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# Epstein–Barr Virus Markers in a Series of Burkitt's Lymphomas from the West Nile District, Uganda*

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**Abstract**—*In an epidemiological survey in the West Nile District of Uganda, 70 pathologically confirmed BL cases were detected over a 5-yr period; this corresponded to an annual incidence rate of 1.6 per 100,000 general population or about 5 per 100,000 children in the age group 5–14 yr. Of the confirmed cases which were examined by EBV/DNA molecular hybridization, 96% were found to contain an average of 38 EBV genome equivalents per tumour cell, whereas none of the examined unconfirmed cases did. Duplicate hybridization assays in two laboratories were in close agreement. Serological testing showed that 91% of the confirmed BL cases had elevated EBV/VCA titres (≥160) and 64% were EA(D)-positive (≥10). Most of the cases with high EBV/genome content had high VCA titres, but there was a poor correlation between the two parameters among all cases. This study confirms that in high BL incidence areas the association between EBV and this lymphoma is almost constant, whereas it is exceptional in low-incidence areas. This further supports the aetiological implication of EBV in the endemicity of this tumor in equatorial Africa.*

## INTRODUCTION

THAT Epstein–Barr virus (EBV) plays an aetiological role in African Burkitt's lymphoma (BL) is supported by the regular association between the virus and the tumour (for recent review, see [1]). Firstly, BL patients usually have higher levels of EBV antibodies than any control groups selected in the same area [2, 3]. The virus–tumour association is also documented at the cellular level, where each tumour cell regularly contains several copies of the EBV genome and expresses EBV nuclear antigen(s) [4–6]. Epidemiological evidence for an aetiological role of the virus in BL recently resulted from a long-term prospective study in the West Nile District of Uganda [7, 8], where it was found that EBV/VCA antibody titres were significantly elevated in future BL patients several years before the tumour developed.

However, lymphomas histologically and cytologically indistinguishable from BL have been found outside the endemic areas [9, 10], although at a much lower incidence rate [11, 12]. It appears that these sporadic cases have a relationship with the EBV which is somewhat different from that of the African BL: more than 90% of the African BL [4, 5, 13, 14] carry the EBV genome in the tumour cells whereas less than 20% of the American BL-like lymphomas do [15–17]. The difference between the two types of BL is also expressed serologically. As mentioned, the African cases have invariably higher EBV antibody levels than

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their controls, and this is sometimes [18] the case for American BL patients as well, although exceptions have been reported [19, 20].

In order to add more information about the relationship between BL and EBV in different parts of the world, we present here the results of simultaneous serological testing and hybridization assays of a large series of BL patients which were collected during a prospective study [7] in Uganda.

## PLACE AND METHOD OF BL DETECTION

The West Nile District is a tropical lowland situated in the northwestern corner of Uganda, south of the Sudan between the rivers Nile and Zaire (see map in Fig. 1). The altitude of the hilly area varies from about 660 m above sea level up to about 1700 m in the southwest corner of the district. The area is relatively fertile and the rainfall (the annual average was 54 inches for the period 1955–1976; E. H. Williams, unpublished data) is sufficient to sustain farming throughout the district. The population numbered 573,762 in 1969 (Census of Uganda) and the annual growth rate is estimated at about 3% per year (UN Statistical Yearbook, 1978). The vast majority of the people are subsistence farmers who grow maize, millet and cassava for food crops and some tobacco as a cash crop. Malaria is hyperendemic to holoendemic in the area [21] and surveys conducted in connection with the present study confirmed that 60–70% of the children under 10 yr of age have malarial parasites in their blood at any moment. About 90% of the parasites encountered in the West Nile District are *Plasmodium falciparum*, the remainder being *Plasmodium malaria*.

BL registration in the West Nile District has been carried out since 1964 by Dr E. H. Williams at Kuluva Hospital. In 1971 the International Agency for Research on Cancer initiated a prospective study of BL in the district with the specific objective of testing the hypothesis that EBV is an aetiological factor in BL [7, 8]. The serum collection which formed the basis for the prospective study was confined to 5 of the 10 counties which make up the West Nile District (see map in Fig. 1). Active BL detection was, however, carried out in the entire district and the present report deals with cases detected in all 10 counties, including the 16 'pre-bled BL' cases from the prospective study which were published earlier [7, 8].

Although the prospective study was initiated in 1971, it was not until 1973 that the collection of frozen biopsies were included in the field work. At the beginning of 1979 the project in the West Nile District had to be terminated due to the civil


*Fig. 1. Map of the West Nile District showing county borders. Shaded areas were included in the prospective study.*

disturbances in Uganda. From 1973 to 1978 active BL detection was carried out by detection teams which regularly visited all hospitals and health centres in the district to trace patients with symptoms and signs of BL. Patients suspected of having BL were brought into Kuluva hospital and examined by H. E. Williams, who also collected specimens for pathology and virology studies. The case detection was very active and it is believed that nearly all BL cases occurring in the West Nile District during these years were actually discovered. Some cases which came spontaneously to Kuluva hospital from neighbouring Zaire and Sudan during this period were also included in the study since they came from areas which are ethnically and ecologically similar to the West Nile District. Pathology specimens required for diagnosis were given priority over the frozen specimens needed for the hybridization assay.

*Pathology examination*

All patients suspected of having BL were subjected to a diagnostic pathology examination. The necessary biopsies, tumour imprints and needle aspirates were taken before any treatment. They were examined locally by EHW, who directed the treatment of the patients. All pathology specimens were subsequently for-

warded to DHW for final assessment. Sections of tissue embedded in paraffin wax and resin-embedded tissue cut at 1 μm were examined together with imprint preparations and BL identified on the basis of established light microscopic criteria [22, 23].

*EBV/DNA hybridization*

Whenever sufficient tumour tissue could be obtained from a BL patient, part of this was placed in liquid nitrogen and forwarded to IARC by air in insulated containers. After some time of storage in liquid nitrogen at the IARC specimen bank, the frozen specimens were forwarded to the laboratory in either Stockholm or Freiburg for EBV/DNA hybridization; for some cases where abundant tumour tissue was available, material was sent to both laboratories. The two laboratories used different techniques for the detection of EBV/DNA in the biopsies. The Stockholm laboratory used filter hybridization with $^{32}$P-labelled EBV-cRNA as described by Lindahl *et al.* [13]. The laboratory at Freiburg used DNA-reassociation kinetics with $^{3}$H-labelled EBV/DNA as a probe as described by Nonoyama *et al.* [5] and Bornkamm *et al.* [24]. If only small amounts of DNA were available, filter hybridization was more sensitive than reassociation kinetics. The hybridization was done 'blindly' in so far as the final pathology diagnosis of the tumour was not known to the laboratory at the time of testing. The results were given in terms of the number of genome-equivalents per cell; in addition, the laboratory in Freiburg indicated the detection limit of the test by stating in each case the lowest number of genomes which could be detected with the amount of DNA available.

*Serological testing*

A serum sample was collected from each examinee by vein puncture before treatment. The sera were kept in liquid nitrogen at the project centre in the West Nile District for a few weeks and then forwarded to IARC, Lyon, where the sera were tested for the following anti-EBV antibodies: viral capsid antigen (VCA); early antigen (EA); and nuclear antigen (EBNA).

Anti-VCA and anti-EA (D) and (R) antibody titres were evaluated by indirect immunofluorescence techniques as previously described [25, 26]. Anti-EBNA titres were measured by the anticomplement immunofluorescence technique described by Reedman and Klein [27].

## RESULTS

*BL cases*

During the 6 yr (1973–1978) of case collection, a total of 70 BL suspects were detected in the West Nile District. In addition, 14 cases from neighbouring Zaire, 1 from Sudan and 1 from Uganda outside the West Nile District, who reported spontaneously to the project during this period, were included in the study. On the basis of the population figures published in the Uganda Census of 1969, the mid-year population of the West Nile District for the period 1973–1978 was estimated at 730,000. By referring the 70 cases detected in the district to this base population, an average annual BL incidence rate of 1.6 per 100,000 is arrived at. Most of the BL cases (approx. 85%) in the West Nile District occurred in the age group 5–14 yr, which constitutes 27% of the total population (UN Demographic Year Book, 1972). Thus the BL incidence rate was about 5 per 100,000 children aged 5–14 yr.

Of the 86 suspected BL cases which were available for the study, the pathologist confirmed the BL diagnosis in 74 cases (Table 1) but not in the 12 others. The reasons for not confirming the BL diagnosis were as follows (see also Appendix I): 6 cases were found to be other childhood tumours, *viz.* 2 retinoblastomas, 2 rhabdomyosarcomas, 1 Wilms tumour and 1 embryonic tumour; for 2 cases, there was no evidence of malignant cells; and for the remaining 4 there was insufficient material for diagnosis.

Details regarding age and sex of the patients, anatomical location and pathological and virological data are given in Appendix I.

*Table 1. Number of confirmed and unconfirmed BL cases, number examined by EBV hybridization and number found positive and negative in each category, West Nile District, 1973–1978*

| No. of BL suspects examined by pathologist | BL diagnosis | | EBV hybridization | | EBV positive | EBV genome negative |
|---|---|---|---|---|---|---|
| 86 | confirmed: | 74 | done: | 53 | 51 | 2 |
| | | | not done: | 21 | | |
| | not confirmed: | 12 | done: | 5 | 0 | 5 |
| | | | not done: | 7 | | |

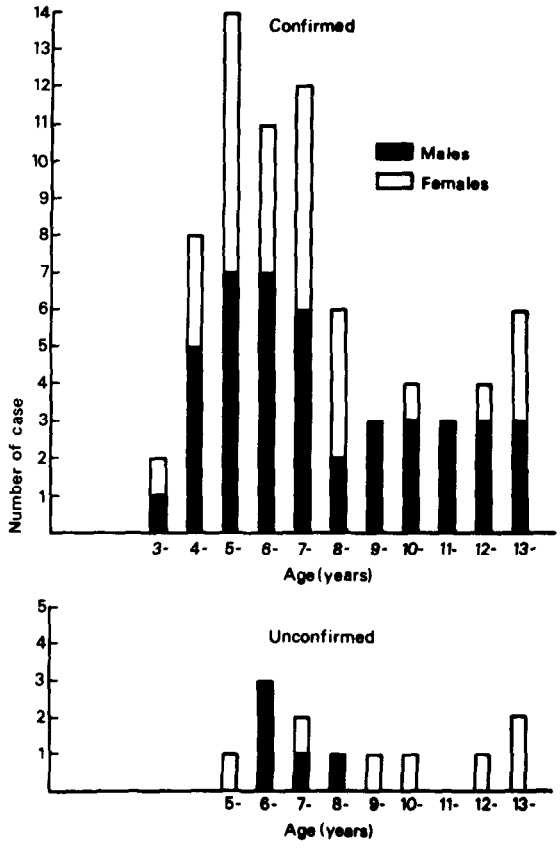


*Fig. 2. Age and sex distribution of confirmed and unconfirmed BL suspects from the West Nile District of Uganda.*

The age and sex distribution of the 74 confirmed and 12 unconfirmed cases are compared in Fig. 2. The BL patients ranged in age from 3 to 18 yr (mean age, 7.5 yr) and there were more boys than girls (ratio male:female, 1.4). The unconfirmed cases were slightly older (mean age, 9 yr) and more were girls than boys.

*EBV/DNA detection*

Both confirmed and unconfirmed BL tumours were examined for the presence of EBV genome. However, as mentioned earlier, the tumour specimens were, in some cases, so small that the EBV genome count could not be estimated. This was the case in 2 tumours (Nos 247 and 260), where the absence of EBNA in the tumour imprints caused us to label these as EBV/DNA negative. Table 1 shows that 51 (96%) of the 53 pathologically confirmed BL tumours which were examined by hybridization contained the EBV genome, whereas none of the 5 unconfirmed tumours (which were tested) did. Details regarding the number of genome equivalents found in the tumour cells are given in Appendix I.

In addition to case No. 247, another confirmed case (No. 223) failed to reveal the presence of EBV/DNA in the tumour cells. No. 247 was a 6-yr-old boy with an orbital tumour and with EBV antibody titres as follows: VCA 40, EA <10 and EBNA 40. No. 223 was an 8-yr-old boy with an abdominal tumour with the following anti-EBV titres: VCA 80, EA <10, EBNA 640.

Fifteen tumours were large enough to each yield two specimens for EBV genome testing, and one specimen was sent to each of the two laboratories. The results of their independent hybridization assays are cross-tabulated in Table 2, from which it can be seen that there was a very good agreement between the two laboratories. Not only did both find that all 15 tumours contained the EBV genome, but they also found very similar genome counts.

*Serology*

Sera were collected from all the 86 BL suspects included in the present investigation and all were

*Table 2. BL tumours cross-tabulated by results of EBV genome counts in two laboratories*

| | | No. of EBV genome equivalents per cell | | | | | |
|---|---|---|---|---|---|---|---|
| Freiburg: | 0–9 | 10–19 | 20–29 | 30–39 | 40–49 | 50+ | All |
| Stockholm: | 0–9 | | | | | | |
| | 10–19 | 2 | 1 | | | | 3 |
| | 20–29 | 1 | 1 | | 1 | | 3 |
| | 30–39 | | | 3 | 1 | | 4 |
| | 40–49 | | | | 1 | 3 | 4 |
| | 50+ | | | | | 1 | 1 |
| | All | 3 | 2 | 3 | 3 | 4 | 15 |

No. of EBV genome equivalents per cell.

*Table 3. Cross-tabulation of EBV antibody results by size of VCA and EA(D) titres, separately for confirmed and unconfirmed BL suspects, West Nile District, Uganda*

| Diagnosis | EA titres | VCA titres <10 | 10 | 20 | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 | >2560 | All |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Confirmed BL | <10 | | 1 | | 2 | 3 | 1 | 2 | 7 | 6 | 3 | 1 | 26 |
| | 10 | | | | | | | 1 | 2 | 2 | 4 | 1 | 10 |
| | 20 | | | | | | | | 1 | 3 | 3 | | 7 |
| | 40 | | | | | | | 1 | | 1 | 2 | | 4 |
| | 80 | | | | | | | 1 | 1 | 3 | 2 | | 7 |
| | 160 | | | | | | | | 2 | 1 | 3 | | 6 |
| | 320 | | | | | | | 1 | 1 | 1 | 1 | | 4 |
| | 640 | | | | | | | | | | | 2 | 2 |
| | 1280 | | | | | | | | | | | 1 | 1 |
| | 2560 | | | | | | | | | 1 | 1 | 5 | 7 |
| | >2560 | | | | | | | | | | | | |
| | All | — | 1 | — | 2 | 3 | 1 | 6 | 14 | 18 | 19 | 10 | 74 |
| Not confirmed | <10 | | 1 | | 1 | 1 | 1 | 1 | 2 | 1 | 1 | | 9 |
| | 10 | | | | | | | | | | | | |
| | 20 | | | | | | | | | | | | |
| | 40 | | | | | | | | | | | | |
| | 80 | | | | | | | | | | | | |
| | 160 | | | | | | | | | | | | |
| | 320 | | | | | | | | | 2 | | | 2 |
| | 640 | | | | | | | | | | | 1 | 1 |
| | 1280 | | | | | | | | | | | | |
| | 2560 | | | | | | | | | | | | |
| | >2560 | | | | | | | | | | | | |
| | All | — | 1 | — | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 1 | 12 |

tested for EBV antibodies (VCA, EA and EBNA) at the IARC. Titres measured for each of the three antibodies are shown in Appendix I for each individual. In Table 3 the VCA and EA titres are cross-tabulated. It can be seen that 67/74 (91%) of the confirmed BL cases have elevated VCA titres (≥160) vs 8/12 (67%) of the unconfirmed cases. In the general child population in the West Nile District, about 35% of the 6- to 9-yr-old children have EBV/VCA titres above 160 [28]. The geometric mean (GMT) of the VCA titres shown in Table 3 is 1152 for the confirmed and 534 for the unconfirmed cases. The GMT of positive VCA titres in the general child population aged 5–9 yr in the West Nile District has previously been found to be 134 [28]. Table 3 further shows that 48/74 (64%) of the confirmed BL cases have positive EA titres (≥10) whereas only 3/12 (25%) of the non-confirmed cases have such titres. In the general child population aged 4–10 yr in the West Nile District, about 10% have positive EA titres [28]. In Table 3 all cases, confirmed as well as unconfirmed, which have VCA titres below 320 have negative EA titres. Although some of the unconfirmed cases in Table 3 may in fact be BL, it appears that the presence of EA antibodies in a patient depends more on his level of VCA antibodies than on whether or not he has BL, as pointed out previously by de-The [29].

*Correlation between EBV genome equivalents and EBV titres*

A cross-tabulation of results by EBV/VCA titres and by average number of EBV genomes per cell is shown in Fig. 3 for the 53 confirmed and the 5 unconfirmed cases which were subjected to both EBV hybridization and EBV antibody testing. Here the genome counts were taken from either laboratory (Stockholm or Freiburg), using whichever result was highest. It can be seen that there is some correlation between the two different measures of EBV association: nearly all cases without detectable EBV genome have relatively low VCA titres (<320) whereas most of those with high genome counts (≥20) have VCA titres of 320 or above, going up to 2560; the scatter of titre is, however, so wide at each level of genome count that it is impossible to predict the size of any of the two variables from knowledge about the other.

## DISCUSSION

The epidemiological findings presented here confirmed that the West Nile District of Uganda is an area with a relative high incidence of BL.

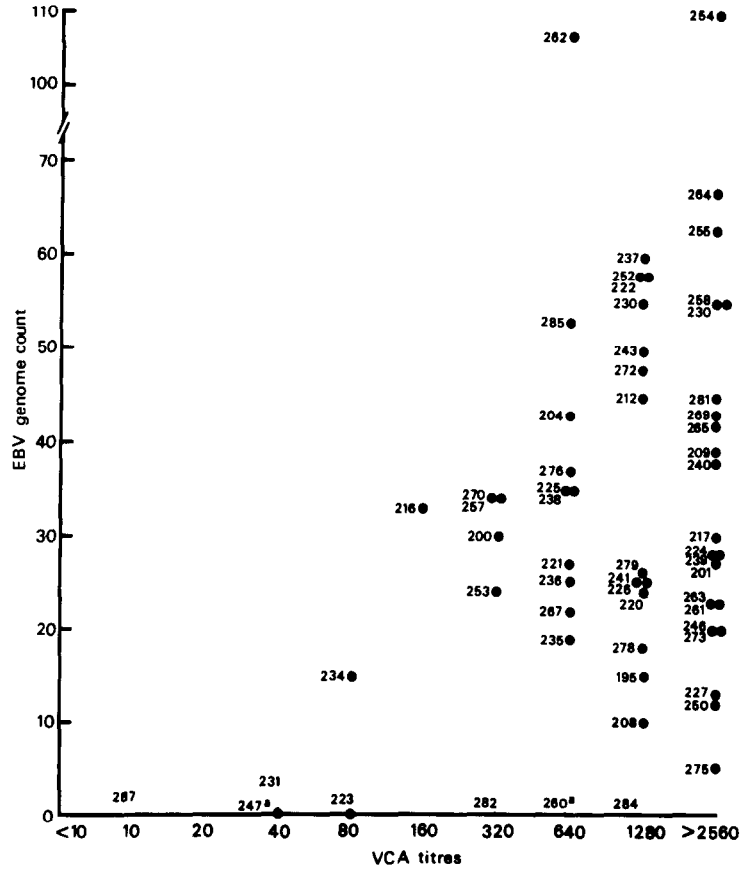


*Fig. 3. Distribution of 58 BL suspects by EBV/VCA antibody titre and by number of EBV genome equivalents per tumour cell; West Nile District, Uganda.* n = 58. *Serial No. followed by* ● = *confirmed BL; serial No. followed by* × = *unconfirmed case. (a) EBNA is tumour-negative.*

During the years 1973–1978 the annual incidence rate was about 5 per 100,000 children aged 5–14 yr or about 1.6 per 100,000 total population. This incidence is of the same order of magnitude as found in other areas of tropical Africa, where an incidence rate of 2.8 was reported in the general population from Tanzania [30] and one of 0.5 from Ghana [31]. In earlier years, from 1961 to 1975, the annual incidence rate of BL was as high as 2.8 [32] per 100,000 general population in the West Nile and there is thus an indication that the incidence may have been declining there in recent years. Nevertheless, the findings of viral BL markers presented in this report still reflect conditions prevailing in a high incidence area.

The EBV/DNA hybridization results found in the present study are shown in Table 4 in comparison with those obtained in similar studies of other BL tumours from East Africa. It can be seen that the results from the West Nile District are in close agreement with those obtained elsewhere in Africa, both with respect to the proportion of BL which carry the EBV genome and with respect to the average number of genome equivalents per tumour cell. It thus seems firmly established that about 96% of the East African BL

*Table 4. Results of EBV-DNA studies of African Burkitt's lymphoma*

| Reference | No. of cases studied | No. EBV-positive | Percentage of EBV-positive cases | Mean No. of EBV genome equivalents/cell |
|---|---|---|---|---|
| [4] | 10 | 10 | 100 | — |
| [5] | 20 | 19 | 95 | 40.4 |
| [13] | 27 | 26 | 96 | 39.1 |
| [14] | 15 | 14 | 93 | 38.8 |
| This study | 53 | 51 | 96 | 34.7 |
| Total | 126 | 121 | 96 | 38.2 |

carry the EBV genome at an average rate of about 38 genome equivalents per tumour cell. Furthermore, the serological findings confirmed that the level of EBV antibodies is highly elevated in African BL patients compared to the levels found in neighbourhood children.

Does the 4% EBV genome-negative BL in Uganda reflect experimental error or the true existence of an EBV-free form of BL in Africa? An error of the order of 4% might well occur merely by chance as a result of mistakes in such procedures as the taking and labelling of specimens or the diagnosis of the tumour. It would be necessary to arrange repeated examinations of many supposedly genome-negative tumours to ascertain that those found free of EBV/DNA are truly BL. It may appear unlikely that error could have been the cause of the finding of the two EBV genome-negative BL tumours in this study, since both EBV markers (viral DNA and serological profile) were absent in the two discordant cases. However, if it happened that these two tumours were not really BL, the absence of EBV markers would be explained.

Experimental error apart, it seems that the most likely interpretation of the present findings is that two forms of BL occur in Africa: a common 'endemic' EBV-associated form and a rare 'sporadic' non-EBV-related form which constitutes about 4% of the African tumours. The sporadic form occurs everywhere in the world and makes up about 80% of all BL tumours in the U.S.A.

It has been suggested [33, 34] that the 'endemic' form of BL develops in at least two stages: firstly, EBV initiates the carcinogenic process early in life [35] by stimulating B-lymphocytes. At a later stage a non-random cytogenetic abnormality occurring in a clone of stimulated cells promotes tumour development. These cytogenic changes seem to be independent of the presence of EBV in the affected cells and are of the three following types: t(8;14), t(2;8) or t(8;22) [36]. Recent experiments suggest that these chromosomal translocations, which involve Ig-locus-carrying chromosomes in all cases, may be a crucial event in the pathogenesis of lymphomas [37, 38], even though initiating factors may differ depending on geography. What is now needed in order to further elucidate this problem is a more accurate study of non-African BL, especially of the non-EBV-associated cases.

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## REFERENCES

1. DE-THE G. The epidemiology of Burkitt's lymphoma: evidence for a causal association with Epstein–Barr virus. *Epidemiol Rev* 1979, 1, 32–54.
2. HENLE G, HENLE W, CLIFFORD P *et al.* Antibodies to Epstein–Barr virus in Burkitt's lymphoma and control groups. *JNCI* 1969, **43**, 1147–1157.
3. HENLE G, HENLE W, KLEIN G *et al.* Antibodies to early Epstein–Barr virus induced antigens in Burkitt's lymphoma. *JNCI* 1971, **46**, 861–871.
4. ZUR HAUSEN H, SCHULTE-HOLTHAUSEN H, KLEIN G *et al.* EB-virus DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature* 1970, **228**, 1056–1058.
5. NONOYAMA M, HUANG CH, PAGANO JS, KLEIN G, SINGH S. DNA of Epstein–Barr virus detected in tissue of Burkitt's lymphoma and nasopharyngeal carcinoma. *Proc Natl Acad Sci USA* 1973, **70**, 3265–3268.
6. KLEIN G. Studies on the Epstein–Barr virus genome and the EBV-determined nuclear antigen in human malignant disease. *Cold Spring Harbor Symp Quant Biol* 1975, **XXXIX**, 783–790.
7. DE-THE G, GESER A, DAY NE *et al.* Epidemiological evidence for causal relationship between Epstein–Barr virus and Burkitt's lymphoma from Ugandan prospective study. *Nature* 1978, **274**, 756–761.
8. GESER A, DE-THE G, LENOIR G, DAY NE, WILLIAMS EH. Final case reporting from the Ugandan prospective study of the relationship between EBV and Burkitt's lymphoma. *Int J Cancer* 1982, **29**, 397–400.
9. BURKITT D. Burkitt's lymphoma outside the known endemic areas of Africa and New Guinea. *Int J Cancer* 1967, **2**, 562–565.
10. LEVINE PH, CHO BR, CONNELLY RR *et al.* The American Burkitt's lymphoma registry: a progress report. *Ann Intern Med* 1975, **83**, 31–36.
11. COSSMAN J, BERARD CW. Histopathology of childhood non-Hodgkin's lymphomas. In: GRAHAMPOLE, ed. *Non-Hodgkin's Lymphomas in Children.* New York, Masson, 1980, 13.

12. PHILIP T, LENOIR G, BYRON PA *et al.* Burkitt-type lymphoma in France: identification among non-Hodgkin's malignant lymphomas in Caucasian children. *Br J Cancer* 1982, **45**, 670–678.
13. LINDAHL T, KLEIN G, REEDMAN BM, JOHANSSON B, SINGH S. Relationship between Epstein–Barr virus (EBV) DNA and the EBV-determined nuclear antigen (EBNA) in Burkitt's lymphoma biopsies and other lymphomo-proliferative malignancies. *Int J Cancer* 1974, **13**, 764–772.
14. OLWENY CLM, ATINE I, KADDU-MUKASA A *et al.* Epstein–Barr virus genome studies in Burkitt's and non-Burkitt's lymphomas in Uganda. *JNCI* 1977, **58**, 1191–1196.
15. PAGANO JS, HUANG DH, LEVINE P. Absence of Epstein–Barr viral DNA in American Burkitt's lymphoma. *N Engl J Med* 1973, **289**, 1395–1399.
16. ANDERSSON M, KLEIN G, ZIEGLER JL, HENLE W. Association of Epstein–Barr viral genomes with American Burkitt's lymphoma. *Nature* 1976, **260**, 357–359.
17. ZIEGLER JL, ANDERSSON M, KLEIN G, HENLE W. Detection of Epstein–Barr virus DNA in American Burkitt's lymphoma. *Int J Cancer* 1976, **17**, 701–706.
18. LEVINE PH, O'CONOR GT, BERARD CW. Antibodies to Epstein–Barr virus in American patients with Burkitt's lymphoma. *Cancer* 1972, **30**, 610–615.
19. LEVINE PH, ABLASHI DV, BERARD CW, CARBONNE PP, WAGGONER DE, MALAN L. Elevated antibody titres to Epstein–Barr virus in Hodgkin's disease. *Cancer* 1971, **27**, 416–421,
20. HIRSHAUT Y, COHEN MH, STEVENS DA. Epstein–Barr virus antibodies in American and African Burkitt's lymphoma. *Lancet* 1973, **ii**, 114–116.
21. KAFUKO GW, BAINGANA N, KNIGHT EM, TIBEMANYA J. Association of Burkitt's tumour and holoendemic malaria in West Nile District, Uganda: malaria as a possible etiological factor. *East Afr Med J* 1969, **47**, No. 7.
22. WRIGHT DH. Burkitt's lymphoma: a review of the pathology, immunology and possible etiological factors. *Pathol Annu* 1971, 337–363.
23. BERARD CW, O'CONOR GT, THOMAS LB, TORLONI H. Histopathological definition of Burkitt's tumour. *Bull WHO* 1969, **40**, 601–607.
24. BORNKAMM GW, STEIN H, LENNERT K, RÜGGEBERG P, BARTELS H, ZUR HAUSEN H. Attempts to demonstrate virus-specific sequences in human tumours. IV. EB viral DNA in European Burkitt's lymphoma and immunoblastic lymphadenopathy with excessive plasmacytosis. *Int J Cancer* 1976, **17**, 177–181.
25. HENLE G, HENLE W. Immunofluorescences in cells derived from Burkitt's lymphoma. *J Bacteriol* 1966, **91**, 1248–1256.
26. LENOIR G, TOVEY MG, LAVOUE M-F. Induction of Epstein–Barr virus early antigen by phytohaemagglutin in the presence of 5-iodo-2'-deoxyuridine: application of EBV serology. *J Immunol Methods* 1980, **34**, 23–29.
27. REEDMAN BM, KLEIN G. Cellular localization of an Epstein–Barr virus (EBV)—associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int J Cancer* 1973, **11**, 499–520.
28. DE-THE G, DAY NE, GESER A *et al.* Sero-epidemiology of the Epstein–Barr virus: preliminary analysis of an international study. In: *Oncogenesis and Herpesviruses II.* Lyon, IARC Scientific Publications, 1975, Vol. 11, 3–16.
29. DE-THE G. Epidemiology of Epstein–Barr virus and associated diseases in man. In: ROIZMAN B, ed. *The Herpes Viruses.* New York, Plenum Press, Vol. 1A, in press.
30. BRUBAKER G, GESER A, PIKE MC. Burkitt's lymphoma in the North Mara District of Tanzania 1964–70: failure to find evidence of time-space clustering in a high risk isolated rural area. *Br J Cancer* 1973, **28**, 469–472.
31. BIGGAR RJ, NKRUMAH FK. Burkitt's lymphoma in Ghana; urban–rural distribution, time-space clustering and seasonality. *Int J Cancer* 1979, **23**, 330–336.
32. WILLIAMS EH, SMITH PG, DAY NE *et al.* Space-time clustering of Burkitt's lymphoma in the West Nile District of Uganda; 1961–1975. *Br J Cancer* 1978, **37**, 109–122.
33. MORROW RH, GUTENSOHN N, SMITH PG. Epstein–Barr virus–malaria interaction models for Burkitt's lymphoma: implications for preventive trials. *Cancer Res* 1976, **36**, 667–669.
34. DE-THE G. Multistep carcinogenesis, Epstein–Barr virus and human malignancies. In: *Viruses in Naturally Occurring Cancers.* Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Laboratory, 1981, Vol. 7, 11–21.
35. DE-THE G. Is Burkitt's lymphoma related to perinatal infection by Epstein–Barr virus? *Lancet* 1977, **i**, 335–338.
36. BERNHEIM A, BERGER R, LENOIR G. Cytogenetic studies on African Burkitt's

lymphoma cell lines: t(8;14), t(2;8) and t(8;22) translocations. *Cancer Genet Cytogenet* 1981, **3**, 307–315.

37. LENOIR G, PREUD'HOMME JL, BERNHEIB A, BERGER R. Correlation between immunoglobulin light chain expression and variant translocation in Burkitt's lymphoma. *Nature* 1982, **298**, 474–476.
38. KLEIN G, LENOIR G. Translocations involving Ig-locus-carrying chromosomes: a model for genetic transposition in carcinogenesis. *Adv Cancer Res* 1982, **37**, 381–387.

## APPENDIX I. DEMOGRAPHIC, PATHOLOGICAL AND VIRAL DATA FOR 86 BL SUSPECTS FOUND IN THE WEST NILE DISTRICT, UGANDA

| BL No. | Place of origin* | Sex/age | Site of tumour | Date of diagnosis | Pathology diagnosis† | EBV serology EA VCA | EA DR | EA D | EBNA | DNA hybridation‡ Stockholm | DNA hybridation‡ Freiburg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 195 | Koboko | F 03½ | maxil. orbit. cran. nerve | 24.01.73 | consistent with BL | 1280 | 20 | <10 | 1280 | 12 | 15 |
| 200 | Maracha | M 09 | l. maxil. | 04.05.73 | Burkitt's lymphoma | 320 | 80 | <10 | 80 | 30 | ne |
| 201 | Terego | M 09 | maxil. mandible | 08.05.73 | Burkitt's lymphoma | 2560 | <10 | <10 | 80 | nd | 27 |
| 202 | Maracha | F 05½ | mandible, ovary | 09.05.73 | Burkitt's lymphoma | 320 | 320 | <10 | 1280 | nd | nd |
| 203 | Aringa | M 03½ | maxillary | 23.06.73 | Burkitt's lymphoma | 640 | <10 | <10 | 40 | nd | nd |
| 204 | Aringa | M 06 | maxillary | 10.07.73 | Burkitt's lymphoma | 640 | <10 | <10 | 20 | nd | 43 |
| 205 | Ayivu | F 07 | orbits, maxil, abd. | 06.06.73 | diagnosis not possible | 1280 | 320 | 160 | 160 | nd | nd |
| 206 | Ayivu | M 14 | face, neck, knee | 07.08.73 | Burkitt's lymphoma | >2560 | 1280 | 10 | 160 | nd | nd |
| 207 | Maracha | F 07 | thyroid, liver, ovary | 03.08.73 | Burkitt's lymphoma | 1280 | 10 | <10 | 640 | nd | nd |
| 208 | Vura | F 07 | r. neck LN | 24.08.73 | Burkitt's lymphoma | 1280 | 80 | 10 | 160 | nd | 10 |
| 209 | Zaire | F 06 | maxil, orbit, ovary | 04.09.73 | Burkitt's lymphoma | 2560 | 160 | <10 | 640 | 39 | 33 |
| 210 | Terego | F 12 | maxil. chest wall | 06.09.73 | Burkitt's lymphoma | >2560 | 640 | 1280 | 40 | nd | nd |
| 211 | Terego | M 05 | neck LN, abdomen | 20.09.73 | Burkitt's lymphoma | 2560 | 20 | 40 | 1280 | nd | nd |
| 212 | Terego | F 07 | groin, abdomen | 21.09.73 | probable BL | 1280 | 320 | 10 | 320 | nd | 45 |
| 213 | Aringa | F 03 | maxil. orbit, ovary | 21.09.73 | Burkitt's lymphoma | 1280 | <10 | <10 | 2560 | nd | nd |
| 214 | Koboko | F 04 | ovaries | 03.10.73 | Burkitt's lymphoma | >2560 | 2560 | 40 | 160 | nd | nd |
| 216 | Terego | F 06 | maxil. cran. nerve, abd. | 05.12.73 | Burkitt's lymphoma | 160 | <10 | <10 | 1280 | nd | 33 |
| 217 | Vura | M 05½ | mandible | 29.01.74 | Burkitt's lymphoma | >2560 | 640 | 1280 | 5120 | nd | 30 |
| 218 | Maracha | M 07 | mandibles, paraplegia | 13.02.74 | Burkitt's lymphoma | >2560 | 10 | <10 | 40 | nd | nd |
| 219 | Zaire | M 14 | mandible, paraplegia | 12.02.74 | probable BL | 40 | <10 | <10 | 160 | nd | nd |
| 220 | Ayivu | F 05 | maxil. orbit | 09.04.74 | consistent with BL | 1280 | <10 | <10 | 10 | 24 | 15 |
| 221 | Maracha | F 06 | orbit | 11.09.74 | Burkitt's lymphoma | 640 | 80 | <10 | 640 | nd | 27 |
| 222 | Terego | M 04½ | maxillaries | 16.09.74 | Burkitt's lymphoma | 1280 | 20 | <10 | 2560 | nd | 58 |
| 223 | Koboko | M 08 | abdomen | 05.10.74 | Burkitt's lymphoma | 80 | <10 | <10 | 640 | <1 | ne |
| 224 | Zaire | F 04 | orbit, liver | 28.01.75 | Burkitt's lymphoma | 2560 | 160 | <10 | 320 | nd | 28 |
| 225 | Ayivu | M 03½ | maxillary | 11.02.75 | Burkitt's lymphoma | 640 | 160 | <10 | 80 | nd | 35 |
| 226 | Terego | F 10 | orbit, maxil. mand., liver | 21.02.75 | Burkitt's lymphoma | 1280 | 160 | 10 | <5§ | nd | 25 |
| 227 | Ayivu | M 05 | mandible, liver | 28.02.75 | probable BL | >2560 | 2560 | <10 | 2560 | nd | 13 |
| 228 | Aringa | M 16 | orbit, mandible | 17.03.75 | Burkitt's lymphoma | 80 | <10 | <10 | 160 | nd | ne |
| 229 | Maracha | F 10 | orbit, nose | 29.04.75 | embryonal rhabdomyosarc. | 160 | <10 | <10 | 160 | nd | nd |
| 230 | Ayivu | M 06 | maxil. orbit, liver | 21.05.75 | Burkitt's lymphoma | 1280 | <10 | <10 | 5 | nd | 55 |
| 231 | Jonam | F 13 | sacrum | 04.05.75 | papillary carcinoma | 40 | <10 | <10 | 5 | <1 | ne |
| 232 | Bukyoro | F 14 | orbits, maxil. mand. abd. | 11.06.75 | possible BL | 640 | <10 | <10 | 160 | nd | ne |
| 233 | Ayivu | M 05 | orbits, jaws, liver | 05.07.75 | Burkitt's lymphoma | 640 | <10 | <10 | 160 | nd | nd |
| 234 | Maracha | M 08 | maxil. bone marrow | 05.07.75 | probable BL | 80 | <10 | <10 | 160 | 0–12 | 15—ne |
| 235 | Terego | M 10 | mandible | 12.07.75 | Burkitt's lymphoma | 640 | 20 | <10 | 40§ | nd | 19 |
| 236 | Zaire | M 04 | maxillary | 05.07.75 | Burkitt's lymphoma | 640 | 10 | <10 | 320 | nd | 25 |
| 237 | Padieri | F 13 | thyroid, ovaries | 04.08.75 | Burkitt's lymphoma | 1280 | 80 | <10 | 160 | nd | 60 |

**APPENDIX I continued**

| BL No. | Place of origin* | Sex/age | Site of tumour | Date of diagnosis | Pathology diagnosis† | EBV serology VCA | EA DR | EA D | EBNA | DNA hybridation‡ Stockholm | DNA hybridation‡ Freiburg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 238 | Zaire | F 04 | maxillary, ovaries | 11.08.75 | Burkitt's lymphoma | 640 | 10 | <10 | 640 | nd | 35 |
| 239 | Zaire | M 11 | mand. thyroid, retrop. | 19.08.75 | Burkitt's lymphoma | 2560 | 40 | 40 | 320 | nd | 28 |
| 240 | Koboko | M 06 | 4 jaws | 25.08.75 | Burkitt's lymphoma | 2560 | 160 | 320 | <5 | nd | 38 |
| 241 | Aringa | M 06 | jaws, orbit | 28.08.75 | Burkitt's lymphoma | 1280 | <10 | <10 | 40 | 25 | ne |
| 242 | Ayivu | M 12 | mandible | 09.09.75 | consistent with BL | ⩾2560 | 20 | <10 | 320 | nd | nd |
| 243 | Zaire | F 05 | orbits, thyroid, abd. | 29.09.75 | Burkitt's lymphoma | 1280 | <10 | <10 | 2560 | nd | 50 |
| 244 | Aringa | M 06 | mandible | 12.10.75 | not BL | 80 | <10 | <10 | 160 | nd | ne |
| 245 | Sudan | F 04½ | maxil. nose, lip | 18.10.75 | Burkitt's lymphoma | 2560 | 10 | <10 | 160 | ne | ne |
| 246 | Maracha | F 05 | 4 jaws, ovary | 24.11.75 | Burkitt's lymphoma | ⩾2560 | 20 | <10 | 1280 | 4 | 20 |
| 247 | Terego | M 05 | orbits, liver | 13.11.75 | Burkitt's lymphoma | 40 | <10 | <10 | 40 | nd | ne |
| 248 | Jonam | M 06 | orbit, testic. liver | 09.12.75 | Burkitt's lymphoma | 320 | 10 | <10 | 80 | nd | ne |
| 249 | Koboko | M10 | parotid, paraplegia | 02.12.75 | Burkitt's lymphoma | 2560 | 40 | <10 | 5 | nd | nd |
| 250 | Koboko | F 07 | mandible | 31.12.75 | Burkitt's lymphoma | ⩾2560 | 80 | 10 | 80 | 12 | ne |
| 251 | Zaire | F 19 | abd. liver, ovary | 04.02.76 | trans. cell ca./Wilm's tm | 2560 | <10 | <10 | 10 | nd | ne |
| 252 | Maracha | M 07 | maxillary | 17.03.76 | Burkitt's lymphoma | 1280 | 80 | 80 | 80 | 45 | 58 |
| 253 | Padieri | M 05 | maxillary, liver | 02.06.76 | Burkitt's lymphoma | 320 | <10 | <10 | 640 | 24 | nd |
| 254 | Zaire | F 18 | orbit, forehead | 09.06.76 | probable BL | 2560 | <10 | <10 | 160 | 110 | nd |
| 255 | Zaire | F 07 | liver, ovary, parapleg. | 10.06.76 | Burkitt's lymphoma | 2560 | 10 | <10 | 160 | 63 | nd |
| 256 | Padieri | F 12 | ? | 21.06.76 | diagnosis not possible | 640 | <10 | <10 | 160 | ne | nd |
| 257 | Koboko | M 06 | jaw, liver | 31.08.76 | Burkitt's lymphoma | 320 | 40 | <10 | 2560 | 34 | nd |
| 258 | Ayivu | M 04 | mandible, liver | 12.10.76 | Burkitt's lymphoma | ⩾2560 | ⩾2560 | <10 | 640 | 55 | nd |
| 259 | Ayivu | M 11 | 4 jaws | 03.11.76 | Burkitt's lymphoma | 10 | <10 | <10 | 320 | ne | nd |
| 260 | Maracha | M 06 | orbit, palate | 19.11.76 | retinoblast./neuroblast. | 640 | <10 | <10 | 2560§ | nd | ne |
| 261 | Koboko | M 04 | orbit, paraplegia | 23.11.76 | Burkitt's lymphoma | 2560 | 10 | <10 | 640 | 23 | nd |
| 262 | Aringa | F 05 | neck LN | 26.11.76 | Burkitt's lymphoma | 640 | <10 | <10 | 640 | 107 | 102 |
| 263 | Terego | M 06 | maxillary, clavicle | 30.11.76 | probable BL | ⩾2560 | 2560 | ⩾2560 | 2560§ | nd | 23 |
| 264 | Ayivu | M 12 | maxillary | 12.01.77 | probable BL | >2560 | 2560 | ⩾2560 | 40 | 46 | 67 |
| 265 | Zaire | M 07 | maxillary, orbit | 21.01.77 | Burkitt's lymphoma | >2560 | <10 | <10 | 640 | 42 | nd |
| 266 | Terego | M 07 | mandible | 19.06.77 | Burkitt's lymphoma | 640 | <10 | <10 | 80 | ne | nd |
| 267 | Zaire | M 07 | mandible | 05.09.77 | Burkitt's lymphoma | 640 | 160 | 80 | 640 | 22 | nd |
| 268 | Terego | F 08 | jaws, CNS | 27.06.77 | Burkitt's lymphoma | 1280 | 2560 | <10 | 1280 | nd | nd |
| 269 | Vura | M 12 | mandible | 13.10.77 | Burkitt's lymphoma | ⩾2560 | 10 | <10 | 160 | 22 | 43 |
| 270 | Ayivu | F 05 | mandible, maxillary | 28.11.77 | Burkitt's lymphoma | 320 | <10 | <10 | 10 | 30 | 34 |
| 271 | Jonam | F 09 | ovaries | 14.10.77 | diagnosis not possible | 1280 | 320 | <10 | 20 | nd | nd |
| 272 | Terego | F 08 | maxil. CNS, orbit, liver | 22.12.77 | Burkitt's lymphoma | 1280 | <10 | <10 | 640 | 36 | 48 |
| 273 | Koboko | F 06 | max. orbit, ovaries, kidney | 15.01.78 | Burkitt's lymphoma | 2560 | 80 | 10 | 640 | 20 | nd |
| 274 | Maracha | M 07 | orbit | 03.02.78 | probable retinoblastoma | >2560 | 640 | 640 | 1280 | nd | nd |
| 275 | Ombachi | F 08 | maxillary | 18.04.78 | consistent with BL/Ewing? | 2560 | 320 | 640 | 640 | 5 | nd |

**APPENDIX I continued**

| BL No. | Place of origin* | Sex/age | Site of tumour | Date of diagnosis | Pathology diagnosis† | EBV serology VCA | EA DR | EA D | EBNA | DNA hybridation‡ Stockholm | DNA hybridation‡ Freiburg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 276 | Zaire | M 07 | maxillaries | 25.04.78 | consistent with BL | 640 | <10 | <10 | 40 | 37 | 35 |
| 277 | Zaire | M 09 | maxillary | 08.06.78 | Burkitt's lymphoma | 1280 | 20 | <10 | 640 | ne | nd |
| 278 | Aringa | M 10 | neck LN | 17.08.78 | Burkitt's lymphoma | 1280 | 40 | <10 | 160 | 10 | 18 |
| 279 | Zaire | M 11 | orbit, mandible | 19.08.78 | Burkitt's lymphoma | 1280 | 10 | <10 | 320 | 21 | 26 |
| 280 | Maracha | M 05 | orbit, mandible, liver | 22.08.78 | Burkitt's lymphoma | ⩾2560 | 1280 | <10 | 320 | 10 | 21 |
| 281 | Madi | F 08 | orbit, maxillary | 06.04.78 | Burkitt's lymphoma | >2560 | 2560 | 320 | 160 | 43 | 45 |
| 282 | Ayivu | M 08 | eye | 09.09.78 | diagnosis not possible | 320 | <10 | <10 | 160 | <1 | nd |
| 284 | Okoro | F 05 | maxillary, cheek | 26.10.78 | embryonal rhabdomyosarc. | 1280 | <10 | <10 | 160 | <1 | ne |
| 285 | Aringa | F 07 | maxillaries | 07.11.78 | Burkitt's lymphoma | 640 | 320 | 20 | 640 | 49 | 53 |
| 287 | Madi | M 06 | maxillary, orbit | 05.12.78 | not BL | 10 | <10 | <10 | 320 | <1 | ne |

*Place of origin: county in West Nile/Uganda or Sudan or Zaire.

†Pathology diagnosis: by Professor D. Wright.

‡nd = not done: ne = not enough DNA.

§Results of EBNA testing of tumour cells: BL 226, positive; BL 235, positive; BL 247, negative; BL 260, negative; BL 263, positive.