Introduction: Burkitt’s Lymphoma in Africa

Richard H. Morrow
Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115

Since I am listed both as Co-chairman and as first speaker for this session on Burkitt’s lymphoma, I would like to take the opportunity to present several miscellaneous types of information. Some information is new and not fully analyzed as yet, whereas some is old but not published as yet. It is my hope that this discussion can be focused upon factors other than the Epstein-Barr virus in the pathogenesis of Burkitt’s lymphoma.

If the Epstein-Barr virus is a necessary factor in the development of Burkitt’s lymphoma, then there must be other factors necessary as well. The most commonly invoked “other factor” is chronic malaria, and I hope to precipitate discussion about how we might go about investigating the role of malaria. For the time being, however, I should like to review other types of evidence concerning Burkitt’s lymphoma that may either cloud or clarify the chain of events that culminate in the development of Burkitt’s lymphoma.

There are three main pieces of information to relate. The first concerns preliminary information about the epidemiology of Burkitt’s lymphoma in the Mengo districts of Uganda from 1959 to 1968. The second concerns socioeconomic factors that were a spin-off from our other studies in Uganda, and the third concerns the results of antibody levels to infectious agents other than the Epstein-Barr virus that were carried out by Dr. John Sever and Dr. Brian Henderson, the results of which have not been published. Following this, Dr. Peter Smith brings us up to date on the evidence for continued clustering in the West Nile District.

After discussion of the epidemiological studies in Uganda, Dr. John Ziegler presents some of the newer findings from the Uganda Cancer Institute concerning the clinical and immunological aspects of Burkitt’s lymphoma. This review of Burkitt’s lymphoma in Africa is followed by some of the findings in American Burkitt’s lymphoma and by some of its new laboratory aspects.

Since the first part of the program is directly related to Uganda, it is useful to have some background information concerning Uganda. Uganda, more or less in the center of Africa, is a country of extraordinary variation; it is varied geographically, ethnically, and in the basic ways of life among its many peoples. Mr. Burkitt has previously reviewed the variations in altitude, population densities, malaria, and Burkitt’s lymphoma for Uganda. In brief, as one goes from the semimountainous southwest of Uganda with its high altitude and little malaria and moves northward, there is decreasing altitude and increasing malaria and Burkitt’s lymphoma. Taken on a district by district basis within Uganda, the incidence of Burkitt’s lymphoma closely parallels that of malaria. The highest rates of Burkitt’s lymphoma are in the north where malaria is hyperendemic, but where population density is the lowest, medical facilities are fewest, and case ascertainment is almost certainly the poorest.


This study is based on all patients registered in the Kampala Cancer Registry as having Burkitt’s lymphoma whose onset of illness occurred in East or West Mengo Districts in the 10-year period from January 1, 1959, to December 31, 1968. One hundred thirty patients are included in this preliminary report. The diagnosis was based on microscopic examination of biopsy or postmortem tissue in 118 patients, and an additional 12 included were based on clinical diagnosis alone.

East and West Mengo Districts, which include the capital city of Kampala, had a population of nearly 2 million in 1969. Population growth was very rapid over the 10 years of the study with considerable immigration to the area from other parts of Uganda and from neighboring countries as well. The area is classed as mesoendemic for malaria. The Mengo Districts have the best medical facilities available in Uganda, and case ascertainment of Burkitt’s lymphoma is probably better than most other districts in Uganda.

The most important finding was that of a steady decline in incidence rate of Burkitt’s lymphoma during the decade from about 30 per million per year early in the decade to about 9 per million per year in 1966–1968 in children under the age of 16. In contrast, the rates in northern Uganda have remained at about 50 to 100 per million per year. The decline in incidence varied by ethnic group and was significantly greater in the Baganda born in the Mengo Districts than in non-Bagandans, who were usually immigrant to the Districts. There was also an increasing population of females registered over the decade, changing from about a 2:1 ratio of males to females early in the decade to about a 1:1 ratio in 1966–1968—probably a reflection of an increased awareness (and, therefore, an increased case ascertainment) that Burkitt’s lymphoma often presents as an ovarian or abdominal tumor. Another finding was that the age distribution varied by ethnic group; the median age of patients immigrant from areas higher in altitude than
Mengo was significantly older than that of the Baganda patients, whereas the median age of patients immigrant from areas generally lower in altitude was younger. The average annual incidence rate in the northeastern counties of the Mengo Districts was consistently higher than in the other countries. Furthermore, the relative incidence was highest for the southwestern counties early in the decade and then it declined, whereas the relative incidence in the northeastern counties increased significantly during the decade. However, the Knox method for demonstration of time-space clustering was not significant for any specific time or space intervals.

The results of this study support the hypothesis that malaria plays an important role in the pathogenesis of Burkitt’s lymphoma, and some evidence suggests that malaria may be the stimulus that sets off malignant transformation of Epstein-Barr virus-infected cells rather than the reverse.

**Socioeconomic Factors in Burkitt’s Lymphoma.** Richard H. Morrow, Aloisius Kisuule, and Josea Mafigiri. Harvard School of Public Health, Boston, Massachusetts 02115 [R. H. M.], and Makerere University Medical School, Kampala, Uganda [A. K., J. M.]

During the course of our investigation of Burkitt’s lymphoma in Uganda, we collected information concerning the family and environmental circumstances in which the tumor occurred. The basic approach was to visit the home of each patient with confirmed Burkitt’s lymphoma, to ask questions of the family, to inspect the home, and to investigate the surroundings. At the time of the visit the nearest neighbor with a child of the same sex and age (+ 1 year) was selected to serve as a control. Selection of the neighbor was based on a prearranged pattern of homes to seek one in which an appropriately age- and sex-matched child could be found. Every effort was taken to avoid the many kinds of bias that can arise in the selection of such a control.

The study population consisted of those patients diagnosed and treated at Mulago Hospital in 1967 and 1968 whose home could be visited. Fifty-six patients with matched controls were so included.

The major conclusions from the study were as follows: (a) Burkitt’s lymphoma patients had more siblings than their age- and sex-matched, nearest neighbor controls; (b) Burkitt’s lymphoma patients had a higher percentage of sibling deaths than did their controls; (c) Burkitt’s lymphoma patients had more people sharing the rooms in which they slept than did their controls; (d) there was no difference in distributions by birth order between Burkitt’s lymphoma patients and their controls; (e) The families of Burkitt’s lymphoma patients lived in poorer houses than did their controls; and (f) the families of Burkitt’s lymphoma patients moved their residences more frequently than did their controls.

The general pattern strongly reinforces the importance of environmental factors in the etiology of Burkitt’s lymphoma.

**Antibody Levels to Infectious Agents Other than Epstein-Barr Virus in Burkitt’s Lymphoma Patients.** Richard H. Morrow, John L. Sever, and Brian E. Henderson. Harvard School of Public Health, Boston, Massachusetts 02115 [R. H. M.]; NIH, Bethesda, Maryland 20014 [J. L. S.]; and University of Southern California Medical School, Los Angeles, California [B. E. H.] 90033

Since the demonstration of a relationship between Epstein-Barr virus and Burkitt’s lymphoma, the question has been repeatedly raised as to whether patients with Burkitt’s lymphoma might not be generally more susceptible to any virus and/or might be more reactive nonspecifically in terms of humoral antibody response to any agent. Complement-fixing antibody levels were determined on sera from 42 patients with Burkitt’s lymphoma and from 27 controls from Mulago Hospital for the following agents: adenovirus, herpes simplex, varicella, cytomegalovirus, ECHO, reo 1, influenza A and B, mumps, rubella, rubella (by hemagglutination inhibition), lymphocytic choriomeningitis, psittacosis, and toxoplasmosis. The controls were not individually matched, but the distribution by age and sex was comparable. In this series, no Burkitt’s lymphoma patients had antibodies to reo 1 and to mumps than did the controls (44% versus 15% and 48% versus 12%, respectively). There were no other significant differences. Probably the most important conclusion to be drawn from this work is that patients with Burkitt’s lymphoma are not importantly different from others in terms of either their exposure to a wide array of infectious agents or their basic humoral antibody response to these agents. (An interesting aside, however, in this series: patients with Burkitt’s lymphoma had a significantly lower titer response to measles than did the controls. This finding deserves confirmation.)

We also carried our serological tests to 14 arboviruses in a variety of Burkitt’s lymphoma patients and controls, but significant differences were not found.

**Subsequent Discussion**

The vigorous discussion that followed was largely centered on the relationship of malaria to Burkitt’s lymphoma. Dr. Feorino (National Center for Disease Control) pointed out that they had tested the same sera for malarial antibodies that Dr. Henderson had tested for arbovirus antibodies. In this series there were no differences between Burkitt patients and controls in their malarial antibodies and, indeed, about 25% of Burkitt’s lymphoma patients tested apparently had no antibodies to malaria. On the other hand, Dr. Ziegler mentioned that, in the studies of malaria in the Lymphoma Treatment Center, no one was actually negative using the IFA and complement fixation tests, but the distribution of titers tended to be higher in the Burkitt patients. He emphasized that there were serious reservations concerning the interpretation of the data because of the difficulties in the selection of controls. The immunological response to malaria is extraordinarily complex; results vary not only by test method and according to the antigen used but also according to presence or absence of parasitemia,
the species of plasmodia, the time since antimalarial treatment, and various undefined host factors. It was agreed that much of the variation would be quantitative and not qualitative. There are serious problems with the definition of negative with all the serological tests for malaria because of nonspecific reactions in the low range. In reality, no one seriously questions the fact that everyone has had malaria in hyper- and holoendemic areas. The important questions revolve around how different people respond differently to malaria, in what ways these different responses may be of importance and, finally, how these differences in host response may be measured.

There was considerable discussion as to whether Epstein-Barr virus infection or malaria comes first in the pathogenesis of Burkitt’s lymphoma or, indeed, whether it matters. Although the immigrant data might be interpreted to support the idea that malaria touched off a malignant transformation in people already infected with Epstein-Barr virus, it was pointed out that Burkitt’s lymphoma could well develop in those few children who escaped primary infection in their first 2 years of life and who then became infected with Epstein-Barr virus at a slightly later age after the host defenses had been at least partially mobilized against malaria.

There was further discussion about the problems involved in attempting to invoke 2 virtually ubiquitous diseases to account for a relatively rare cancer, particularly one that can behave in an apparently epidemic fashion. Some suggested the need to invoke a 3rd factor such as C-type particles or a peculiar host immune deficiency, but a 3rd factor might not be required. Instead one might hypothesize a series of odd happenings in relation to the timing of one infection to the other or in terms of a particular stage of preparedness on the part of the host. The important question was raised as to whether the association of malaria to Burkitt’s lymphoma, based chiefly on geographical coincidence, could not better be attributed to ecological coincidence than to malaria itself. Actually, most early emphasis was placed on the mosquito rather than malaria. The only epidemiological study that might directly implicate malaria rather than the vector has been a comparison of sickle trait in Burkitt patients with controls. People with sickle trait are not protected from being bitten by mosquitoes nor are they protected from malarial infection, but they are protected against the lethal effects of overwhelming *Falciparum malaria* in early childhood and from the intense reticuloendothelial stimulation that sometimes progresses to big spleen disease.

Although the results to date are in the right general direction, they are still equivocal. Of some interest is that studies done by Dr. F. Nkrumah in Ghana indicate that hemoglobin C may be providing some protection against Burkitt’s lymphoma. These studies are being pursued but are not yet conclusive.

Theoretically, holoendemic malaria does not drift from place to place within the areas so designated. However, it seems reasonable that infections with Epstein-Barr virus behave in an epidemic fashion just as do other ubiquitous viral infections such as measles and polio. If one accepts that time-space clustering does occur at some places at some times, then it might be better to look upon Epstein-Barr virus infection as the factor related to the clustering. Nevertheless, there may be important variations in transmission of malaria, even in holoendemic areas, that we do not have adequate tools to measure. Certainly, there is variation in holoendemic areas. Of speculative interest is that there may be more Burkitt’s lymphoma in areas of Northern Uganda where most malaria is holoendemic than in Ghana where malaria is holoendemic. There are serious confounding problems in Ghana, however, where there is widespread use of chloroquine. It may be that, despite a high transmission of malaria there, the effects are actually ameliorated and function more like hypo- or mesoendemic malaria.

Peter Smith presented the latest evidence on time-space clustering in the West Nile and made it clear that there seemed to be important changes in the tendency for Burkitt’s lymphoma to cluster in the West Nile. From 1961 to 1965 the evidence for clustering was highly significant. Continued follow-up in 1966 to 1967 showed that there was still highly significant shifting in time and space of Burkitt’s lymphoma in that area. There is no evidence that these were the result of artifacts in case ascertainment or population shifts. However, from Years 1968 to 1971, during which time there have been medical teams systematically providing surveillance of the area, the clustering has virtually disappeared. Using the same time-space intervals that provided the highest degree of significance previously, the evidence for continued clustering is weak and of marginal significance. Also, attempts to find space-time clustering in Acholi, Lango, and Mengo Districts in Uganda and North Mara in Tanzania have not been fruitful. The number of Burkitt’s tumor cases in West Nile has remained quite stable since 1966 with 15 to 20 cases per year, but there has been an increase in the number of young females being diagnosed as compared to previously. There is no satisfactory interpretation of the end of time-space clustering in the West Nile. Perhaps there was something odd in 1961–1965, but there is no supporting evidence. In conclusion, although in a statistical sense there is still evidence for continuing space-time clustering in the West Nile, the evidence is actually not strong and it is much more difficult to consider the evidence useful in causal epidemiology.

For the present it would seem that all the descriptive epidemiological studies of Burkitt’s lymphoma that would be useful in Africa have been done. Emphasis should now be placed upon the testing of specific hypotheses in an analytical fashion. The long-term cohort study being carried out in the West Nile, specifically investigating in a prospective fashion the relationship of Epstein-Barr virus to Burkitt’s lymphoma, is the type of investigation that must be done. That study is progressing very well with more than 19,000 sera collected from children. The 1st Burkitt’s lymphoma patient has occurred among those who have been bled, right on schedule. The other area that requires careful analytical studies at this time are studies concerned with the relationship of malaria to Burkitt’s lymphoma. I would hope that,
as a result of this workshop, we will be able to stimulate work both in the laboratory and in the field on the role of malaria in carcinogenesis.

Clinical and Immunological Aspects of Burkitt's Lymphoma

Dr. John Ziegler provided an excellent general review of the clinical and immunological aspects of Burkitt's lymphoma. Details on the clinical features will be reported in the near future. He reviewed the latest information in 4 general areas: (a) criteria for diagnosis; (b) clinical features of the disease; (c) immunological aspects; and (d) new information about the natural history of the disease.

He first discussed the criteria for diagnosis with emphasis on the histological features. Patients in whom the histological pattern deviates from the strict criteria for Burkitt's lymphoma cells toward either a lymphosarcoma-like picture on the one side or reticulum cell sarcoma on the other side tend to deviate in their clinical symptomatology and response to chemotherapy.

Although patients with Burkitt's lymphoma present with a wide range of clinical symptoms, 60 to 70% of patients seen at the Lymphoma Treatment Center did have jaw involvement. A revision of the staging definitions is in progress, but the basic concept of staging continues to be useful. The major changes in classification involve (a) patients formerly categorized in Stage 3 but whose abdominal tumor can be resected and who have an excellent prognosis, and (b) Stage 4 patients with central nervous system involvement, some of whom have a reasonable prognosis. Patients with central nervous system symptoms continue to be a major therapeutic challenge, but there has been a substantial improvement in outlook as a result of combined intrathecal and systemic chemotherapy. Several Stage 4 patients have now maintained a long-term remission.

The 3rd area reviewed was concerned with the immunological aspects of Burkitt's lymphoma. A number of studies have been carried out to investigate the immune competence of patients with Burkitt's lymphoma. In aspects of both humoral and cell-mediated immunity, Burkitt's lymphoma patients appear to be more robust from the controls tested. Burkitt's lymphoma patients tend to have lower IgM levels than the controls, confirming findings reported from West Africa. Also, there is a decreased antibody response to polysaccharide antigens. In general, Burkitt patients provided a more feeble response to the Vi antigen for Escherichia coli than did the controls. This diminished response correlated with the reduced IgM level. There were no other particular correlations of the IgM levels with any aspect of the clinical picture of Burkitt's lymphoma. Otherwise, Burkitt's tumor patients were similar in their humoral antibody response to the controls; thus these findings of decreased IgM level and response to Vi antigen appear to be isolated defects on the part of Burkitt's lymphoma patients.

As to cell-mediated immunity, patients with Burkitt's lymphoma usually responded to dinitrochlorobenzene in a fashion similar to controls. However, there was some deficiency in their response to a series of recall skin test antigens, and these did indeed relate to the likelihood of future relapse. The 3rd area concerned with cell-mediated immune response was the patient's response to autologous tumor extract. In general, patients with tumor or patients in relapse do not respond to skin testing with tumor extract, whereas a high percentage of patients in remission do respond. Furthermore, those who respond tend to remain in remission, while those whose skin test reverts to negative tend to relapse.

Preliminary results in the use of Bacillus Calmette-Guérin for immunological prophylaxis in patients who have achieved remission are generally encouraging, but it is too soon to be confident of significant results. Dr. Ziegler and Dr. Bluming have shown that the Mathé method does tend nonspecifically to potentiate delayed hypersensitivity reactions.

The 4th area Dr. Ziegler reviewed concerned the natural history of the disease and the new evidence for different types of relapse patterns. Apparently, there are 2 essentially distinct types of pattern: (a) early relapse occurring less than 10 weeks after complete remission has been achieved, and (b) late relapse occurring after that period of time. About one-third of the patients go into sustained remission and have no relapses; another one-third develop early relapse; and the remaining one-third have the late relapse pattern. Patients having early relapse tend to relapse in the same site, frequently have central nervous system involvement at relapse, have a poor response to further chemotherapy, and have a poor prognosis. On the other hand, those with a late relapse tend to relapse at a new site, rarely have central nervous system involvement, respond to chemotherapy as though they had a new tumor, and their overall prognosis seems little affected by the fact that they have had a relapse. Early relapse could be explained by regrowth of the original tumor, resistance to chemotherapy, and/or resistance or inaccessibility to other host factors such as immunological control. Late relapse, on the other hand, does not fit this explanation on a kinetic basis. Rather, late relapse may be a manifestation of the reemergence of a latent tumor which has been held in check for a prolonged period. This possibility receives some support from the demonstration of altered tumor-associated immunity preceding the occurrence of late relapse. Alternatively, late relapse may be a manifestation of tumor reinduction, possibly brought about by persistence of the same agent(s) which induces the primary tumor. This latter possibility is supported by the clinical behavior of the tumor and by some evidence that late-relapse tumors originate from a different clone of tumor cells than the initial tumor. An interesting report by Dr. Fialkow would provide strong confirmation for the reinduction theory. In general, Burkitt's tumors have been found to be monoclonal in terms of the type of glucose 6-phosphate dehydrogenase activity in tumor cells. The test is only useful in female patients heterozygous for glucose 6-phosphate dehydrogenase, but in all patients tested to date biopsies from different tumor sites in the same patient have yielded results consistent with the monoclonal hypothesis. The 1 exception was in a girl who had a late relapse. In this
case, the initial biopsy showed an A-type phenotype, whereas at relapse B-type was found.

One other difference in patients with varying relapse patterns was the difference in the Epstein-Barr virus early antigen titers. In general, there is a good correlation between the presence of restricted or R-type early antigen and the appearance of a later relapse. The Henles have more to say about this point later.

**Discussion of Clinical and Immunological Aspects of Burkitt’s Lymphoma**

In the ensuing discussion there were a number of comments concerning the theory of reinduction as an explanation for late relapse. Apparently, there were some problems concerning the patient on which Fialkowski reported in that the patient had received multiple transfusions, providing the possibility that the origin of one clone was not from the patient. Dr. G. Henle spoke of a patient in which cell culture of the tumor produced IgM, but cells from a biopsy at late relapse produced no γ-globulin, indicating that perhaps 2 different cell types were involved. Although there are other interpretations of these findings, the suggestion that reinduction is taking place remains an exciting possibility. It was pointed out that, if the reinduction theory is true, it minimizes both the Epstein-Barr virus and the malaria theories, at least in their simpler forms. Dr. Ziegler pointed out that several patients at the Lymphoma Treatment Center developed late relapses after months of continued chloroquine antimalarial prophylaxis, and thus the immediate stimulus for reinduction could not be malarial parasitemia. Dr. de Thé observed that, if reinduction of the disease did occur in the presence of antibody against Epstein-Barr virus, the prospective study discussed earlier should not demonstrate Burkitt’s lymphoma solely in children who escaped early Epstein-Barr virus infection and where antibody negativity is assumed to be an important factor in the etiology of Burkitt’s lymphoma.

Dr. Manaker again brought forth the thesis that it may well be necessary to invoke a 3rd factor to account for the pathogenesis of Burkitt’s lymphoma, and he specifically suggested that the factor might well be the much discussed C-type particle. The difficulty for the time being, of course, is that there is as yet no marker indicating the presence of C-type particles for clinical or epidemiological studies.

The next major item of discussion centered upon the relationship of the early antigen to relapse. Dr. W. Henle discussed the results for a series of patients from Nairobi, demonstrating several different patterns of response and relapse. Although definitions of relapse used in the Nairobi study were somewhat different from those at the Lymphoma Treatment Center, the general picture was quite clear. Those patients that were going to relapse and had relatively poor prognosis were those that had the high anti-R early antigen. Dr. W. Henle pointed out that the D and R antigens are intracellular and that antibodies to them cannot directly affect the cell. However, they might well be acting as enhancing bodies interacting with some other component of the cell-mediated immune response. It is clear that working out the mechanisms involved will be of great fundamental importance.

Dr. Gregory O’Conor closed the session with an all-purpose slide demonstrating potential interrelations between Burkitt’s Lymphoma, malaria, and Epstein-Barr virus. Despite the fact the slide had been developed 3 years ago, it still proved to be a fit summary of the session’s discussions.