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Metabolic effects of acute measles in chronically malnourished Nigerian children

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Abstract

We hypothesized that acute measles infection imposes severe metabolic demands on malnourished children. Nigerian rural communities, characterized by severe poverty and extensive malnutrition, served as site for this study. Sixty-five children (mean [±SD] age 2.67 ± 1.96 years) with measles and a randomly selected equal number of children (age 2.83 ± 1.23 years) from the same communities but measles-free were studied. Both groups were serologically negative for human immunodeficiency virus. The percentages of nonmeasles group who were underweight and wasted as exemplified by weight for age (WAZ) and weight for height (WHZ) scores less than −2.0 SD were 43% and 23%, respectively. Comparative values for the measles group (66% and 54% respectively) were significantly ($P < 0.01$ or 0.001) different. Compared to the controls, measles-infected children had significantly ($P < 0.001$) higher plasma cortisol level, marked hyporetinemia (plasma retinol 0.62 ± 0.24 μmol/L) and prominent reduction ($P < 0.002$) in the sum of serum essential amino acids. Measles promoted a TH_1 to TH_2 cytokine shift, with severe depletion of plasma interleukin (IL)–12, a key cytokine in the development of cell mediated immunity. IL-6, a key stimulator of hepatic acute phase protein response, was prominently ($P < 0.002$) increased in plasma in measles-infected children. Glucocorticoids exert effects on cytokine expression, as well as on cytokine receptor expression and cytokine-regulated biological responses. They enhance synergistically, the effects of IL-1 and IL-6 type cytokines on many acute phase proteins. Because of the prominent increase in circulating level of cortisol in acute measles, glucocorticoid treatment for associated sepsis may pose serious problems. Additionally, glucocorticoids antagonize several effects of retinoids at cellular and transcriptional levels, thus suggesting that hypercortisolemia may increase the requirement for retinoids.

Keywords: Measles; Malnutrition; Hyporetinemia; Hypercortisolemia; Serum cytokines

1. Introduction

Measles virus, a negative-stand ribonucleic acid virus (family *Paramyxoviridae*), has two envelope glycoproteins, the hemagglutinin and fusion proteins, which mediate receptor binding and membrane fusion, respectively [1]. A complementary regulatory molecule (CD46) expressed on all nucleated cells in humans, and a human signaling lymphocyte activation molecule (SLAM, also known as CD150) are the two recognized cell surface receptors for measles virus [1,2].

Measles, one of the top 10 killers of children worldwide, remains endemic in most parts of sub-Saharan Africa. In 1998, about 30 million cases were reported worldwide, with 880,000 measles-related deaths, 85% of which occurred in Africa and southeast Asia [3,4]. It is a catabolic disease, and elicits profound immunosuppression that can last up to 6 months after the acute phase, even when there is no detectable virus [5,6]. The complications of measles include pneumonia, diarrhea, blindness, malnutrition, and severe stomatitis (frequently associated with *Herpes simplex* and *Candida* infections), which may in some cases evolve into oro-facial gangrene (noma or cancrum oris) [4,7]. Before the development of pharmacological corticosteroids, plasma from patients with measles infection was found to produce a remission of nephrotic syndrome [8,9]. This suggested the presence of high levels of immunosuppressors such as cortisol and other factors in the plasma of these individuals.

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Pre-existing malnutrition promotes the severity of measles in children, and in the face of a heavy burden of co-infection the mortality rate can exceed 25% [4,6]. The hormonal system influences the physiological response to protein-energy malnutrition, since it determines the rate and direction of flow of substrates and energy [10]. Among other changes, the plasma level of free active cortisol is elevated in protein-energy malnutrition because of a reduction in the circulating level of corticosteroid-binding globulin, a hepatic protein that, under physiologic conditions, transports more than 90% of plasma cortisol [10,11]. There is also good evidence that the distinct diurnal rhythm exhibited by cortisol secretion in healthy individuals is abolished in malnourished ones [11]. The latter impacts negatively on several immune parameters, for example, synthesis of the pro-inflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor–α (TNF-α), interleukins (IL)–1 and 12, and others with production patterns inversely related to the normal cortisol rhythm [12].

Studies of the influence of malnutrition per se on circulating levels of the pro- and anti- inflammatory cytokines have yielded conflicting results [13,14], and this is due in part to the confounding effects of infections [15]. Infectious illnesses such as measles influence metabolism of nutrients in ways far more complex than simple depletion of the cellular stores of the relevant nutrients, and involve the interactions of several inflammatory mediators and hormones [15,16]. We hypothesized that measles in the chronically malnourished child would exacerbate the existing hypercortisolemia already evident in the latter [10,11], a situation favoring a shift in $TH_1$to TH_2 cytokine profile and severely compromised immune responsiveness [13,16,17].

2. Methods and materials

This study was carried out with the prior approval of the Institutional Review Board, University of Maryland School of Medicine, and the Ministry of Health, Sokoto State, Nigeria. The project was classified as high risk by the Institutional Review Board because of severe state of debilitation often seen in malnourished children with measles. The village chiefs in charge of the relevant rural communities also gave their consent to the study. Informed consent was obtained from the children's parents or legal guardians in the presence of a neutral primary healthcare worker. In all cases, the child's dissent prevailed over parental permission.

2.2. Subjects

As part of our ongoing studies of noma (oro-facial gangrene), a frequent complication of severe measles in malnourished African children [7,18], we investigated the metabolic effects of this viral infection during an epidemic of the disease in rural communities in Sokoto State, Nigeria. We had earlier demonstrated that in these communities, at least 45% of the children have long-term malnutrition and poor health status as exemplified by the height-for-age *Z*-scores [7]. The patients were examined and enrolled in the study within 1 week of the onset of a generalized maculopapular rash. Clinical diagnosis of measles was based on the criteria established by the World Health Organization [19]. Randomly selected, age-matched children who were from the same socioeconomically deprived rural communities and were without overt signs of measles or other infections served as the control group. The control children were not healthy and differed from the experimental group only in the absence of measles. All the children were screened serologically for human immunodeficiency virus (HIV)–1 and HIV-2 infection, and those testing positive were eliminated from the study.

Sokoto State is located in the northwest corner of Nigeria and is bordered on the north by the Republic of Niger. The estimated population of Sokoto State in 1997/1998 was 2.8 million, with children constituting about 45% of the population. Prevalence of low birth weight in rural Northern Nigerian communities is estimated to be 20% [20,21]. In Nigeria, the infant mortality rate is 114/1000 live births, and mortality among children <5 years of age is as high as 300/1000 live births in several rural communities. Measles immunization coverage of the rural communities was low ($<30\%$). The principal health problems were malnutrition, measles, pneumonia, malaria, diarrhea, and tuberculosis.

2.3. Anthropometry and nutritional evaluation

Body weight was measured to the nearest 50 g. Children <3 years of age were weighed using a balance-beam scale, and those >3 years on a standing-beam scale. Length/height was measured to the nearest 0.1 cm. For children <3 years of age, height was measured as supine length, and for those >3 years, standing height was determined using a stadiometer attached to a wall. Assessment of nutritional as well as health status was carried out using low weight for height, low height for age, and low weight for age as indices of wasting, stunting, and body mass relative to chronological age respectively [22]. In most cases, wasting is a reflection of a recent and severe process of weight loss, often resulting from a severe disease and/or acute starvation [23], whereas low height for age among children 2–3 years of age is an indication of a continuing process of growth failure [22,23]. Height for age (HAZ), weight for height (WHZ), and weight for age (WAZ) *Z* scores [standard deviation (SD) scores] were calculated using EPI 2000 program from the Centers for Disease Control and Prevention (Atlanta, GA). This software program uses the National Center for Health Statistics reference values [24]. Anthropometry of healthy, socioeconomically privileged Nigerian children compares very favorably to the standards of the National Center for Health Statistics [25]. In this study, we chose the *Z* score cut-off points of -2 SD, -3 SD, and -4 SD of the refer-

ence median to reflect moderate, severe, and critical malnutrition/poor health respectively.

Age was determined from birth records issued by the Primary Health Care Centers when available and more importantly, from interviews with mothers or legal guardians, using validated local calendar of important ceremonial or cultural events occurring in recent years as a guide. Usually, the improvised local calendar was constructed after a focus group discussion with parents in the relevant communities. In a few instances, the reported timing of dental eruption in Nigerian children was used as an additional guide [26]. Children whose ages could not be ascertained with any reasonable degree of certainty were treated for any health problems and excluded from the study. Also excluded were children with manifest congenital disorders.

2.4. Biochemical studies

2.4.1. Sample collection

Venous blood was collected into plain and heparinized tubes from each subject during the period 8–10 AM. Because of the high-risk nature of the study, no more than 5 mL of blood was collected from each child, and only at one encounter. Care was taken to protect the blood samples from undue exposure to light, heat, and air. Under field conditions, the blood-filled vacutainer tubes were retained in an ice-cooled, opaque container until centrifuged ($2000 \times g$ for 10 min), usually within 30 to 60 minutes after collection, to separate the plasma and serum, which were then divided into aliquots for storage at −70°C [7]. The aliquot for cortisol assay was stored in a siliconized tube, and the aliquot for retinol assay was wrapped in aluminum foil to shield it from light.

2.4.2. Retinol assay

The measurement of plasma concentration of retinol was done as previously described [7] using the Beckman high-performance liquid chromatography (HPLC; Beckman System Gold, 166 Detector, Beckman Instruments). All-trans-retinol (Sigma, St. Louis, MO) served as the standard. For the interpretation of vitamin A status, plasma levels <0.35 μmol/L were considered suggestive of deficiency, and levels ranging from 0.35 to 1.05 μmol/L classified as low to marginal [4,27].

2.4.3. Other assays

Concentrations of free amino acids in serum were measured by reverse-phase HPLC after precolumn derivatization with phenylisothiocyanate [28].

Plasma cortisol level was measured by radioimmunoassay. Immunochem (coated tube) ^{125}I cortisol radioimmunoassay kit was used (ICN Biomedicals, CA; catalog no. 07-221102, sensitivity 4.1352 nmol/L). In the assay, the antibody was covalently bound to the inner surface of a polypropylene tube. Thus, antibody bound antigen was also bound to the tube. To quantitate the antigen, the radioactive and nonradioactive forms of the antigen competed for binding sites on the antibody. In the presence of more nonradioactive antigen, less radioactive antigen remained bound to the tube. The unbound, free antigen was aspirated. Iodine-125 (^{125}I) radioactivity in the coated tube was determined in a gamma counter. Levels of cortisol in the samples were then determined graphically from a standard curve that was run with each assay. The reference ranges for plasma cortisol levels were 193–690 nmol/L (mornings) and 55–248 nmol/L (evenings).

Circulating cytokines were analyzed using enzyme linked immunoassay (ELISA)/enzyme amplified sensitivity immunoassay (EASIA) kits from Biosource, CA, as described by the manufacturers. The Spectra count plate reader (Packard Bioscience Co., CT) with a linearity of up to 3.0 absorbance was used. In each of the immunoassay kits, a blend of monoclonal antibodies (oligoclonal system) directed against fixed epitopes of the cytokine was used. Standards or samples containing the cytokine of interest, reacted with the capture monoclonal antibody (mAb-1) coated in the microtiter well, and with another monoclonal antibody (mAb-2) labeled with horseradish peroxidase. After formation of the sandwich complex (mAb1-cytokine-mAb2) during the incubation period, the plate was washed to remove unbound enzyme-labeled monoclonal antibodies. The amounts of bound enzyme-labeled antibodies were then measured using a chromogenic reaction. The chromogenic solution added was tetra-methyl benzidine (TMB + H_2O_2). The reaction was stopped after the specified incubation period using a solution of 2N H_2SO_4. Substrate turnover was measured as absorbance at 450 nm. A standard curve for the cytokine was prepared with each assay, and data reduction software (I smart) used to calculate the cytokine concentration.

2.5. Statistical analysis

Results are expressed as the mean ± SD; in some cases, the median value is indicated. Statistical analysis was performed using Sigma Stat version 2.0 (Jandel Corp., CA). Comparisons between the malnourished children with and without acute measles are generally carried out using the Mann-Whitney rank sum test for all the variables. In the case of the anthropometric data, the χ^2 test is used to compare the differences between frequencies (percentages). A difference is considered statistically significant at $P < 0.05$.

3. Results

Although there was no statistically significant difference between the mean ages of the control and measles groups of children, the latter children were generally younger than their control counterparts, with median ages of 2.0 years and 3.0 years, respectively (Table 1). This observation is very

Table 1
Anthropometric data of the study population*

Item†	Village control (n = 65)	Measles group (n = 65)
Age, y (mean ± SD)	2.83 ± 1.23	2.67 ± 1.96
Median	3.00	2.00
WAZ		
% <− 2.0SD	43.1‡	66.2‡
% <− 3.0SD	9.2§	44.6§
% <− 4.0SD	6.2	15.4
WHZ		
% <− 2.0SD	23.1§	53.9§
% <− 3.0SD	9.2	23.1
% <− 4.0SD	1.5	7.7
HAZ		
% <− 2.0SD	32.3	43.1
% <− 3.0SD	12.3	18.5
% <− 4.0SD	3.1	6.2

* Both the measles and village control groups were drawn from the same communities. Children with measles were generally younger, with a median age of 2.0 y compared with control value of 3 y.

† WAZ = weight-for-age Z score; WHZ = weight-for-height Z score; HAZ = height-for-age Z score. The range of HAZ scores in the control group was (+) 6.03 → (−) 3.95, with a mean ± SE of (−) 1.26 ± 0.22 and a median of (−) 1.40. Comparative values for the measles group were (+) 8.28 → (−) 5.84, (−) 1.90 ± 0.30, and (−) 1.63 respectively.

‡ Difference significantly different ($P < 0.01$).

§ Difference significantly different ($P < 0.001$).

Table 2
Serum amino acid levels in the study population*

Amino acids†	Village control (n = 46)	Measles group (n = 22)	P value‡
Threonine	81.0 ± 32.8	67.2 ± 26.3	
Valine	179.7 ± 53.7	159.0 ± 45.0	
Methionine	29.0 ± 20.9	21.5 ± 6.6	
Isoleucine	58.9 ± 18.9	47.5 ± 14.9	0.035
Leucine	150.9 ± 44.0	124.4 ± 43.1	0.014
Phenylalanine	111.4 ± 32.1	95.4 ± 32.7	
Tryptophan	39.0 ± 17.2	24.6 ± 13.6	0.002
Lysine	158.4 ± 55.1	120.0 ± 34.9	0.005
Arginine	126.6 ± 63.6	94.0 ± 41.0	0.042
Histidine	70.5 ± 32.9	48.1 ± 17.6	0.005
Glutamic acid/glutamine	218.8 ± 69.0	235.1 ± 74.2	
Serine	178.4 ± 47.1	154.6 ± 33.0	
Asparagine	81.1 ± 29.2	61.8 ± 24.6	0.010
Glycine	320.3 ± 99.3	277.8 ± 101.1	
Taurine	143.5 ± 50.7	137.5 ± 59.4	
Alanine	514.5 ± 184.3	438.0 ± 199.8	
Proline	233.2 ± 75.5	208.5 ± 59.0	
Tyrosine	72.4 ± 21.8	62.9 ± 22.7	0.035
Ornithine	109.8 ± 49.8	96.8 ± 42.9	
ΣEAA	1001.5 ± 270.7	801.6 ± 202.8	0.002
ΣBCAA	387.6 ± 109.7	330.9 ± 96.8	0.026
Glycine/valine ratio	1.78	1.75	
FMR	2.11	2.09	

* Data are expressed as the mean ± SD in μmol/L.

† ΣEAA = sum of essential amino acids (includes histidine and arginine); ΣBCAA = sum of the branched chain amino acids (valine, leucine, isoleucine); FMR = Fischer molar ratio [(valine + leucine + isoleucine)/(phenylalanine + tyrosine)].

‡ P values reported for only the amino acids significantly different between the two study groups.

relevant to the interpretation of our anthropometric data (Table 1), particularly the HAZ scores, in as much as linear growth faltering of underprivileged children observed in many developing countries starts at about 3–4 months after birth and is substantially complete by 18–22 months of age [29,30]. Between 22 and 40 months of age, linear growth in the group exhibits a slight "catch-up" relative to rates in the well nourished, healthy reference children [29].

3.1. Anthropometry

Stunting, a reflection of failure to grow, was very widespread in the village children studied, and the percentage of children affected was not significantly different between children with measles and those not infected (Table 1). The range of HAZ scores obtained in the control children was +6.03 → −3.95, with a mean ± SE of −1.26 ± 0.22 and a median of −1.40. Comparative values for the measles group were +8.28 → −5.84, −1.90 ± 0.30, and −1.63 respectively (Table 1). Wasting, as reflected by the low WHZ scores, was more severe in the children with measles than in their noninfected village counterparts, with as many as 53.9% of the former group showing a WHZ score less than −2.0 SD compared with only 23% in the latter group. Body weight relative to chronological age was severely compromised in the noninfected village children, with as many as 43% and 9.2% of those studied showing WAZ scores less than −2.0 SD and –3.0 SD, respectively (Table 1). Comparative figures for the children with acute measles infection were 66.2% and 44.6%, and these values were significantly different ($P < 0.01$ or 0.001) from the noninfected group. As many as 10 of the 65 children with measles (15%) had WAZ scores less than −4.0 SD.

3.2. Free amino acids in serum

Table 2 summarizes the mean concentrations of serum free amino acids in the village children with and without acute measles infection. Compared with the village children without measles (Table 2), the viral infection was characterized by a significant reduction ($P < 0.002$) in the sum of the essential amino acids. Most prominently affected were Trp, Ile, Leu, Lys, Arg, His, and the semi-essential amino acid, tyrosine. Among the dietary nonessential amino acids, measles infection produced nonsignificant reductions in the concentrations of glycine, alanine, and serine, but a statistically significant ($P < 0.01$) reduction in the level of asparagine compared with findings in their malnourished noninfected village counterparts. The glycine/valine and Fischer molar ratios were not different between the two study groups.

Table 3
Plasma levels of vitamin A and cortisol in the study population

Item*	Village group	Measles group
Vitamin A (μmol/L ± SD)	1.45 ± 0.68	0.62 ± 0.24†
Cortisol (nmol/L ± SD)	695.07 ± 233.69	1292.62 ± 688.27†

* For vitamin A, samples from 32 children in the village group and 39 in the measles group were analyzed, For cortisol, the n values for the village group and measles group were 29 and 35, respectively.

† Statistically different ($P < 0.001$) from the corresponding control value.

3.3. Plasma retinol and cortisol

Compared with the noninfected village children, the measles infected group demonstrated a prominent reduction (−57%) in mean plasma retinol concentration (Table 3). The difference was statistically significant ($P < 0.001$). In the noninfected group, 31% of the children had plasma retinol level <1.04 μmol/L, 6% had values <0.35 μmol/L. None of the measles infected children had retinol concentration >1.04 μmol/L, and 20% of the children had levels <0.35 μm/L.

Mean plasma cortisol concentration in the malnourished village children without measles was 695.07 ± 233.69 nmol/L, close to the upper limits of the normal reference range (193–690 nmol/L for mornings). Acute measles was associated with a statistically significant ($P < 0.001$) increase in plasma cortisol concentration (Table 3). Unpublished data from our laboratory indicated that the timing of blood collection, whether morning or evening, had no significant effect on the plasma free cortisol levels, particularly in the measles group. In effect, for most of the children, particularly those with acute measles, blood levels of glucocorticoids were high relative to the reference ranges for 24 hours every day.

3.3. Cytokine levels in plasma

Table 4 summarizes the observed plasma concentrations of various cytokines assayed in the children. Within each experimental group there was marked individual variability in the levels of the various cytokines. In addition, for some of the cytokines, limited numbers and quantities of plasma samples were available for analysis. Nonetheless, certain trends were observed. The most pronounced effect of measles infection was on IL-12, the secretion of which appeared to be virtually ablated (Table 4). Compared with the village control group, measles produced statistically significant reduction in plasma concentrations of IL-12, IL-l-β, and IL-8, and an increase in the levels of IL-6 and soluble tumor necrosis factor receptor–p55. The concentration of IFN-γ was nonsignificantly reduced in measles, whereas that of IL-10 showed a nonsignificant increase.

Table 4
Plasma concentrations of cytokines in the study population*

Item†	Village group	Measles group	P value
IL-6	10.26 ± 10.87	27.19 ± 23.58	<0.022
IL-8	275.93 ± 262.62	48.29 ± 39.34	<0.011
IL-10	10.69 ± 4.81	12.80 ± 6.48	
IL-12	221.88 ± 169.91	3.85 ± 3.83	<0.001
IL-1ra	560.04 ± 391.55	441.56 ± 310.49	
IL-1β	29.67 ± 22.25	2.69 ± 2.68	<0.001
IFNγ	6.18 ± 5.89	3.95 ± 4.19	
sTNFR-p55	2696.12 ± 923.44	3450.12 ± 1762.59	<0.018
STNFR-p75	8378.05 ± 5793.52	8059.03 ± 6198.34	

* Data expressed as pg/mL ± SD.

† Interleukin (IL); IL-1ra (interleukin-1 receptor antagonist); Soluble tumor necrosis factor receptor (sTNFR-p55 and sTNFR-p75); IL-1β (interleukin 1β); interferon gamma (IFNγ). For the cytokines, the numbers of subjects (n) studied in the measles group were 35, 34, 9, 7, 7, 19, 21, 14, 14 for sTNFRp55, sTNFRp75, IL-12, IL-8, IL-6, IL-1ra, IL-10, IFNγ and IL-1β, respectively. The corresponding numbers for the village group were 64, 62, 45, 26, 13, 10, 13, 14, and 17 respectively.

4. Discussion

Ideally, this type of study should be carried out longitudinally, using the same children before and during an episode of acute measles infection. This approach is practically impossible in predominantly rural, socioeconomically deprived communities where follow-up of patients is difficult, and probably raises some ethical issues.

Many metabolic, immune, and hormonal alterations characterize the body's reaction to the stress of acute measles, and the severity would vary with the host's nutritional status among other factors. In the communities where our study was carried out, the children had poor dietary intakes and lived under deplorable hygienic conditions that promoted high prevalences of various endemic infectious and parasitic diseases [7]. Earlier studies by others have shown that the growth patterns of healthy, well-fed, socioeconomically privileged Nigerian children compare favorably with the standards of the United States National Center for Health Statistics [25]. Use of these standards for comparative purposes was therefore justified in our study. The level of stunting in the children studied (Table 1) was disturbingly high and was consistent with reported observations in similar West African [31] and Nigerian [32,33] communities. A recently published independent study shows that the prevalence of stunting in northern Nigerian rural children varies from 50% to 53%, depending on the community [33]. The linear growth retardation in such communities is attributed in part to malnutrition [29], but also to the continuous burden of chronic immunostimulation by environmental antigens [34,35], as well as low birth weight [36]. As shown in Table 1, height for age did not change with a brief episode of acute measles. Using the severity index proposed by de Onis [22], the prevalence of wasting which was already serious in the village children, became critical in those infected by the measles virus (Table 1), a finding

reflecting the profound catabolic effect of the disease [37]. Gastrointestinal protein loss in malnourished Nigerian children with acute measles is reported to be equivalent to a mean absolute albumin loss of about 1.7 g/day [38].

The Fischer molar ratio [(sum of the branched chain amino acids)/(sum of aromatic acids excluding tryptophan)] calculated for the malnourished children with and without acute measles (Table 2) was closer to the value of 2.45 reported in subjects with classic dengue than to the value of 3.26 noted in normal individuals [37]. This could be due to pre-existing chronic malnutrition in both groups of children studied by us. Dengue is an acute viral infection transmitted by mosquitoes (*Aedes aegypti* and *Aedes albopictus*) and characterized by sudden onset of high fever. The marked reduction in total serum amino acids, particularly the essential and semi-essential amino acids, in children with acute measles (Table 2), was reminiscent of reported findings during the febrile phase of sand fly fever infection in adults [39].

Cytokine networks constitute the key controlling factors in the inflammation and immune reactions occurring in infections; a salient feature is hypercortisolemia, which persists as long as cytokines are secreted [16]. The very prominent hypercortisolemia in malnourished rural Nigerian children, particularly in those with superimposed acute measles infection (Table 3), was in all probability due mainly to impaired hepatic synthesis of corticosteroid-binding globulin rather than to increased synthesis of the hormone [10,11,16]. Other studies have shown significantly elevated urinary excretion of 17-hydroxycorticosteroids in previously healthy Egyptian children with measles, and much higher than levels in children with chicken pox, mumps, or poliomyelitis [40]. Increased circulating levels of cortisol are also found in many conditions that range from infections to idiopathic diseases [41]. Among the conditions are HIV and acquired immunodeficiency syndrome (AIDS) [42], malaria [43], tuberculosis [44], sepsis [45], and vaccination against several infections including measles [46]. Although glucocorticoids participate in the maintenance of hemodynamic stability during a severe illness [16,47], the pathophysiological roles of profound changes in levels of endogenous corticosteroids in inflammation and immunomodulation are still poorly understood [48]. The complex functional significance of glucocorticoids in relation to the cytokines and hepatic acute phase protein response has been examined in several studies [12,13,47,49]. A role has been proposed for hypercortisolemia in the progressive reduction in type 1 cytokines and an increase in type 2 cytokines, which characterize HIV infection and AIDS [42]. Hypercortisolemia also enhances cytokine receptor expression, augments the common signal transducer (gp 130), and induces apoptosis of T-lymphocytes [49,50]. Glucocorticoids may suppress inflammation by stimulating synthesis of several anti-inflammatory proteins (e.g., lipocortin-1) and cytokines (IL-1ra, IL-10), but inhibiting transcription of such cytokines as IL-1, IL-2, IL-12, TNF-γ, and tumor growth factor (TGF)–β as well as some chemokines (IL-8, RANTES, endothelial adhesion molecules) [49]. Studies in rats have shown that glucocorticoids promote depletion of stored retinol from the liver [51], the primary site of whole body vitamin A storage [52].

Acute measles was associated with low plasma vitamin A concentration compared with levels in the malnourished group without measles virus (Table 3). An earlier study in Zaria, Nigeria, showed that measles infection in children <3 years of age reduced serum retinol concentration by more than 30%, with the effect more pronounced in malnourished individuals [27]. Like hypercotisolemia, hyporetinemia is frequently observed in many other infections including malaria, AIDS, tuberculosis, respiratory diseases, and diarrheal diseases [15,43,53,54]. Predictors of serum retinol in children with infections are not clear, but there are suggestions that the hyporetinemia may be a secondary consequence of cytokine-induced inhibition of production of retinol-binding protein and transthyretin [15,27]. Experimental animal studies suggest that inflammation-induced hyporetinemia represents a redistribution of tissue vitamin A resulting from impaired hepatic synthesis of retinol-binding protein [55]. With regard to measles specifically, studies of the infection in Zambian children (majority of whom were <60 months of age, and more than 33% had chronic undernutrition) showed that 80% had subclinical vitamin A deficiency (serum retinol <0.7 μmol/L) and 50% of these children had severe deficiency (serum retinol <0.35 μmol/L), with serum levels of retinol-binding protein and transthyretin paralleling the reduction in serum retinol concentration [56]. In these Zambian children, WAZ scores and the serum levels of retinol, retinol-binding protein, and transthyretin were significantly correlated with one another.

The problems inherent in the quantitation and interpretation of circulating cytokines notwithstanding [57], our results (Table 4) confirmed the well documented ablation of IL-12 in acute measles infection, which promotes dominance of the type 2 cytokine pathway [6]. IL-12 is a key inducer of differentiation of uncommitted T helper (T_H) cells toward the T_{H1} pathway, and thus regulates cell-mediated immunity. Also significantly reduced in the plasma of children with measles (Table 4) were levels of IL-8 (a potent chemotactic and an activating peptide for leukocytes, macrophages, and intraepithelial lymphocytes). The inhibitory effects of glucocorticoids on production and release of IL-1β, IL-12, and interferon (IFN)–γ are well documented [50]. IL-1β secretion, like that of TNF-α, is generally a short-lived phenomenon, and these cytokines may not be readily detectable in the plasma of patients [57]. Compared with the control groups (Table 4), measles elicited a 2- to 3-fold significant increase ($P < 0.02$) in plasma level of IL-6, a potent pleiotropic cytokine and an acute phase reactant whose plasma concentration is often correlated to the severity of stress and inflammation [45]. IL-6 is considered a key stimulator of hepatic acute phase protein response [45,58]. Glucocorticoids up-regulate high-affinity IL-6 re-

ceptors and gp 130 in hepatocytes, and this may explain the synergy between glucocorticoids and the hepatic acute phase protein response to IL-6 [58]. An additional observation in the children with measles was a significantly increased ($P < 0.018$) plasma concentration of soluble tumor necrosis factor receptor–p55. It is believed that the inflammatory effects of TNF are mediated by the slow p55–60 TNF receptor, which is expressed in the vascular system, whereas the fast p75-80 TNF receptor is the predominant receptor type in activated lymphocytes and monocytes and is involved in the immunostimulatory effects of TNF [59].

Of some research interest to us is the potential relevance of hypercortisolemia to the hyporetinemia and the therapeutic benefits of vitamin A supplementation in measles and other infections [15,19]. Glucocorticoids are known to antagonize many of the effects of retinoids at cellular and transcriptional levels [16,17], thus raising the possibility that over-abundance of the former could increase the requirement for the latter [16,49]. Unlike the glucocorticoids, retinoids increase CD4/CD8 ratio of circulating T-lymphocytes, and promote synthesis of IL-2, IFN-γ, and TGF-β, among other differential functions [17]. Glucocorticoids are reported to decrease the expression of retinoic acid receptors. Retinoic acid is a transcriptionally active metabolite of retinol and is believed to function as a signal of the body's need for vitamin A [60]. Like the glucocorticoid receptor, retinoic acid receptors bind to cyclic AMP responsive element binding protein (CBP), and there appears to be some competition for binding sites on CBP [17,49]. As suggested by Ross [61], the strong similarity of amino acid sequences between the DNA-binding portions of the receptors for steroid hormones, vitamin D, retinoic acid, and thyroxine raises the possibility of some competition by the ligands of the steroid hormone superfamily of receptors. It is also necessary to examine the relevance of severe hypercortisolemia in measles to reactivation of latent viruses, particularly the herpes viridae, and the prominent mortality and post-measles complications often encountered in malnourished African children. The significantly increased endogenous production of glucocorticoids in measles in the face of prominently elevated levels of pro-inflammatory cytokines also raise serious questions about use of exogenous glucocorticoid in the treatment of sepsis and septic shock associated with the viral infection [50].

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