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Plasma immunoglobulin levels in Nigerian infants in the first year of life

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Summary

Serial estimations of plasma immunoglobulins IgG, IgM and IgA were undertaken in the first year of life of healthy Nigerian infants by the single radial immunodiffusion method. High levels of IgG present at birth dropped below half their values in the first month of life and remained low for about four months after which a sustained rise was observed although birth levels were not attained at 1 year of age. IgM was low at birth and IgA was absent but was detached at one month. Both IgM and IgA rose rapidly in the first four months of life which is in keeping with immunological competence and primary antibody response to various subclinical infections in infancy.

Résumé

Des calculs des immunoglobulins IgG, IgM, et IgA ont été faits en série pendant la première année de vie des enfants nigérians de bonne santé, en se servant de la seule méthode radiale immunodiffusion. Des niveaux elevés de IgG qui étaient présents à la naissance ont baissé à moins de la moitié de leur valeur pendant le premier mois de vie et ils ont restés bas pour quatre mois environs, après lequel on a constaté une augmentation soutenue bien que les niveaux de la naissance n'étaient pas atteinte à l'age d'un an. IgM était bas à la naissance et IgA était absent mais il s'est fait remarquer à l'age d'un mois. Le IgM et le IgA sont montés rapidement pendant les quatre premiers mois de vie et cela est en harmonie avec la compétence immunologique et avec la réponse primaire d'anticorps aux infections sub-cliniques variés à l'enfance.

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Introduction

The first year of life is extremely hazardous for the survival of the African infant and mortality rate is very high during this period. The mode of response of these infants to infections and antigenic stimuli has not been fully established as has been done for their Caucasian and American counterparts. Basic knowledge about the humoral as well as the cell-mediated defence mechanisms is incomplete and calls for further work.

The humoral antibody system has been investigated by the evaluation of plasma immunoglobulin levels in some West African Communities (Rowe *et al.*, 1968) and also in relation to specific infections in Nigerians (McFarlane & Voller, 1966, McFarlane, 1966, Williams & McFarlane, 1970, Sagoe, 1970). Comparative quantitation of immunoglobulins in paired maternal and cord blood in Nigerians was also undertaken at Ibadan. (McFarlane & Udeozo, 1968). However, baseline values in healthy children are not available and for this reason a study of the ontogenic development of the major plasma immunoglobulins (IgG, IgM and IgA) was undertaken in healthy Nigerian infants in the first year of life. The pattern obtained would elucidate some aspects of the humoral antibody system and the related immunological competence of this defence mechanism. It will also contribute significantly to establishing normal values for plasma immunoglobulins in this age group which is an essential information in the understanding of the immunologic profile of the African child.

Materials and methods

Normal full term infants weighing not less than 2000 g were chosen for the study. They were born to healthy mothers either at the University College

Hospital, Ibadan or in one of two other hospitals in the town. The first samples of blood were obtained within 48 h of birth from a heel prick into heparinized tubes. Cord blood was not used. The infants were seen again and bled at 1, 3, 4, 6, 9 and 12 months of life respectively. Blood was obtained by heel prick within the first 4 months and by venipuncture in the older infants. The plasma was separated by centrifugation soon after, with one or two drops of 0·1 M sodium azide added as preservative. The plasma was stored at 4°C till needed for batch analysis. Sick babies who had fever from any cause, e.g. malaria, or who suffered from measles, gastroenteritis, mumps or respiratory infections were excluded from the study. This elimination of sick ones coupled with the reluctance of mothers to bring healthy babies for frequent bleeding necessitated the selection of a second group of healthy babies to augment the study. These were healthy infants brought to Welfare Baby Clinics for routine inoculations or advice about diet, weaning etc. Those selected for the study had been similarly innoculated with BCG, oral polio vaccine and triple antigen as children in the group studied. They were matched for the age of those who defaulted or excluded from the study, and bled once only. It was difficult to match for sex and this was not successfully done. Twenty samples were obtained for each period of study.

All twenty cases chosen for the longitudinal study seen and bled at birth were successfully followed up to the third month after when there was gradual decrease in numbers due to default and exclusions because of ill-health. Completely serialized blood samples were obtained in eight out of twenty infants with whom the study was commenced. These children were apparently in 'good health' in their first year of life during the period at which blood samples were obtained. Default rate and exclusions were highest between the ages of 4–6 months.

The three major classes of plasma immunoglobulins (IgG, IgM and IgA) were quantified by the single radial immunodiffusion method in agar gel. (Mancini, Carbonara & Heremans, 1965). Commercial anti-IgG and anti-IgM antisera from Wellcome Research Laboratories, Beckenham, England, were used in the preparation of immunodiffusion plates in our laboratory. The specificity of antisera used were verified by immunoelectrophoresis against whole Nigerian human serum. Single precipitin lines were obtained with anti-IgG and anti-IgM antisera in the appropriate positions in the electrophoretic field. Large glass slides measuring 8·2 cm × 8·2 cm provided plates with forty-nine wells and enabled twenty-one samples of plasma to be estimated in duplicate at the same time under the same experimental conditions. Commercial immunodiffusion plates (Hyland laboratories) were used for the quantification of serum IgA. Commercially obtained standards of immunoglobulins (IgM, IgG and IgA) were obtained from Behringwerke Ag and used as constant reference sera throughout the study.

The immunodiffusion plates were placed in moist plastic chambers and diffusion allowed to take place at 4°C for 18 h. Four estimations were performed on each sample and close correlation was sought in values obtained. Two diameters of the precipitin rings at right angles were read on a viewbox which provided oblique illumination against darkened background using a fine-scale comparator lens.

Standard curves were prepared by plotting the ring diameters against reference immunoglobulin concentrations on a 2-cycle semi-logarithm scale (ring diameters on the horizontal arithmetic scale and concentrations of corresponding reference serum on vertical logarithmic scale), and from this the concentrations of the unknown samples were determined. A separate standard curve was incorporated into each immunodiffusion plate, but the same batch oi reference sera was used for all the samples tested.

The error of the technique for standardization of the three immunoglobulins on paired plates as measured by the coefficient of variation of the diameter determinations was 2·5%.

Results

The values in Tables 1, 2 and 3 show the range of plasma immunoglobulins (G, M and A) obtained in healthy Nigerian infants, from birth through the first year of life whilst Figs 1, 2 and 3 graphically present the same data. The values obtained from those who completed the longitudinal study were similar to those from single blood samples of other healthy children of the same age. No significant difference was noticed.

IgG: Table 1

The major fraction of the immunoglobulins was the IgG. At birth the range was 1150 mg–2550 mg% but this fell dramatically to less than half of this value at the age of 1 month in almost all cases. This

TABLE 1. Serum IgG levels in Nigerian children during the first year of life in g%

	Age (months)						
	Birth	1	3	4	6	9	12
Mean (g%)	1·65	0·62	0·49	0·43	0·66	0·82	1·26
s.d.	0·31	0·13	0·08	0·10	0·12	0·12	0·28
s.e.	0·07	0·03	0·02	0·02	0·03	0·03	0·06
n*	20(20)	20(20)	20(20)	20(14)	20(11)	20(10)	20(8)
Range	1·15–2·55	0·36–0·88	0·33–0·61	0·26–0·58	0·45–0·82	0·61–0·97	0·61–1·94
95% Con. limits	1·51–1·79	0·56–0·68	0·45–0·53	0·39–0·47	0·60–0·72	0·76–0·88	1·14–1·38

* Figures in parenthesis are numbers of children studied longitudinally.

TABLE 2. Serum IgM levels in Nigerian children during the first year of life in mg%

	Age (months)						
	Birth	1	3	4	6	9	12
Mean	10·07	30·80	42·05	56·00	58·15	66·80	79·45
s.d.	3·42	10·63	9·60	7·32	11·69	12·44	18·00
s.e.	0·76	2·38	2·15	1·64	2·62	2·79	4·02
n*	20(20)	20(20)	20(20)	20(14)	20(11)	20(10)	20(8)
Range	6·2–17·0	7·0–46·0	23·0–70·0	45·0–70·0	33·0–85·0	51–100	40–110
95% Con. limits	8·58–11·56	26·17–35·49	37·84–46·26	52·79–59·21	53·02–63·29	61·35–72·25	71·57–87·33

* Figures in parenthesis are numbers of children studied longitudinally.

TABLE 3. Serum IgA levels (mg%) in Nigerian children during the first year of life

	Age (months)						
	Birth	1	3	4	6	9	12
Mean	—	18·0	25·6	47·9	73·2	81·2	85·7
s.d.	—	8·4	10·2	12·2	20·4	22·0	20·1
s.e.	—	1·9	2·3	2·7	4·6	4·9	4·5
n*	—	19(20)	20(20)	20(14)	20(11)	20(10)	20(8)
Range	—	1·4–31·6	10·6–42·1	31·6–53·3	42·1–107·0	21·8–126·2	33·6–126·2
95% Con. limits	—	14·2–21·8	21·1–30·1	42·6–53·3	64·3–82·1	71·6–90·9	76·9–94·5

* Figures in parenthesis are numbers of children studied longitudinally.

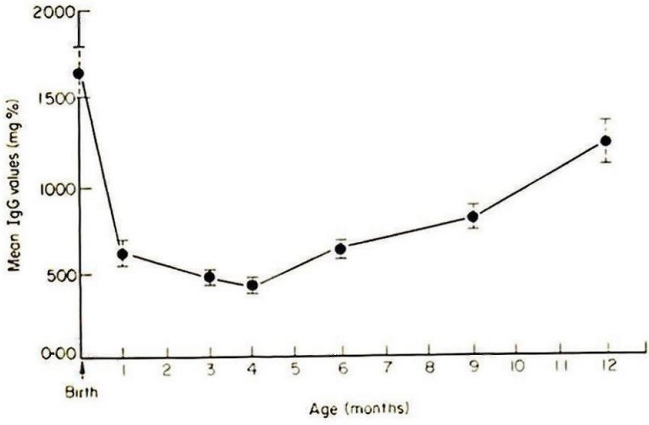

FIG. 1. IgG values in the first year of life, mean values and 95% confidence limits.

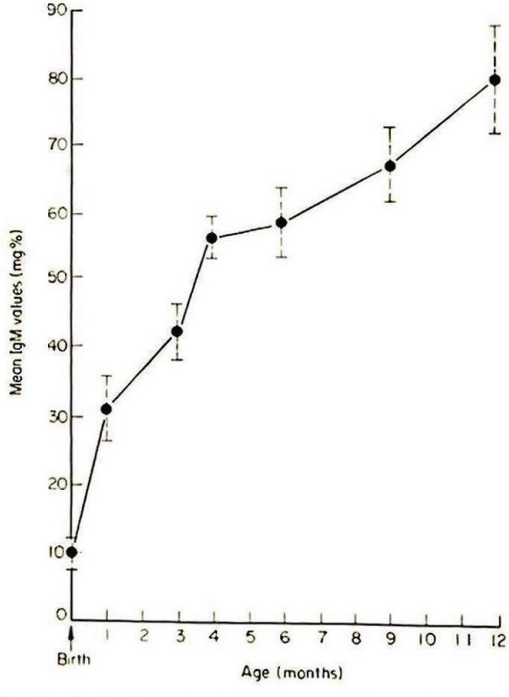

FIG. 2. IgM values in the first year of life, mean values and 95% confidence limits.

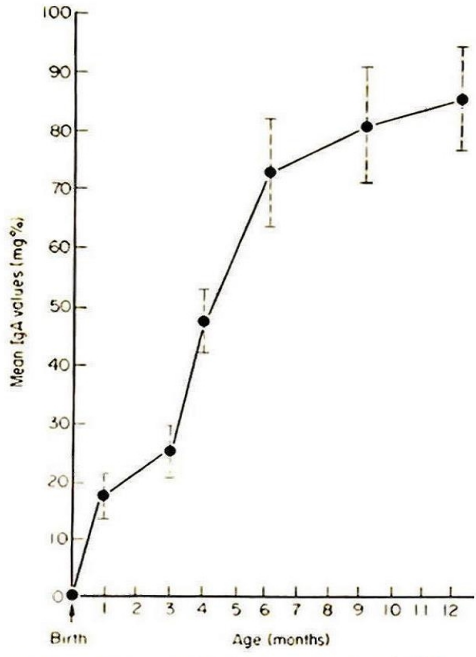

FIG. 3. IgA values in the first year of life, mean values and 95% confidence limits.

downward trend was maintained, but far less precipitously, till the fourth month of life after which a steady rise in values was observed. The lowest values obtained ranged from 0·26–0·58 g%, and birth levels were not attained at 1 year of age.

IgM: Table 2

Low levels of plasma IgM were present at birth and ranged between 6·2–17·9 mg%. A fairly rapid rise was observed in the first 4 months of life after which it became more gradual and reaching 40–110 mg% at 1 year of age.

IgA: Table 3

This class of immunoglobulin was not detected at birth in any of the children but was present at one month of age in all of them. Production was rapid in the first 6 months of life and at one year of age the values ranged between 33·6–126·2 mg%.

Discussion

The high level of plasma IgG found in the infant at birth and its dramatic fall in the first month of life may be attributed to three important factors. (i) Most of the plasma IgG was maternal in origin and was transferred transplacentally to the infant. This was in turn being rapidly catabolised by the infant (Brambell, 1958). (ii) High levels of plasma IgG are known to predispose to its rapid fractional catabolism (Fahey & Robinson 1963). (iii) Haemodilution factors are known to occur in the first month of life as a result of rapid blood volume expansion.

After the initial rapid fall, blood levels remained relatively low till about the fourth month of life when a steady rise was observed. The infant at this state would seem to have taken up the synthesis of its own immunoglobulin G and various antibodies mediated by it.

Although cells capable of producing IgM immunoglobulin have been found in fetuses just over 14 weeks old, production remains low in the absence of appropriate stimulation. Being a macromolecular immunoglobulin, no transplacental transfer is known to occur and levels at birth indicate the fetus own native production. Studies to ascertain whether the maternal IgM crosses the placenta into the fetus

were undertaken by Gitlin *et al.* (1964) and van Furth *et al.* (1966). They administered radioactive labelled IgM to mothers in the last trimester of pregnancy. They failed to demonstrate transplacental transfer into the baby. It is however known that certain intrauterine infections such as rubella, malaria or syphillis stimulate high fetal IgM production in utero, and this has been used to confirm such conditions in the mother during pregnancy. The initial antibody response to infections generally in the newborn period is of the IgM class (Smith & Eitzman 1964, Stiehm, Ammann & Cherry (1966). It may be concluded therefore that the fairly rapid rise in the level of this immunoglobulin in the first 4 months of life reflects the primary response of the Nigerian infants to various antigenic stimuli from the common infections known to occur.

Failure to detect IgA at birth would suggest that it was either absent or present in such low levels that we could not detect it by our methods. Its appearance at the age of 1 month would indicate that production in significant quantities was dependent upon external stimuli after birth.

Conclusion

This study establishes for the first time the levels of plasma immunoglobulins in healthy Nigerian newborn infants and the ontogeny of three major classes (G, A and M) within the first year of life. Similar work has been done in the Caucasian children and other ethnic groups and some important similarities and differences are noted.

In the Nigerian newborn, the level of plasma IgG (1150–2550 mg%) is significantly higher than that found in Caucasian or American children (740–1374 mg%) and probably reflects the higher values of this immunoglobulin in Nigerian mothers. There was however a similar fall in plasma concentrations within the first month of life in both groups although is more profound in the Nigerian infant (30% of birth levels). This is expected to fall in view of the normal half life of plasma IgG which is 21–25 days. Maternal IgG antibodies are believed to protect the infant during the early months of life against measles, poliomyelitis, malaria and chickenpox especially in mothers who have been exposed to these infections or in cases of active immunization. After 4 months of age plasma IgG levels begin to rise as the infant begins to synthesize its own, thereby initiating active protective mechanisms.

A slightly higher rate of IgM synthesis is noticed from relatively low levels recorded at birth and confirms the absence of significant intrauterine infections in normal Nigerian infants. Although absent at birth the pattern of IgA production is similar to that of IgM.

These results show that the Nigerian infant possesses innate capability of producing immunoglobulins required for mounting humoral antibody responses early in life—activation of this system being an important prerequisite for survival in the tropics. This system is crucial for the augmentation of maternal protection which is known to be effective in early life. Recent experience of infections with measles, and malaria in children under the age of 6 months indicate that maternal protection is not absolute in some Nigerian infants and further investigations are required.

All of the infants in this study were wholly breast fed for at least the first month of life after which the introduction of artificial feeds (cow's milk) was variable. Some started at 4 weeks and others later. We are not aware of any significant difference that this might make to plasma immunoglobulin levels. In the present social set up in Nigeria, babies entirely breast fed for 3 months are very rare to come by and we may assume that the method of feeding in this series represent the average normal practice in Nigeria today. Most of them were partially weaned off the breast by 1 year of age on to pap and other semi-solid diet supplemented with artificial milk, proteins and vegetables.

Further work is necessary to relate the changes in social customs, infections, overall health conditions, immunizations and other factors to the immunological profile of the Nigerian infant. This study was designed to investigate some aspects of the humoral antibody system in the crucial first year of life during which mortality is still unacceptably high.

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