21 Bouchard C, Deprés J-P, Tremblay A. Exercise and obesity. *Obesity Research* 1993;1:133-47.
22 MacDougald OA, Hwang C-S, Fan H, Lane MD. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 1995 **92**:9034-7.
23 Cusin I, Sainsbury A, Doyle P, Rohner-Jeanrenaud F, Jeanrenaud B. The *ob* gene and insulin. A relationship leading to clues to the understanding of obesity. *Diabetes* 1995;**44**:1467-70
24 Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, *et al.* Transient increase in *obese* gene expression after food intake or insulin administration. *Nature* 1995;**377**:527-9.
25 Schwarz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. *Diabetes* 1996;**45**:531-5.
26 Weigle DS, Bukowski TR, Foster DC, Holdermans S, Kramer JM, Lasser G, *et al.* Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. *J Clin Invest* 1996;**96**:2065-70.
27 Hamilton BS, Paglia D, Kwan AY, Deitel M. Increased *obese* mRNA expression in omental fat cells from massively obese humans. *Nature Med* 1995;1:953-6.
28 Lönnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (*ob*) gene in adipose tissue of human subjects. *Nature Med* 1995;1:950-3.

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Cell mediated immunity after measles in Guinea-Bissau: historical cohort study

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Abstract

***Objective*—To investigate whether children who have had measles have reduced general cell mediated immunity three years later compared with vaccinated children who have not had measles.**

***Design*—Historical cohort study.**

***Setting*—Bissau, Guinea-Bissau.**

***Subjects*—391 children aged 3-13 years who were living in Bissau during a measles epidemic in 1991 and still lived there. These included 131 primary cases and 139 secondary cases from the epidemic and 121 vaccinated controls with no history of measles.**

***Main outcome measures*—General cell mediated immunity assessed by measurement of delayed type hypersensitivity skin responses to seven recall antigens. Anergy was defined as a lack of response to all antigens.**

***Results*—82 out of 268 cases of measles (31%) were anergic compared with 20 of the 121 vaccinated controls (17%) (odds ratio adjusted for potential confounding variables 2.2 (95% confidence interval 1.2 to 4.0); P = 0.009). The prevalence of anergy was higher in secondary cases (33% (46/138)) than in primary cases (28% (36/130)), although this difference was not significant. Anergy was more common in the rainy season (unadjusted prevalence 31% (91/291)) than in the dry season (11% (11/98)) (adjusted odds ratio 4.8 (2.2 to 10.3)). This seasonal increase occurred predominantly in the cases of measles.**

***Conclusions*—Reduced general cell mediated immunity may contribute to the higher long term mortality in children who have had measles compared with recipients of standard measles vaccine and to the higher child mortality in the rainy season in west Africa.**

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Introduction

Measles kills more than one million children in developing countries each year.[1] This estimate is based on the numbers of deaths in the acute phase of the disease and does not take account of the longer term effects of measles. In west Africa, where acute death rates for measles are particularly high, a phenomenon of "delayed mortality" has been described. Children who survive acute measles (the first month) are more likely to die during the subsequent months than are vaccinated children of a similar age.[2,3] Studies of the pattern of exposure to measles virus have shown that acute and delayed death rates are higher in secondary cases (those infected by someone living in the same house) than in primary cases (those infected by someone outside the house).[4-6] Many communities in west Africa are polygamous and have large extended families, which leads to extreme overcrowding in houses. Children are therefore intensively exposed to measles at home, and this results in a high proportion of secondary cases.

The mechanism underlying delayed death is not known. One possibility is that general cell mediated immunity may be depressed for many months after measles, which increases the risk of other infections. Anergy, or loss of delayed type hypersensitivity on skin testing with antigens such as tuberculin, is well recognised during acute measles.[7] Although most studies have found that this does not persist for longer than a few months,[8,9] one study from South Africa suggested that it might last as long as one year.[10] An alternative hypothesis to explain the difference in long term mortality between children with measles and vaccinated children is that measles vaccination stimulates general immunity, thus protecting against other infections.[6,11,12]

We investigated the relation between measles and subsequent cell mediated immunity in children in Guinea-Bissau, where measles is particularly severe. The aims were, firstly, to see whether children who had measles had lower cell mediated immunity three years later compared with children who had been vaccinated and not had measles, and, secondly, to see whether cell mediated immunity was more impaired in secondary than primary cases of measles.

Subjects and methods

The study area was on the outskirts of Bissau, the capital of Guinea-Bissau, and comprised two semirural districts, Bandim 1 and Bandim 2, and an urban district, Belem. The houses are multifamily dwellings of mud brick, and polygamy is common. The Papel is the largest of the ethnic groups living in the study area.

Measles has been studied in Bissau since 1979. Epidemics have occurred every few years, and cases of measles have been ascertained by clinical examination or interviews with mothers during the epidemics. In Guinea-Bissau mothers can recognise measles infection reliably.[13,14] Measles vaccination has also been documented. Most vaccinated children have received the standard Schwarz vaccine, but a few have received medium or high doses of Edmonston-Zagreb vaccine.

A major measles epidemic occurred between October 1990 and June 1991. Measles cases were ascertained by daily contact with the health centre and paediatric department of the hospital, by contact tracing whenever a new house with measles cases was detected, by ongoing weekly morbidity surveys in some houses in the study area, by demographic survey of all houses every three months, and by four specific measles surveys during and after the peak of the epidemic. The timing of the rash of multiple cases in each house was documented to determine whether children were primary or secondary

cases. Cases who developed a rash 0-6 days after the primary case in the same house were considered to be coprimary cases. Cases who developed a rash more than six days after the primary case were classified as secondary cases.[5]

Children were eligible for this study if they were aged 0-9 years during the 1991 epidemic and were still living in the study area in 1994. Controls were selected from children who had been vaccinated before the 1991 epidemic and had no documentation of measles. Cases of measles were selected from the 1991 epidemic. After exclusion of cases infected outside Bissau or at the hospital, a total of 707 cases in children aged 0-9 years were ascertained during the epidemic; 51 of these children died in the acute phase (within one month) and 37 died over the next three years. Thus 619 cases of measles were, to our knowledge, still alive. However, many of these children had moved before 1994 or were temporarily living outside Bissau for the duration of the study. In all, 131 of the 304 primary cases (43%) and 139 of the 315 secondary cases (44%) were identified and included in the study. Overall, 65% (175/270) of cases of measles had been seen by a doctor or nurse in the epidemic. The remaining cases had been diagnosed on the basis of a history from mothers or guardians. Each week, after selecting secondary cases, a similar number of primary cases and controls was chosen from the same broad age bands (0-2, 3-5, 6-9 years in 1991) and from the same house as the secondary cases or from neighbouring houses. Mothers or guardians were asked for permission to include their child in the study, and none refused. Cases included clinical vaccine failures—that is, children who had measles despite having been vaccinated 21 days or more before a measles rash developed. For children exposed to measles infection at home, the clinical vaccine efficacy was 87% (range 77-93%) for children under 3 years of age. However, measles vaccination coverage was high in the study area and therefore a substantial proportion of the cases in this study had received vaccine before contracting measles.

Children were assessed at home between May and September 1994. Height, head circumference, and mid-upper arm circumference were measured to the nearest millimetre with a portable stadiometer and steel tape measure. Weight was measured with electronic scales (Seca, Birmingham) to the nearest 100 g. Children were examined for splenomegaly and the presence of a BCG scar. Thick and thin blood films were taken from fingerprick samples to count malaria parasites from a subset of children during the rainy season, which begins in June.

Delayed type hypersensitivity was assessed with the Multitest CMI (Merieux, Lyons). This instrument allows seven recall antigens and a glycerin control to be administered simultaneously in a standardised fashion and gives reproducible results.[15] It has been used in epidemiological studies of children in developed and developing countries.[16-20] The antigens include proteus, trichophyton, candida, tetanus, diphtheria, streptococcus, and tuberculin. The kits were stored in a refrigerator before use.

Table 1—*Prevalence and size of positive reactions to each antigen in 389 children*

Antigen	No (%) of subjects with reaction size ≥ 2 mm	Median diameter (range) of induration (mm)
Proteus	2 (1)	2.3 (2.0-2.5)
Trichophyton	27 (7)	2.5 (2.0-4.5)
Candida	127 (33)	3.0 (2.0-5.0)
Tetanus	149 (38)	4.5 (2.0-11.0)
Diphtheria	123 (32)	4.0 (2.0-12.0)
Streptococcus	2 (1)	2.3 (2.0-2.5)
Tuberculin	173 (44)	4.0 (2.0-17.0)

The instrument was applied to the paravertebral skin of the upper back,[17] and reactions were assessed blind to the history of measles 48 (range 45-50) hours later by only one of us (CBH) throughout the study. The presence and limits of induration were determined using the ballpoint technique.[21] The perpendicular diameters of induration were measured to the nearest millimetre. A positive reaction was defined as an induration with a mean diameter of ≥2 mm (Merieux). Subjects were considered to be anergic if the reactions to all antigens were negative. A sample of 28 children (20 cases and eight controls) who were seen in May were retested at the end of the study.

At the end of the assessment a questionnaire was administered to the mother or guardian. This asked about ethnic group; the child's health, including current respiratory symptoms; illness in the previous two weeks; chloroquine treatment in the previous month; past illnesses; duration of breast feeding; birth order; and mother's age and schooling. Fourteen children who had been selected as controls had a history of measles according to the mother when interviewed in 1994. Some cases of measles might not have been documented previously if children had measles infection while temporarily out of the study area. Therefore these 14 children were excluded from the analyses.

Background data available for subsets of children included arm circumference measured under 3 years of age before the epidemic; birth weight if born in hospital in Bissau; information about the house and living conditions from a recent survey during 1993-4; and documentation of diphtheria, tetanus, and pertussis vaccination.

STATISTICAL METHODS

Descriptive analyses and linear regressions were performed using the SPSS/PC+ package. Exact confidence intervals for differences in proportions were obtained using the CIA package.[22] Arm circumferences before the epidemic were adjusted for age and sex within the population studied, and z scores were calculated using multiple linear regression. Cell mediated immunity was analysed by examining anergy using multiple logistic regression and the number of positive reactions using Poisson regression (EGRET). The repeatability of the number of positive reactions in the same individuals was assessed with a paired *t* test. In addition, a score of reactivity was calculated by adding the size of all positive reactions (Merieux). Scores were compared between groups by the Mann-Whitney U test.

Results

Overall, 131 primary cases, 139 secondary cases, and 121 vaccinated controls were included in the study. The age range was 3-13 years (mean 7.2 (SD 2.3) years). Multitest data were complete for 389 children. There were no positive reactions to the glycerin control. Table 1 shows the prevalence and the median size of induration of positive reactions to each antigen. Positive reactions were more common and larger to candida and the three vaccine related antigens, tetanus, diphtheria, and tuberculin. In all, 109 children (28%) had one positive reaction, 80 (21%) had two, and 98 (25%) had three or more. One hundred and two children (26%) were anergic.

There were no significant differences between anergic and non-anergic children in current anthropometric results, birth weight, arm circumference before the epidemic, or prevalence of BCG and diphtheria, tetanus, and pertussis vaccinations (table 2). After adjustment for age and sex, cases were 0.7 kg heavier (95% confidence interval for difference 0.1 to 1.3 kg) and had larger arm circumferences (difference 0.4 cm

Table 2—*Mean (SD) anthropometric results and prevalence (numbers (percentages) of subjects) of BCG and diphtheria, tetanus, and pertussis vaccination according to anergic state*

	Anergic group		Non-anergic group	
	No of subjects	Value	No of subjects	Value
Current height (cm)*	102	115.9 (4.7)	287	116.9 (5.4)
Current weight (kg)*	102	20.5 (2.6)	287	20.5 (3.0)
Current arm circumference (cm)*	102	16.6 (1.4)	287	16.4 (1.3)
Birth weight (g)	33	3158 (473)	109	3170 (489)
z Score of arm circumference before 1991 epidemic	31	0.11 (1.07)	88	−0.04 (0.97)
BCG vaccination:	102		287	
Recorded		63 (62)		184 (64)
Scar present		75 (74)		215 (75)
No of doses of DTP vaccine:	74		227	
≥1		57 (77)		182 (80)
≥4		37 (50)		111 (49)

DTP = diphtheria, tetanus, and pertussis.
*Adjusted for age and sex.

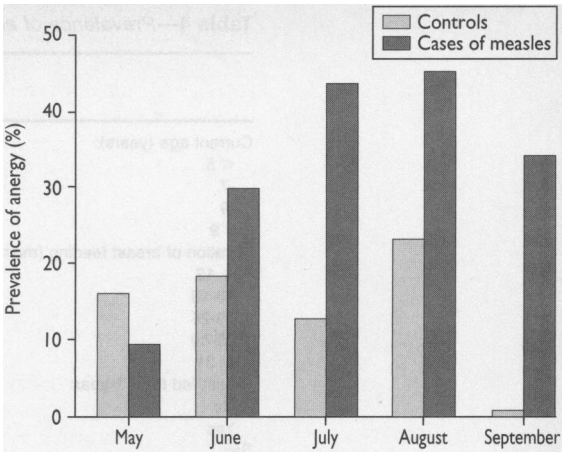

Fig 1—*Unadjusted prevalence of anergy by month of testing and history of measles in 389 children aged 3-13 years from Bissau*

(0.1 to 0.7 cm)) than vaccinated controls but did not differ significantly with respect to height, birth weight, or arm circumference before the epidemic (table 3). Cases were significantly less likely than controls to have a record of BCG vaccination (difference in prevalence 17% (7% to 27%)) and to have a BCG scar (difference in prevalence 10% (2% to 19%)), but there was no significant difference in the number of doses of diphtheria, tetanus, and pertussis vaccine between the two groups.

Ethnic group, place of birth, and the age and years of schooling of the mother did not differ significantly by measles history or anergic state. Overall, 13% (13/101) of anergic children had splenomegaly compared with 7% (19/282) of non-anergic children (difference in prevalence 6% (−1% to 13%)). The two groups did not differ in the prevalence of malaria parasitaemia (33% (13/39) and 31% (22/71) respectively) in the subset of children who had fingerprick blood samples. Illness in the previous two weeks was reported for 46% (46/100) of anergic children and 36% (101/284) of non-anergic children (difference in prevalence 10% (−1% to 22%)).

Table 4 shows the prevalence of anergy according to measles history and other variables. The prevalence of anergy decreased with increasing age and was lower in those breast fed for more than a year. It was higher in girls, in children who coughed more than others, and in children who had taken chloroquine in the previous month. The prevalence of anergy increased during the study.

Anergy was more common in primary and secondary cases of measles than in vaccinated controls. After adjustment for other variables in table 4, secondary cases were significantly more likely to be anergic than were vaccinated controls. Primary cases had an intermediate risk of anergy, but this was not significantly different from that of controls or secondary cases. The adjusted odds ratio for primary and secondary cases combined was 2.2 (1.2 to 4.0; P = 0.009). This did not change when six controls were excluded who had received the high dose Edmonston-Zagreb vaccine and not standard Schwarz vaccine. Overall, 109 of the 270 cases were clinical vaccine failures. When these children were excluded the adjusted odds ratio for all cases was unchanged. The odds ratios for primary and secondary cases were 1.5 (0.7 to 3.4) and 3.0 (1.4 to 6.4) respectively.

The rise in anergy during the study was most noticeable between the end of the dry season in May and the beginning of the rainy season in June. The adjusted odds ratio for anergy in the rainy season (June to September) compared with the dry season (May) was 4.8 (2.2 to 10.3). The seasonal increase in anergy was predominantly in measles cases (fig 1). The odds ratio in controls was 1.3 (0.3 to 5.1) and in cases 9.6 (3.4 to 27.1) (P = 0.02 in test for interaction).

As all measles cases were from one epidemic, age at measles infection was highly correlated with current age, and we therefore could not determine whether age

Table 3—*Mean (SD) anthropometric results and prevalence (numbers (percentages) of subjects) of BCG and diphtheria, tetanus, and pertussis vaccination according to measles history*

	Vaccinated controls		Primary cases		Secondary cases		All cases	
	No of subjects	Value	No of subjects	Value	No of subjects	Value	No of subjects	Value
Current height (cm)*	121	116.9 (5.1)	131	116.4 (5.6)	139	116.6 (5.2)	270	116.5 (5.4)
Current weight (kg)*	121	20.0 (2.4)	131	20.5 (2.8)	139	20.9 (3.4)	270	20.7 (3.1)
Current arm circumference (cm)*	121	16.2 (1.2)	131	16.6 (1.3)	139	16.6 (1.5)	270	16.6 (1.4)
Birth weight (g)	50	3183 (485)	44	3096 (470)	48	3216 (496)	92	3159 (485)
z Score of arm circumference before 1991 epidemic	42	−0.13 (1.01)	38	−0.05 (1.05)	39	0.18 (0.91)	77	0.06 (0.98)
BCG vaccination:	121		131		139		270	
Recorded		91 (75)		80 (61)		77 (55)		157 (58)
Scar present		99 (82)		103 (79)		90 (65)		193 (71)
No of doses of DTP vaccine:	111		96		95		191	
≥1		91 (82)		79 (82)		70 (74)		149 (78)
≥4		61 (55)		53 (55)		35 (37)		88 (46)

DTP = diphtheria, tetanus, and pertussis.
*Adjusted for age and sex.

Table 4—*Prevalence of anergy in children aged 3-13 years in Bissau by measles history and other variables*

	Unadjusted prevalence (No (%))	Univariate odds ratio (95% confidence interval)	Adjusted odds ratio (95% confidence interval)*
Current age (years):			
⩽ 5	36/90 (40)	1.0	1.0
-7	30/113 (27)	0.5 (0.3 to 1.0)	0.5 (0.2 to 0.9)
-9	19/98 (19)	0.4 (0.2 to 0.7)	0.3 (0.1 to 0.6)
> 9	17/88 (19)	0.4 (0.2 to 0.7)	0.3 (0.2 to 0.7)
Duration of breast feeding (months):			
⩽ 12	7/18 (39)	1.0	
13-18	16/86 (19)	0.4 (0.1 to 1.1)	
19-24	56/206 (27)	0.6 (0.2 to 1.6)	
25-30	14/42 (33)	0.8 (0.3 to 2.5)	
⩾ 31	5/23 (22)	0.4 (0.1 to 1.7)	
Breast fed for > 1 year:			
No	7/18 (39)	1.0	1.0
Yes	91/357 (25)	0.5 (0.2 to 1.4)	0.3 (0.1 to 0.9)
Sex:			
Boys	44/196 (22)	1.0	1.0
Girls	58/193 (30)	1.5 (0.9 to 2.3)	1.5 (0.9 to 2.5)
Coughs more than other children:			
No	81/336 (24)	1.0	1.0
Yes	19/49 (39)	2.0 (1.1 to 3.7)	2.3 (1.1 to 4.7)
Chloroquine in previous month:			
No	61/269 (23)	1.0	1.0
Yes	39/115 (34)	1.8 (1.1 to 2.8)	1.8 (1.0 to 3.0)
Month of test:			
May	11/98 (11)	1.0	1.0
June	29/110 (26)	2.8 (1.3 to 6.0)	3.5 (1.5 to 8.2)
July	22/68 (32)	3.8 (1.7 to 8.5)	5.0 (2.0 to 12.7)
August	37/98 (38)	4.8 (2.3 to 10.1)	7.0 (2.9 to 16.5)
September	3/15 (20)	2.0 (0.5 to 8.1)	4.0 (0.8 to 19.1)
Measles history:			
Vaccinated control	20/121 (17)	1.0	1.0
Primary case	36/130 (28)	1.9 (1.0 to 3.6)	1.8 (0.9 to 3.6)
Secondary case	46/138 (33)	2.5 (1.4 to 4.6)	2.7 (1.4 to 5.3)

*Adjusted for other variables in table in 374 subjects with complete information.

at measles infection was related to anergy independently of current age. When subjects were stratified by age the increase in anergy in measles cases was similar in all age groups. Age at measles vaccination was unrelated to anergy.

The mean number of positive reactions to the antigens in the children overall was 1.6 (1.3) (range 0 to 5). In a Poisson regression analysis the mean number of reactions was examined by measles history after adjustment for potential confounding variables (table 5). The mean number of reactions was 38% lower (95% confidence interval of difference 26% to 48%) in the rainy season than in the dry season and 18% lower (2% to 30%) in all measles cases compared with vaccinated controls. The reduction in primary and secondary cases was similar. After exclusion of vaccine failures the reduction in the mean number of positive reactions was 16% (−8% to 34%) in primary cases and 25% (4% to 41%) in secondary cases.

Table 5—*Poisson regression analyses of mean number of positive reactions by measles history and other variables*

	No of subjects	Rate ratio (95% confidence interval)*
Current age (years):		
⩽ 5	90	1.0
-7	110	1.35 (1.05 to 1.73)
-9	92	1.77 (1.39 to 2.26)
> 9	82	1.38 (1.07 to 1.79)
Breast fed for > 1 year:		
No	18	1.0
Yes	356	1.31 (0.86 to 2.00)
Sex:		
Boys	191	1.0
Girls	183	0.83 (0.70 to 0.98)
Coughs more than other children:		
No	328	1.0
Yes	46	0.95 (0.74 to 1.24)
Chloroquine in previous month:		
No	262	1.0
Yes	112	0.82 (0.68 to 1.00)
Month of test:		
May	96	1.0
June	103	0.68 (0.55 to 0.84)
July	66	0.69 (0.54 to 0.88)
August	95	0.51 (0.40 to 0.65)
September	14	0.59 (0.37 to 0.94)
Measles history:		
Vaccinated control	118	1.0
Primary case	126	0.83 (0.68 to 1.01)
Secondary case	130	0.82 (0.67 to 1.01)

*Adjusted for other variables in table in 374 subjects with complete information.

Reactivity scores were calculated by adding the size of all positive reactions. The scores were significantly lower in measles cases (median 4.0) than in vaccinated controls (median 6.0) (Mann-Whitney U test P = 0.003). There was no significant difference in score between primary and secondary cases (Mann-Whitney U test P = 0.9).

Twenty eight children (10 primary cases, 10 secondary cases, and 8 controls) from one district who had been tested in May were retested in September. The mean decrease in the number of positive reactions was 0.5 (95% confidence interval for reduction 0.0 to 1.0) (test for difference from zero, P = 0.04). The same reduction in the number of positive reactions was seen in measles cases and controls.

Discussion

In Guinea-Bissau we found that children who had measles infection had significantly lower general cell mediated immunity three years later than vaccinated children who had not had measles. Measles cases were more than twice as likely as vaccinated controls to be anergic on skin testing with seven recall antigens, and they had a lower mean number of positive reactions and a lower reactivity score. Secondary cases were more likely to be anergic than primary cases, particularly

when unvaccinated cases were examined, although this difference was not significant.

BIAS AND CONFOUNDING

Two thirds of measles cases were confirmed by a doctor or nurse. The remainder were ascertained from the history given by the mother or guardian. Serological studies in Guinea-Bissau have shown that, although some infections may be missed by mothers, a positive history is nearly always correct.[13 14] We therefore believe that the degree of misclassification of measles infection in this study will have been small. We cannot exclude the possibility that the association between measles and anergy was biased by losses from the 1991 population through migration or death. However, if this were so measles infection would have to be associated with a lower prevalence of anergy in children who had migrated or died, which seems unlikely.

Anergy was more common in younger children and in girls, which is consistent with results from studies in developed countries.[16 17] The increase in anergy in children who had recently taken chloroquine may reflect the immunosuppressive effects of the drug.[23] Anergy was less common in children who had been breast fed for more than a year, and there is some evidence from the United States that cell mediated immunity to tuberculin, assessed in vitro, is enhanced in breast fed infants.[24 25] The relation between measles and anergy remained after adjustment for age, sex, chloroquine, and breast feeding.

Two other potentially confounding factors were nutritional state and vaccination with antigens that might contribute to delayed type hypersensitivity. In other populations prenatal growth retardation, as defined by low birth weight,[26 27] and postnatal growth retardation, as measured by low weight for age[28] and low weight for height,[29] have been linked to impaired delayed type hypersensitivity. However, we found no difference between anergic and non-anergic children in birth weight, arm circumference before the 1991 epidemic, or current anthropometric results after adjusting for age. Deficiency of micronutrients such as vitamin A might lead to depression of cell mediated immunity. Vitamin A deficiency is associated with impaired delayed type hypersensitivity,[30] and clinical deficiency in children is associated with abnormalities in T cells.[31] However, a previous survey of children in the same community found no evidence of clinical deficiencies in vitamins.[32] There were no significant differences in BCG vaccination or number of doses of diphtheria, tetanus, and pertussis vaccine between anergic and non-anergic children.

Infection with HIV is associated with cutaneous anergy as measured by the Multitest.[33] However, this is unlikely to be an important cause of anergy in the age group that we studied. Seroprevalence surveys in 1987 and 1989 in Bissau found that HIV-2 infection but not HIV-1 infection was present in adults, but the prevalence of HIV-2 infection in children aged 4-14 years was less than 1% and there was no evidence for vertical transmission.[34-36]

IMMUNE SUPPRESSION OR STIMULATION

In a retrospective study of this kind we cannot discount the possibility that anergic children were more likely to develop measles, but this does not seem biologically plausible. Measles virus is highly infectious, and subclinical infection is rare. Pre-existing immunological abnormalities are therefore unlikely to modify the risk of clinical infection.

There are therefore two possible explanations for our findings. Firstly, measles caused suppression of general cell mediated immunity that lasted for three years. If this were so we would have expected such suppression to be more profound in secondary cases than in primary cases as secondary cases have a higher delayed mortality.[5 6] The difference in the prevalence of anergy between primary and secondary cases, though not significant, is consistent with this hypothesis, although a smaller study of an earlier epidemic in Bissau found no evidence for persistent suppression of T cell subsets after measles.[37]

Secondly, general cell mediated immunity was enhanced long term in children who had received standard measles vaccine. Studies from Guinea-Bissau have shown that vaccination in early infancy is associated with improved long term survival beyond that expected from protection against measles alone.[11 12] This effect was specific for measles vaccine and not confounded by other vaccines,[12] which is in keeping with our findings for cell mediated immunity.

DOSE MAY BE IMPORTANT

The long term effects of measles vaccine and natural infection on cell mediated immunity may depend on the dose of measles virus. In this respect high dose live attenuated vaccine may be analogous to natural infection. Reductions in CD4:CD8 ratios in Guinea-Bissau[38] and poorer responses to Multitest antigens in Peru[20] have been found two to three years after vaccination in girls who have received high dose vaccine rather than standard low dose vaccine. In Guinea-Bissau high dose vaccine is also associated with delayed mortality over a similar period.[39] The higher prevalence of anergy in secondary cases compared with primary cases is in keeping with the hypothesis that secondary cases are infected with a higher dose of virus than are primary cases.[40] In Senegal secondary cases continue to have significantly higher mortality than primary cases up to three years after acute measles.[6]

SEASONAL EFFECT

A striking finding was the noticeable increase in the prevalence of anergy during the rainy season both in the population and in a subset of children who had repeat measurements. A population based study in Bangladesh has also noted seasonal variation in the prevalence of anergy in children[19] and another study in Guinea-Bissau found that children had lower CD4 counts and CD4:CD8 ratios in the rainy season.[41] We do not know what factors might be responsible for the increase in anergy during the rains or why this increase was predominantly in cases of measles. Malaria and respiratory infections are more common in the rains. Data from other west African countries are conflicting as to whether acute malaria leads to impaired delayed type hypersensitivity.[42 43] A previous study in Bissau found a reduction in the percentage of CD4 T lymphocytes in asymptomatic children with malaria parasitaemia,[44] although we found no relation between parasitaemia and anergy in a subgroup of children. Respiratory infections can cause anergy,[45] but the seasonal effect was independent of reported recent illness and cough.

CONCLUSIONS

We found a significant reduction in cell mediated immunity three years after infection in children who had had measles compared with vaccinated controls. These findings offer an explanation for the divergent survival of these two groups of children in Guinea-Bissau, which has been reported previously[2] and been confirmed in our cohort (unpublished data). If this explanation is correct we would expect cases who have died to have had even more reduced cell mediated immunity, resulting in an increased susceptibility to infections. Prospective studies are needed to test this hypothesis further and to determine what infections are responsible for the long term difference in mortality

Key messages

- West African children who survive measles are at greater risk of dying in the ensuing few years than children who have been vaccinated
- The mechanisms underlying delayed mortality are not understood
- In Guinea-Bissau children who were tested three years after having had measles had lower general cell mediated immunity than vaccinated children
- Reduced cell mediated immunity was more common in the rainy season than in the dry season, particularly in cases of measles
- A reduction in cell mediated immunity may, by increasing susceptibility to other infections, contribute to delayed mortality after measles and to the higher death rates during the rainy season in west African children

between children who have had measles and children who have been vaccinated. Studies from other developing countries have shown that anergy is a significant predictor of diarrhoeal morbidity in children.[18 19 29]

Differences in functioning of the immune system between these two groups of children may persist for longer than three years. In a study of young adults in Bissau we found differences in atopy 15 years after measles.[46] Testing for delayed type hypersensitivity is ideal for assessing the integrity of cell mediated immunity in its entirety in the field and may provide more information than quantitative T cell studies alone.[33] However, more detailed immunological studies are now needed to understand which components of cell mediated immunity were abnormal and responsible for the anergy that we observed.

The death rate from acute diseases, particularly diarrhoea,[47] increases twofold during the rainy season among children in Bissau.[11] If depression of cell mediated immunity contributes to the increase in the morbidity and mortality of children during the rainy season in west Africa then identification of the seasonal factors responsible may be of profound importance for public health.

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1 Global programme for vaccines of the World Health Organisation. Role of mass campaigns in global measles control. *Lancet* 1994;**344**:174-5.
2 Aaby P, Bukh J, Lisse IM, Smits AJ. Measles vaccination and reduction in child mortality: a community study from Guinea-Bissau. *J Infect* 1984;**8**:13-21.
3 Hull HF, Williams PJ, Oldfield F. Measles mortality and vaccine efficacy in rural West Africa. *Lancet* 1983;i:972-5.
4 Aaby P, Bukh J, Lisse IM, da Silva MC. Further community studies on the role of overcrowding and intensive exposure on measles mortality. *Rev Infect Dis* 1988;**10**:474-7.
5 Garenne M, Aaby P. Pattern of exposure and measles mortality in Senegal. *J Infect Dis* 1990;**161**:1088-94.
6 Aaby P, Samb B, Andersen M, Simondon F. No long-term excess mortality after measles infection: a community study from Senegal. *Am J Epidemiol* 1996;**143**:1035-41.
7 Von Pirquet CE. Das Verhalten der kutanen Tuberkulin Reaktion während der Masern. *Deutsche Med Wochenschr* 1908;**34**:1297-300.
8 Whittle HC, Bradley-Moore A, Fleming A, Greenwood BM. Effects of measles on the immune response of Nigerian children. *Arch Dis Child* 1973;**48**:753-6.
9 Tamashiro VG, Perez HH, Griffin DE. Prospective study of the magnitude and duration of changes in tuberculin reactivity during uncomplicated and complicated measles. *Pediatr Infect Dis J* 1987;**6**:451-4.
10 Kipps A, Stern L, Vaughan EG. The duration and the possible significance of the depression of tuberculin sensitivity following measles. *S African Med J* 1966;**40**:104-8.
11 Aaby P, Andersen M, Sodemann M, Jakobsen M, Gomes J, Fernandes M. Reduced childhood mortality after standard measles vaccination at 4-8 months compared with 9-11 months of age. *BMJ* 1993;**307**:1308-11.
12 Aaby P, Samb B, Simondon F, Coll Seck AM, Knudsen K, Whittle H. Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. *BMJ* 1995;**311**:481-5.
13 Aaby P, Knudsen K, Jensen TG, Thaarup J, Poulsen A, Sodemann M, *et al.* Measles incidence, vaccine efficacy and mortality in two urban African areas with high vaccination coverage. *J Infect Dis* 1990;**162**:1043-8.
14 Aaby P, Pedersen IR, Knudsen K, da Silva MC, Mordhorst CH, Helm-Petersen NC, *et al.* Child mortality related to seroconversion or lack of seroconversion after measles vaccination. *Pediatr Infect Dis J* 1989;**8**:197-200.
15 Kniker WT, Anderson CT, Roumiantzeff M. The multi-test system: a standardised approach to evaluation of delayed hypersensitivity and cell-mediated immunity. *Ann Allergy* 1979;**43**:73-9.
16 Corriel RN, Kniker WT, McBryde JL, Lesourd BM. Cell-mediated immunity in schoolchildren assessed by Multitest skin testing. *AJDC* 1985;**139**:141-6.
17 Kniker WT, Lesourd BM, McBryde JL, Corriel RN. Cell-mediated immunity assessed by Multitest CMI skin testing in infants and preschool children. *AJDC* 1985;**139**:840-5.
18 Black RE, Lanata CF, Lazo F. Delayed cutaneous hypersensitivity: epidemiologic factors affecting and usefulness in predicting diarrheal incidence in young Peruvian children. *Pediatr Infect Dis J* 1989;**8**:210-5.
19 Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Malnutrition, cell-mediated immune deficiency and diarrhea: a community-based longitudinal study in rural Bangladeshi children. *Am J Epidemiol* 1993;**137**:355-65.
20 Leon ME, Ward B, Kanashiro R, Hernandez H, Berry S, Vaisberg A, *et al.* Immunologic parameters 2 years after high-titer measles immunization in Peruvian children. *J Infect Dis* 1993;**168**:1097-104.
21 Sokal JE. Measurement of delayed skin-test responses. *N Engl J Med* 1975;**293**:501-2.
22 Gardner MJ, Altman DG, eds. *Statistics with confidence.* London: BMJ, 1989.
23 Salmeron G, Lipsky P. Immunosuppressive potential of antimalarials. *Am J Med* 1983;**75**(suppl):19-24.
24 Schlesinger JJ, Covelli HD. Evidence for transmission of lymphocyte responses to tuberculin by breast-feeding. *Lancet* 1977;ii:529-32.
25 Pabst HF, Godel J, Grace M, Cho H, Spady DW. Effect of breast-feeding on immune response to BCG vaccination. *Lancet* 1989;i:295-7.
26 Chandra RK, Ali SK, Kutty KM, Chandra S. Thymus-dependent lymphocytes and delayed hypersensitivity in low birth weight infants. *Biol Neonate* 1977;**31**:15-8.
27 Ferguson A. Prolonged impairment of cellular immunity in children with intrauterine growth retardation. *J Pediatr* 1978;**93**:52-6.
28 Harland PSEG. Tuberculin reactions in malnourished children. *Lancet* 1965;ii:719-21.
29 Koster FT, Palmer DL, Chakraborty J, Jackson T, Curlin GC. Cellular immune competence and diarrhoeal morbidity in malnourished Bangladeshi children: a prospective field study. *Am J Clin Nutr* 1987;**46**:115-2.
30 Smith SM, Levy NL, Hayes CE. Impaired immunity in vitamin A deficient mice. *J Nutr* 1987;**117**:857-65.
31 Semba RD, Muhilal, Ward BJ, Griffin DE, Scott AL, Natadisastra G, *et al.* Abnormal T-cell subset proportions in vitamin-A-deficient children. *Lancet* 1993;**341**:5-8.
32 Smedman L, Lindeberg A, Jeppsson O, Zetterstrom R. Nutritional status and measles. A community study in Guinea-Bissau. *Ann Trop Paediatr* 1983;**3**:169-76.
33 Gordin FM, Hartigan PM, Klimas NG, Zolla-Pazner SB, Simberkoff MS, Hamilton JD. Delayed-type hypersensitivity skin tests are an independent predictor of human immunodeficiency virus disease progression. *J Infect Dis* 1994;**169**:893-7.
34 Poulsen AG, Kvinesdal B, Aaby P, Molbak K, Frederiksen K, Dias F, *et al.* Prevalence of and mortality from human immunodeficiency virus type 2 in Bissau, West Africa. *Lancet* 1989;i:827-31.
35 Poulsen AG, Kvinesdal B, Aaby P, Lisse I, Molbak K, Dias F, *et al.* Lack of evidence of vertical transmission of human immunodeficiency virus type 2. *J AIDS* 1992;**5**:25-30.
36 Poulsen AG, Aaby P, Gottschau A, Kvinesdal B, Molbak K, Dias F, *et al.* HIV-2 infection in Bissau, West Africa, 1987-1989: incidence, prevalences and routes of transmission. *J AIDS* 1993;**6**:941-8.
37 Aaby P, Lisse IM, Molbak K, Knudsen K, Whittle H. No persistent T lymphocyte immunosuppression or increased mortality after measles infection: a community study from Guinea-Bissau. *Pediatr Infect Dis J* 1996;**15**:39-44.
38 Lisse IM, Aaby P, Knudsen K, Whittle H, Andersen H. Long term impact of high titer Edmonston-Zagreb measles vaccine on T lymphocyte subsets. *Pediatr Infect Dis J* 1994;**13**:109-2.
39 Aaby P, Knudsen K, Whittle H, Thaarup J, Poulsen A, Sodemann M, *et al.* Long-term survival after Edmonston-Zagreb measles vaccination: increased female mortality rate. *J Pediatr* 1993;**122**:904-8.
40 Aaby P, Coovadia H, Bukh J, Lisse IM, Smits AJ, Wesley A, *et al.* Severe measles: a reappraisal of the role of nutrition, overcrowding and virus dose. *Medical Hypotheses* 1985;**18**:93-112.
41 Lisse I, Aaby P, Whittle H, Jensen H, Engelman M. T-lymphocyte subsets in West African children: impact of age, season and sex. *J Pediatr* (in press).
42 Greenwood BM, Bradley-Moore AM, Palit A, Bryceson ADM. Immunosuppression in children with malaria. *Lancet* 1972;i:169-72.
43 Akinwolere OAO, Williams AIO, Akinkugbe FM, Laditan AAO. Immunity in malaria: depression of delayed hypersensitivity reaction in acute Plasmodium falciparum infection. *Afr J Med Sci* 1988;**17**:47-52.
44 Lisse IM, Aaby P, Whittle H, Knudsen K. A community study of T lymphocyte subsets and malaria parasitaemia. *Trans R Soc Trop Med* 1994;**88**:709-10.
45 Kauffman CA, Linnemann CC, Schiff GM, Phair JP. Effect of viral and bacterial pneumonias on cell-mediated immunity in humans. *Infect Immun* 1976;**13**:78-83.
46 Shaheen SO, Aaby P, Hall AJ, Barker DJP, Heyes CB, Shiell AW, *et al.* Measles and atopy in Guinea-Bissau. *Lancet* 1996;**347**:1792-6.
47 Molbak K, Aaby P, Ingholt L, Hojlyng N, Gottschau A, Andersen H, *et al.* Persistent and acute diarrhoea as the leading causes of child mortality in urban Guinea-Bissau. *Trans R Soc Trop Med Hyg* 1992;**86**:216-20.

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