Afr. J. Med. med. Sci. (1987) **16**, 181–186.

Blood lymphocytes and measles viraemia

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Summary

Previously described giant multinucleated cells were observed in phytohaemagglutinin (PHA) cultures of washed leucocytes from blood samples of seventeen (36%) out of forty-seven children with measles or undiagnosed fever in Ibadan. The giant cells were more readily seen in PHA cultures of purified lymphocyte preparations than in total leucocyte suspensions. It was concluded that the giant cells seen in cultures indicate in-vivo infection of some blood lymphocytes of patients with measles and related infections by syncytium-producing virus. The practical application of lymphocyte cultures to the laboratory diagnosis of measles is stressed, and the concept of circulating lymphocytes acting as vehicles for the systemic spread of measles infection is highlighted.

Résumé

Nous avons observé des cellules géantes à noyaux multiples présentes dans les cultures phytohaemagglutinine (PHA) des leucocytes lavés. Ce sont des cultures du prélèvement du sang de dix-sept (36%) sur quarante-sept enfants souffrant de la rougeole ou de la fièvre non-diagnostiquée.

Les cellules géantes étaient plus visibles dans les cultures PHA des préparations lymphocytes purifiées que ceux qui étaient dans des suspensions leucocytes complètes. Nous avons tiré cette conclusion: les cellules géantes visibles dans les cultures sont indicatives de l'infection *in vivo* de quelques lymphocytes dans le sang des malades souffrant de la rougeole et des infections apparentées qui sont causées par des virus producteurs de syncitium. Nous avons mis l'accent sur l'application pratique des cultures lymphocytes de la rougeole aussi bien que sur le fait que des lymphocytes qui circulent contribuent à la diffusion systémique de la rougeole.

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Introduction

Attention was drawn to leucocyte changes in measles half a century ago by Benjamin and Ward (1932) and much later, Peebles (1967) reported association of the virus with leucocytes from measles patients during the viraemic stage of the infection.

Previous studies in West and East Africa showed that multinucleated giant cells can be readily observed by microscopy of 2–3-day cultures of phytohaemagglutinin (PHA)-stimulated leucocytes from measles patients (Osunkoya, Oyedisan & Cooke, 1973; Osunkoya *et al.*, 1974b; Gripenberg & Forbes, 1974). Leucocytes of children with acute infections by unidentified syncytia-producing viruses also showed the in-vitro cytopathic effect (Osunkoya, Ogunjumo & Adeshina, 1974c). It was not possible in these studies to exclude granulocytes and monocytes as the carriers of virus material giving rise to the reported cytopathic effect.

The present study was carried out to determine whether or not blood lymphocyte preparations from children with measles or undiagnosed fever can develop giant cells in PHA cultures equally well as total leucocyte preparations used in the earlier studies.

Patients and methods

Patients

Sixty-five children aged 6 months to 10 years attending the General Out-patients Clinic of

the Oni Memorial Children's Hospital, Ibadan, were selected for the study. There were thirty-five boys and thirty girls, 90% of whom were aged 6 years and below. They were seen during the dry season (November 1982 to April 1983) and selected on the basis of fever (axillary temperature of 38.0°C and above) but no localized diagnostic signs (twenty-one cases), mild upper respiratory infection (thirty-eight cases), and symptoms and signs suggestive of measles prodrome (six cases).

After blood samples were taken on the first visit (day 0), all patients were treated with antimalarials with or without antibiotics depending on the patterns of the fever and immediate drug history of the patient. They were requested to report for re-examination on day 3 and, if indicated, for further follow-up examination on day 5.

Blood samples

The patients were bled by venepuncture for leucocyte cultures, blood smear examination for malaria parasites, and routine haematological investigations (PCV, total and differential WBC and HB genotype). Eight millilitres of blood were collected from each patient in a sterile heparinized universal bottle, and 2 ml in a sequestrine container for the laboratory investigations. The patients and specimens were given serial code numbers and the specimens despatched at ambient temperature to the laboratory where tests were commenced within 2 h of collection. Laboratory studies were carried out blind, i.e. without reference to the clinical background or records of the patients.

Leucocyte cultures

Further observations on leucocyte cultures of children with acute fever were carried out in three series of experiments. In the first series of cultures, total leucocyte suspensions were prepared and phytohaemagglutinin (PHA) cultures set up as previously described (Osunkoya *et al.*, 1974b, c) from blood samples of twelve patients. The culture medium was 10% foetal calf serum in TC 199.

The second series of cultures were carried out on blood samples from sixteen patients. Each heparinized blood sample was divided into two for (i) preparation of total leucocyte cultures as in the above first series of experiments, and (ii) preparation of pure lymphocyte suspension, by differential centrifugation using Lymphoprep as the fractionation fluid. Both types of cell suspensions (total leucocytes and purified lymphocytes) were washed free of plasma in heparinized TC 199 and set up as PHA cultures in 5 ml culture medium.

The third series of cell cultures involved blood samples from nineteen patients. Based on the good impression gained from the second series of cultures, all further cultures were carried out using purified lymphocyte preparations. Lymphocyte preparation from each patient was set up as two parallel 5-ml cultures, one with PHA and the other as control (no PHA) culture.

Cultures were examined on day 3 by microscopy of (i) wet (cover slip) preparations, and (ii) stained smears of centrifuged cell deposit as described in previous studies (Osunkoya *et al.*, 1974b, c). The test (PHA) preparations were examined for giant cells (large and small) and other abnormal features, and compared with control cultures.

Results

Cell cultures of blood samples from a total of forty-seven out of the sixty-nine patients selected for the study were found suitable for diagnostic assessment. The following are highlights of observations made on the forty-seven cases in the three series of experiments mentioned above.

The results obtained from the total leucocyte cultures from the first group of twelve patients are summarized in Table 1. Giant multinucleated cells described in previous studies (Osunkoya *et al.*, 1974b) were present in four of the twelve PHA cultures. One of the four patients with giant cell positive cultures developed frank measles 2 days after blood sampling. Two others did not return for follow-up examination. The fourth patient had malaria parasitaemia when first seen but neither responded to antimalarials nor developed skin rash.

Blood samples of six out of the next group of sixteen patients studied yielded giant cell posi-

Table 1. Giant cells in PHA leucocyte cultures of twelve children with fever

Patient code no.	Giant cells present in leucocyte culture	Relevant clinical data
OMCH 6	+	Frank measles on day 2
7	+	Malaria parasitaemia No response to antimalarial therapy
8	++	Lost to follow-up
9	–	
10	–	
11	–	Malaria parasitaemia
12	–	
13	–	Malaria parasitaemia
14	–	Malaria parasitaemia
15	–	Malaria parasitaemia
16	–	
17	+	Lost to follow-up

tive cultures. As summarized in Table 2, the giant cells were more readily seen in the purified lymphocyte cultures than in total leucocyte cultures set up in parallel from respective blood samples. Of the six positive cell cultures, two were associated clinically with frank measles, two other patients were lost to follow-up and yet another developed conjunctivitis but no rash. There was no clinical evidence of measles in the patient with the sixth giant cell positive culture in this group, during the follow-up period.

The third group of nineteen patients yielded giant cell positive tests in purified lymphocyte cultures of seven patients, as well as an equivocal culture result in one patient (no. 68) with a 2-day history of measles rash (Table 3). There was good correlation of positive giant cell culture and clinical measles in three cases. Three of the remaining giant cell positive cases were lost to follow-up. The seventh positive case developed conjunctivitis, with no skin rash or response to antimalarials.

As summarized in Table 4, the overall

Table 2. Comparison of giant cell positive PHA cultures of (a) total leucocytes and (b) purified lymphocytes from peripheral blood of sixteen children with fever

Patient code no.	Giant cells present		Relevant clinical data
	Total leucocyte culture	Purified lymphocyte culture	
OMCH 18	–	+	Measles on day 4
23	+	–	Well by day 3 on antimalarials No parasitaemia
24	–	++	Measles on day 0 Malaria parasitaemia
26	+	++	Lost to follow-up
29	–	++	Conjunctivitis. No rash
32	–	+	Malaria parasitaemia Lost to follow-up

Table 3. Correlation of measles with giant cells in purified lymphocyte cultures of nineteen pyrexial children*

Patient code no.	Giant cells in PHA culture	Relevant clinical and other information
OMCH 51	+	Lost to follow-up
52	−	
53	++	No response to antimalarials Lost to follow-up after day 2
54	−	
55	−	Measles on day 0 Scanty cells at end of culture
56	++	Measles on day 0
57	++	Measles on day 0
58	+	Conjunctivitis but no rash on day 3
59	−	
60	−	Malaria parasitaemia
61	−	
62	−	
63	++	Measles on day 2
64	−	
65	++	Lost to follow-up
66	−	
67	−	
68	±	2-day history of measles
69	−	Malaria parasitaemia

*Total with positive giant cell test: 7.

No. with good correlation with measles: 3; no. lost to follow-up (? died or improved): 3; no. with conjunctivitis (? subclinical measles): 1.

Table 4. Frequency of giant cells in leucocyte (including lymphocyte) cultures of forty-seven children with fever

Type of PHA culture	No. cases	Giant cells present
Leucocytes	12	4(33%)
Leucocytes and purified lymphocytes	16*	6(37%)
Purified lymphocytes	19	7(37%)

*Leucocyte cultures 2/16: 12% giant cell positive, lymphocyte cultures 5/16: 31% giant cell positive.

frequency of giant cell positive tests in the present study is cell cultures from seventeen out of forty-seven blood samples from children with fever; a frequency of 36.1%. The degree of positivity was scored as + for cultures in which giant cells were present in good numbers, and ++ for cultures in which many giant cells were seen. Equivocal results, in which only one or very occasional multinucleated cells were seen were scored as ±. Cultures were scored as negative (−) when no giant cell was seen by microscopy of slide preparations or smears.

Incidental observations during this study include blood smears positive for malaria parasites in nine of the forty-seven blood samples subjected to PHA culture. Two of these malaria cases (nos 7 and 32) were also positive for giant cells. Although there was no definite clinical evidence of measles in both cases, the laboratory findings constitute good evidence of multiple acute infections in the two patients.

Discussion

We have stressed in previous reports that the test system used and observations made were similar to that of the present study and therefore have good potential of being applied to laboratory diagnosis of measles and respiratory syncytial virus infections in children (Osunkoya *et al.*, 1974b, c). Other workers have also highlighted the need for improved diagnostic tests for this very common type of acute infections in children (WHO, 1981), which in the tropics rank next in incidence, morbidity and mortality only to malaria.

It is long known that measles virus can be propagated in human leucocytes (Berg & Rosenthal, 1961). One of our earlier studies also provides evidence for the presence of measles virus antigens in PHA-induced lymphoblasts obtained from blood samples of measles patients (Osunkoya *et al.*, 1974a). The virus antigens were not detectable by immunofluorescence in small lymphocytes or platelets of the patients, and it was not therefore clear whether other leucocytes (granulocytes and monocytes) were the vehicles for the virus-induced giant cell formation by lymphoblasts *in vitro*. However, the present study has clearly shown that the virus-induced cytopathic change (giant cell formation) can be effected in the presence of a mitogen by small mononuclear cell (lymphocyte) preparations in which granulocytes and monocytes have been eliminated. The implication of this finding is that some lymphocytes of measles patients do contain the virus material, and thereby give rise to giant cell formation during the process of mitogen (PHA)-induced transformation. Although unlikely, it is not possible to exclude blood platelets as vehicles of such virus material, since platelets are invariably present in purified lymphocyte preparations.

Our present observation implicates circulating lymphocytes as vehicles for the spread of measles and related viruses from the portal of entry of such viruses to other parts of the body, especially the skin and lymphoid tissues, where pathological tissue reactions are manifested (White & Boyd, 1973). This concept of viraemia in measles needs to be further explored in terms of the sub-population of lymphocytes that are particularly susceptible to infection by the measles virion. So far circumstantial evidences point to the T subpopulation of lymphocytes, which are known to show a low count in patients with measles (Osunkoya, in press) with corresponding increase in NK cell activity (Greenwood & Whittle, 1981).

The concept of lymphocytes acting as a major vehicle of measles viraemia does not imply that virus material could not be present in plasma of patients. Virus material is indeed present during the late and convalescent stages of the infection, but in association with antibodies. The crucial stage of viraemia in terms of disease (pathological effect) produced by the infective agent is during the very early stage of infection when there is generalized spread of the infective agent. Our previous and present studies have shown that blood lymphocytes are involved in such spread during the prodromal stage of the disease, some days before the appearance of the skin rash that clinically typifies measles (Osunkoya *et al.*, 1974c).

It is difficult, if not impossible, to quantify giant cells in positive tests, either in absolute numbers per unit volume of culture or in relative numbers to other cells in the culture. This present drawback to standardization of the test is engaging our attention as much as the need to develop a microtechnique for the test.

Acknowledgments

The technical assistance of Mrs A. E. Bamgboye and preparation of the typescript by Mrs E. O. Sowunmi are gratefully acknowledged.

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(*Accepted* 8 *December* 1983)