ratios, reduced lymphoproliferation, and increased antibody responses to rabies vaccine, primarily in girls [40, 41]. These alterations support the hypothesis that the immunologic alterations induced by immunization activate type 2 T cell responses, leading to improved antibody production, while suppressing type 1 T cell responses, leading to reduced lymphoproliferation. We found no variation in indicators of immune activation or suppression by sex; however, our numbers were small and we had little power to detect differences. While perturbations of the immature immune system may have an effect on immune responsiveness, many studies have demonstrated that measles vaccine given at 9 months of age [42] results in an overall reduction in childhood mortality.

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