IMMUNOGLOBULIN AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE AS MARKERS OF CELLULAR ORIGIN IN BURKITT LYMPHOMA*

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Knowledge of the number of cells from which a tumor originates can provide important information about its etiology and pathogenesis. For example, clonal origin would be expected from a rare and random oncogenic event, or even if multiple cells were affected initially, but only one clone emerged to form the mature tumor. The problem of tumor cell origin can be approached by studying neoplasms from patients with mosaicism of two or more cell types. Such mosaicism is regularly demonstrated in the cells of mammalian females due to random X chromosome inactivation in early embryonic development. The X-linked glucose-6-phosphate dehydrogenase (G-6-PD)[1] locus is particularly useful as a marker. Thus, females who are heterozygous for the usual B gene (Gd^B) at the G-6-PD locus and a variant allele such as Gd^A have two distinct cell types (1–3). Gd^B is active in one population of cells, which consequently produces type B enzyme; in the other population Gd^A is active, producing the variant A type enzyme. Tumors with a clonal origin in a Gd^B/Gd^A heterozygote should therefore contain either B or A type G-6-PD whereas those with multicellular origin may exhibit both B and A types.

Such studies of G-6-PD types when applied to Burkitt lymphoma adduced evidence that individual Burkitt tumors have clonal origins (4, 5). Limited studies of multiple tumors from the same patients suggested that the entire disease is clonal (4, 5). In one patient, we found that an exacerbation after a long-term remission was not due to reemergence of the originally detected malignant clone, but was probably the result of a "new" induction of disease (6). As such suggestions have major implications for the etiology and pathogenesis of this malignancy, it is important to test their validity with additional cell markers.

Another way of marking cells is to test them for surface-associated antigens. 10–35%

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[1] *Abbreviations used in this paper:* BSS, balanced salt solution; G-6-PD, glucose-6-phosphate dehydrogenase; Gd^A, A allele; Gd^B, B allele; KCC, Kenya Cancer Council.

of blood lymphocytes from normal subjects bear immunoglobulins on their surfaces (7–13). It is thought that these cells are members of the bone marrow-derived (B) subpopulation of lymphocytes, whereas cells without such surface immunoglobulin belong to the thymus-dependent (T) subgroup (14, 15). Surface-associated immunoglobulin on most normal B cells is IgM. Presumably these molecules function as receptors which combine with antigens in immunologically specific reactions (15). Membrane-bound immunoglobulin on a normal B lymphocyte is restricted to one class, one light chain, one IgG subclass, and 1 Gm allotype even in Gm heterozygotes (16).

The presence of cell surface-associated immunoglobulin can be used as a marker of neoplastic lymphoid cells (17, 18). Cells from almost every patient with chronic lymphocytic leukemia bear immunoglobulin, usually IgM, on their surface suggesting a B cell origin for this disease (7–13, 18). Moreover, since a single B cell generally bears only one class of immunoglobulin on its surface (16), the restriction to one class on the surface of chronic lymphocytic leukemia cells is compatible with a clonal origin for that disease. This suggestion receives strong support from findings that the surface-associated light chains in a given patient are of only one type, either kappa or lambda (7, 8, 10, 12, 17–19), and from observations in a patient with IgG-positive leukemia cell surfaces that the IgG was restricted to one subclass and 1 Gm allotype (8). Since IgM has been found on cells from some Burkitt tumors (17, 20), we explored the possibility of using this system as a marker to complement the G-6-PD studies.

Materials and Methods

Preparation of Cell Suspensions.—Biopsy specimens obtained in Nairobi from patients with histologically and cytologically confirmed Burkitt tumors were shipped immediately to Stockholm in Eagle's medium on wet ice. Immunoglobulin tests were always performed within 24 h after surgery. Viable cell suspensions were prepared by mincing and shaking the tissue pieces in tissue culture medium as previously described (17).

Cell Surface-Associated Ig.—Fluorescein-conjugated goat immunoglobulins against human IgM, IgA, IgG, κ-chain, λ-chain, and Fc-fragment of IgG were obtained from Hyland Div., Travenol Laboratories Inc., Costa Mesa, Calif. Conjugated rabbit antilight chain reagent was a gift from Dr. G. Goldstein (University of Virginia). The anti-IgG reagent was found to react in the passive hemagglutination test with IgM-coated erythrocytes. Therefore reactivities observed with this serum are not included in this report. The anti-λ reagent used during a long period of this study was later found to be inactive. Consequently, for the majority of the biopsies no information about the presence of lambda on the cell surface is available. The reagents were used in 1:10 or 1:15, 1:20 or 1:25, and often in 1:40 dilutions. Anti-IgM was further diluted serially to 1:640.

Approximately 5×10^5 cells were centrifuged in small tubes and 0.03 ml of diluted anti-immunoglobulin (anti-Ig) conjugated to fluorescein isothiocyanate was added to the sediment. The mixtures were incubated for 30 min at 37°C and the cells were then washed three times with balanced salt solution (BSS). One drop of 50% glycerol in BSS was added to the cells as a preservative.

A drop of the suspended cells was placed on a slide under a coverslip and 50–100 cells were scored for surface staining with a fluorescent microscope. Cells with the diffuse fluorescence typical of dead cells were disregarded. All cells showing sectorial or any degree of bright green granular fluorescence were counted as positive, even if they had only a few fluorescent spots on their surfaces. In scoring cell suspensions for surface fluorescence, the number of positive cells and the strength of the fluorescent staining were considered. Therefore, in evaluating the staining results consideration was given not only to the percent of positive cells, but also to the

strength and pattern of the staining. The latter was assessed in two ways: one, at low dilutions of anti-Ig sera, by scoring the reactions as weak, moderate, or strong; and, two, by testing dilutions of anti-Ig reagents. Reactions judged to be weak at low dilutions were almost always not present at high dilutions. In evaluation of surface IgM reactivity, a tumor was classified as positive (+) if at least 65% of cells were (+) at a dilution of 1:15 or 1:25 and at least 50% were (+) at a dilution of 1:160.

G-6-PD Isoenzymes.—Blood, other normal tissues, and tumors were obtained, prepared, and the electrophoretic type of G-6-PD determined as previously described (4). In most instances each tumor biopsy specimen was divided into two parts. One part was used for G-6-PD typing and the other part was prepared for histological examination. A rough assessment was made of the relative proportion of tumor cells present and if this was less than 75% of the total cells, the specimen was excluded. In many cases, tumor cell suspensions were also typed for G-6-PD.

RESULTS

Surface-Associated IgM.—Only results obtained on the first studied tumor from each patient are included for the purposes of classifying tumors as IgM(+) or (−). The distribution of tumors according to the percent of IgM(+) cells is essentially bimodal (Fig. 1). In 58 of 95 patients 65% or more cells were IgM(+) at a 1:15 dilution of antiserum. There was excellent correlation between the qualitative assessment of reactivity strength at low dilutions of anti-IgM reagent and the percent of cells which still remained (+) at higher dilutions. The frequency of IgM(+) cells at 1:160 dilution of antiserum declined to less than 50% in only one tumor scored as (+) at 1:15 or 1:25 dilution. Thus, 57 of 95 (60%) tumors were classified as IgM(+) and 40% as IgM(−).

The majority of IgM(−) tumors had 20% or less IgM(+) cells (Fig. 1), but there were three tumors with 30–35% IgM(+) cells and four with 50–60% IgM(+) cells. In all of the former, and, in one of the latter tumors the prevalence of positive cells declined rapidly with increasing dilutions of antiserum indicating weak reactivity. However, three tumors had 50–60% IgM(+) cells at low dilution of antiserum and retained those percentages of positive cells at 1:160 dilution (Table I). Cells from the jaw tumor of patient 1,076 were negative for surface Ig, but approximately 50% of the cells in the suspension from

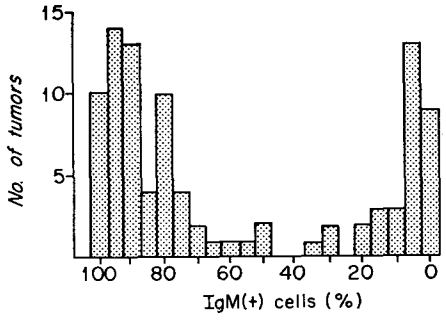

FIG. 1. Percent of cells with surface-associated IgM in Burkitt tumors.

TABLE I

Surface-Associated Immunoglobulin on Cells from Three Burkitt Tumors Initially Scored as IgM(±)

Test antiserum		Percent of cells positive in tumor cell suspension			
Specificity	Dilution	KCC* 1,076		KCC 1,213	KCC 1,364
		Jaw‡	Ovary		
IgA	1:15	0	0	6	20§
Fc	1:10	12	16	38	20
IgM	1:15	14	NS‖	58	57¶
	1:25	0	47**	NS	50¶
	1:40	NS	61**	NS	58¶
	1:160	NS	39**	48	58¶
	1:320	NS	38	NS	60¶
	1:640	NS	16	56	15
κ-chain	1:15	5	62**	68	10
Light chain	1:15	2	52**	NS	NS

* KCC, Kenya Cancer Council.

‡ This tumor was studied a second time with similar results.

§ Very weak fluorescent staining.

‖ NS, not studied.

¶ Great variation in staining pattern; sectorial and dotted fluorescence.

** Strong sectorial reactivity.

the ovarian tumor were positive for kappa as well as IgM. It should be emphasized that the surfaces of these cells showed strong, sectorial, brilliant fluorescence. Thus, the cell suspension from this tumor clearly contained two populations of cells; one, negative for surface-Ig and the other strongly IgM and κ-positive.

Results from the other two tumors summarized in Table I are more difficult to interpret. Surfaces of cells from patient 1,213's tumor reacted with most of the Ig reagents but in all instances the staining was not strong. Furthermore, 25% of the cells in this suspension were not viable indicating that the preparation and test conditions were not optimal. Consequently, it is not possible to classify accurately this tumor as IgM(+) or (−). Cells in the tumor preparation from patient 1,364 exhibited unusually marked variability in staining both quantitatively and qualitatively (e.g., some cells showed sectorial and others, dotted surface fluorescent patterns). Since the expression of surface IgM is dependent on the cell's physiologic status (21), variable condition of the cells in this preparation could be responsible for the variability in staining. Consequently, this tumor could not be classified accurately as IgM(+) or (−).

Studies of serially biopsied tumors showed that IgM(+) tumors remain IgM (+) (Fig. 2). For example, patient J. W.'s tumor was studied 13 times in 22 wk and each time between 90 and 100% of cells were IgM(+). Tumors initially scored as IgM(−) remained IgM(−) (Fig. 2). In vitro, lymphoblastoid cell

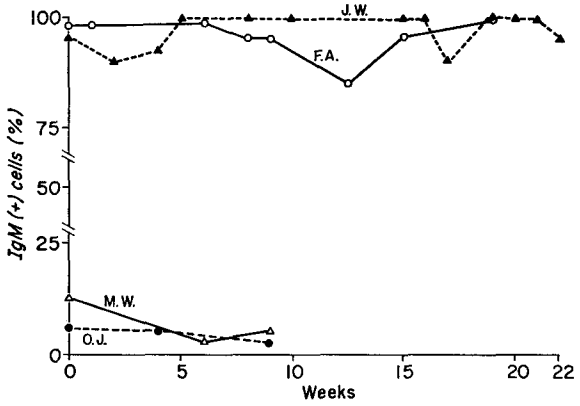

FIG. 2. Percent of cells with surface-associated IgM in serial biopsies of Burkitt tumors.

lines derived from IgM(−) tumors are IgM(−), and cultures established from IgM(+) tumors maintain the marker continuously in culture. For example, a line derived from an IgM(+) tumor in the patient Daudi is still IgM(+) after 5 yr (20). [On one occasion the line was thought to have become IgM(−) (19), but subsequently it was shown that the culture was contaminated. The original line was recovered from the freezer, and still remains IgM(+).]

Surface-Associated Light Chains.—Only two of 38 IgM(−) tumors scored as κ(+). Approximately 40% of the IgM(+) tumors were classified as κ(+), and the remainder as κ(−). Data for five representative κ(+) and five κ(−) tumors are given in Table II. In all κ(+) tumors the pattern of reactivity seen for κ was very similar to that observed for IgM. Thus, in the five κ(+) tumors described in Table II, IgM and κ-reactivities both were strong and sectorial. In the other five tumors, even those few cells recorded as κ(+) had on their surfaces only a few fluorescent dots. This pattern was also seen for the cells recorded as (+) with the other Ig reagents with the exception of anti-IgM.

As in the case of IgM, serial biopsies showed that κ(+) tumors remain κ(+). Thus, patient J. W.'s tumor, studied 13 times in 22 wk (Fig. 2), remained consistently κ(+). Similarly, in vitro, lymphoblastoid cell lines derived from κ(+) tumors maintain that marker. The Daudi line referred to previously is still κ(+) after 5 yr (20).

Other Surface-Associated Ig's.—None of the tumors was IgA(+). Only a few tumors had cells whose surfaces showed unequivocal reactivity with the reagents detecting IgG. Cells in lymphoblastoid lines established from two such tumors maintained surface-associated IgG reactivity thereby confirming the existence of IgG(+) Burkitt tumors.

G-6-PD.—Since the previously reported results on 24 tumors from 12 G-6-PD heterozygotes (4, 5), we have studied 11 more tumors from 7 heterozygotes. One of these tumors was excluded because it was judged to contain less than 75%

TABLE II

Cell-Surface Ig Reactivities in Cell Suspensions from Five Burkitt Tumors Scored as IgM(+), κ (+) and Five as IgM(+), κ (−)

Test antiserum		Percent of cells positive in tumor cell suspensions									
Specificity	Dilution	1,383*	786	19, 171	1,375	1,397	1,202	1,080	1,367	1,229	1,223
IgA‡	1:15	16	26	0	67	50	7	0	0	12	28
Fc‡	1:10	48	NS§	NS	50	60	45	0	5	12	36
IgM‖	1:15	70	100	94	100	100	80	84	81	80	81
κ‖	1:10	80	100	83	100	95	19	0	0	3	0

* Kenya Cancer Council (KCC) patient number except 19,171 which is patient's unit number.

‡ In all cases reactivity with these reagents was weak and consisted of only a few dots. Therefore, none of the cases is considered IgA or Fc(+).

§ NS, not studied.

‖ In all cases IgM reactivity was strong and sectorial and more than 50% of cells were IgM(+) at 1:160 dilution of antiserum. κ-reactivity was strong and sectorial in the first five cases.

tumor cells. Single enzyme types (A or B) were found in 9 of the other 10 tumors. One patient's only tumor had approximately equal amounts of A and B enzymes in specimens judged to contain at least 75% tumor cells and in a cell suspension prepared from the tumor. All cells in the latter were strongly IgM (+) and κ(+) with sectorial staining. Attempts to establish cell lines from this tumor failed. Combined with our previous reports (4, 5), these data show that 33 of 34 tumors from 19 G-6-PD heterozygotes had single enzyme phenotypes (A or B) despite the fact that normal tissues (including blood lymphocytes) always had both A and B types of G-6-PD. Tumors from 11 heterozygotes typed as B, from 7 as A, and from 1 patient as AB.

Patients with Multiple Studied Tumors.—In 17 patients, two or more tumors that were present simultaneously on initial presentation were studied for G-6-P-D and/or surface-associated Ig (Table III). G-6-PD was determined in each of two tumors from seven heteroxygotes and in all seven instances both tumors had a single enzyme of the same electrophoretic type. The probability that this occurred by chance alone is less than 1%. Surface-associated IgM and κ were tested in four tumors from one patient, three tumors from another patient, and in two tumors from 11 patients. With one exception (case 1,076, Tables I and III), multiple tumors from the same patient were either all IgM(+) or IgM(−) and were also concordant for κ. The maxillary tumor from patient 1,076 consisted entirely of IgM(−), κ(−) cells, but the ovarian tumor contained approximately 50% IgM(+), κ(+) cells (Table I). As noted above, this tumor was classified as consisting of two cell populations [IgM(−) and IgM(+)].

Recurrent Tumors.—A tumor was considered to be recurrent rather than persistent if it was noted after what was judged clinically to be a tumor-free interval. 33 recurrent tumors from 26 patients were evaluated for cell surface-asso-

ciated Ig and in 31 instances the phenotypes were concordant for IgM and κ with those found in the initially studied tumors. Data from the two remaining recurrent tumors require detailed comment as the phenotypes seemed discordant with those observed in the initial tumors (Table IV).

On initial presentation, patient 953 had ovarian and right maxillary tumors. Only the former was studied and it was classified as IgM(−), κ(−). A complete remission was induced, but 6 mo after institution of therapy the maxillary tumore recurred and typed as IgM(+),κ(+). A remission was induced but once again the maxillary tumor recurred 6 mo later. This tumor was classified as IgM(−),κ(−). Thus, the first and third studied tumors were concordant, but each was discordant with the second neoplasm. Unfortunately, this patient was not a G-6-PD heterozygote.

The initial maxillary tumor from patient 1,260 was clearly IgM(−),κ(−). A sustained complete remission was induced, but 1½ yr after institution of therapy, tumor recurred in the right neck. Cells from this neoplasm were obtained by a needle biopsy and were sufficient in number to allow only limited testing. The cells reacted strongly with anti-IgM reagent at low dilution. Unfortunately, there was insufficient material to test a 1:160 dilution or with anti-κ reagent.

Eight recurrent tumors from G-6-PD heterozygotes were typed for the enzyme. Five of these were also tested for IgM. The one case in which the G-6-PD phenotypes in an initial and a recurrent tumor were discordant has been reported previously (6). The IgM phenotypes in these tumors were concordant [IgM(+),κ(+)].

In summary, 36 recurrent tumors from 29 patients were typed for membrane-associated IgM and/or G-6-PD isozymes in heterozygotes. Initial and recurrent tumors were discordant for G-6-PD in one patient and in two other cases, probably were discordant for surface-associated Ig.

The duration of remission was defined as the interval between the time of initial therapy and the first observation of recurrent disease. All tumors which recurred before 4 mo were located at previously involved anatomic sites. From 4 mo onward, 10 of 20 recurrences were at sites not previously involved with tumors.

DISCUSSION

In approximately 60% of Burkitt tumors, most of the cells have surface-associated IgM. Some of the IgM(+) tumors are κ(+) and others are κ(−) (e.g., Table II). Since cultured cells from these tumors may retain their surface-associated IgM indefinitely (20), it is produced by the cell bearing it. That the presence or absence of surface-associated IgM reactivity is a specific characteristic of each tumor is indicated by the consistent findings in vitro and in vivo (Fig. 2). These observations indicate that membrane-bound IgM is a good marker for Burkitt cells, permitting a consideration of several questions about the disease's origin and development.

TABLE III

G-6-PD Isoenzymes and Cell Surface-Associated IgM Phenotypes in Multiple Tumors from Burkitt Patients Studied on Initial Presentation

Patient		Biopsy site	Phenotypes		
KCC* no.	Sex		IgM‡	G-6-PD	
				Normal tissues	Biopsy
1,054	F	R. eye	+	AB	B
		L. mandible	+		NS§
		L. neck, anterior	+		NS
		L. neck, posterior	+		NS
788	M	R. submandibular	−	NS	NS
		R. maxilla	−		NS
		L. submandibular	−		NS
899	M	Orbit	−	NS	NS
		Maxilla	−		NS
1,076	F	Ovary	±	B	B
		R. maxilla	−		B
1,106	F	Ovary	−	A–B	B
		L. mandible	−		B
1,140	F	R. ovary	+	A	A
		R. maxilla	+		A
1,143	F	R. ovary	+	A–B	B
		L. maxilla	NS		B
1,165	F	L. ovary	−	B	B
		Omentum	−		B
1,202	M	L. maxilla	+	B	B
		Submandibular	+		B
1,225	F	Breast	+	A–B	B
		L. maxilla	+		B
1,230	F	L. orbit	NS	AB	A
		L. ovary	+		A
1,260	F	R. maxilla	−	AB	A
		R. neck	−		A
1,305	F	L. maxilla	NS	A–B	B
		L. submandibular	−		B

TABLE III—*Continued*

Patient		Biopsy site	Phenotypes		
KCC* no.	Sex		IgM‡	G-6-PD	
				Normal tissues	Biopsy
1,334	F	R. maxilla	−	A	A
		R. submandibular	−		A
19,171	F	Omentum	+	AB	A
		Abdominal wall	NS		A
43,955	F	L. maxilla	+	B	B
		L. submandibular	+		B
45,230	M	R. maxilla	+	B	B
		L. mandible	+		B

* KCC no., Kenya Cancer Council number except for last three patients whose unit numbers are given.

‡ IgM, Cell surface-associated IgM. See text for criteria used to score tumors as + or −.

§ NS, not studied.

TABLE IV

Surface-Associated Ig on Tumor Cells from Two Burkitt Patients in Whom Initial and Recurrent Tumors were Found to be Discordant for IgM

Test antiserum		Percent of cells positive in tumor suspensions				
		KCC* 953			KCC 1,260	
Specificity	Dilution	Ovary 10/7/68	Maxilla 3/17/69	Maxilla 9/15/69	Mandible 12/14/70	Mandible‡ 5/12/72
Fc	1:10	NS§	NS	45	0	0
IgM	1:15	31	86	36	5	100
κ	1:15	23	72	12	8	NS
Light chain	1:15	4	71	NS	NS	NS

* KCC, Kenya Cancer Council.

‡ Puncture biopsy.

§ NS, not studied.

One question is whether Burkitt lymphoma affects T or B lymphoid cells. Presumably, all lymphocytes in man are derived from a common marrow stem cell pool. Differentiated lymphocytes are currently separated into two main classes, B and T. B cells, thought to be the equivalent of bursal-dependent cells in the chicken, synthesize and secrete antibodies. T cells are involved in "cell-mediated" immunity and they may also cooperate with or "help" B cells to produce certain types of antibodies. One of the characteristics distinguishing normal cells is the presence of easily detected surface-associated immunoglobulin on B cells, and its absence on T cells (14, 15). This concept has been extended

to neoplastic lymphocytes in attempts to classify malignant diseases into T or B cell origin according to the presence or absence of surface-associated immunoglobulins. By this reasoning, at least half of Burkitt tumors are of B cell origin. However, recently it has been reported that T cells may also have membrane-associated immunoglobulins although they are less exposed than on the surfaces of B cells (22). Since it is conceivable that malignant transformation of a T or B cell may change its surface exposure of IgM, one cannot exclude T cell origin of IgM(+) Burkitt tumors or B cell origin of IgM(−) tumors.

Studies in G-6-PD heterozygotes of 24 Burkitt tumors without significant "contamination" by normal cells have been reported previously (4, 5). The combined data indicate that 33 of 34 tumors had single enzyme phenotypes (A or B) despite the fact that normal tissues had both A and B enzymes. The one tumor with equal amounts of A and B enzymes in a cell suspension and in tumor specimens that were judged to contain $\geq$ 75% tumor cells may have had multicellular origin. All cells in this tumor were IgM(+),κ(+). The most likely explanation for the single enzyme phenotypes found in the other 33 tumors is that the majority of individual Burkitt tumors have a clonal origin. However, an alternate possibility is that a Burkitt tumor generally develops from many cells each of which has the same type of G-6-PD as the result of chance or of selection based on G-6-PD or based on phenotypes determined by loci linked to G-6-PD. That this is not the case is suggested by study of cell surface-associated IgM, a marker which is completely independent of G-6-PD-phenotype.

The distribution of Burkitt tumors with respect to the proportion of cells bearing surface IgM is essentially bimodal (Fig. 1). Thus, in 92 of 95 tumors, the cell population was either predominantly IgM(+) (60% of tumors) or IgM(−). These findings are compatible with a clonal origin for the majority of Burkitt tumors. Two neoplasms could not be definitively classified. One tumor (Kenya Cancer Council [KCC] 1,076, ovary tumor, Table I) probably contained two cell populations [IgM(+),κ(+) and IgM(−),κ(−)] possibly reflecting the presence of two or more malignant clones. Another neoplasm from this patient was IgM(−),κ(−). However, it is conceivable that the cell suspensions prepared from the tumors were "contaminated" by normal cells. Furthermore, the presence of two clones would not necessarily imply multicelluar origin since it is conceivable that the second clone could have arisen from the first, for example, by marker loss. The likelihood of this possibility is somewhat decreased by the fact that IgM loss has not been observed on prolonged cultivation in vitro.

The validity of the use of surface-associated IgM and κ as markers is suggested by the excellent correlations of these Ig's with G-6-PD phenotypes in multiple tumors from the same patient. In each heterozygous patient, multiple studied tumors were concordant for G-6-PD (i.e., both tumors showed type A or both showed type B), and with one exception (case 1,076, Table III), they were concordant for IgM and for κ. Apparently, in most instances the disease emerges at one focus and then spreads to other parts of the body.

The majority of patients with Burkitt lymphoma have therapeutically induced "complete" remissions, but tumors reappear within 1 yr in about 60% of cases (23, 24). Previously we reported a G-6-PD heterozygote with a recurrent tumor that had an isoenzyme phenotype discordant with the one found in the patient's initial tumor (6). Thus, in this patient, exacerbation of disease did not result from reemergence of the originally detected malignant clone. Using surface-associated IgM as a marker, we detected one or possibly two patients in whom initial and recurrent tumors were discordant for IgM phenotypes. Unfortunately, in one case (1,260, Table IV) the number of available cells from the recurrent tumor was too small to allow complete evaluation. Nonetheless, the virtual absence of IgM(+) cells in the initial tumor and the presence of 100% strongly reactive IgM(+) cells in the recurrence suggests that the tumors were truly discordant. The significance of the discordance found in the first recurrent tumor in the second case (953, Table IV) is somewhat confused by the fact that a second recurrence was concordant with the initial tumor. This might have been due to erroneous classification of the first recurrence, but it is possible that the malignant clone in this tumor was different from the one in the original neoplasm and that the second recurrence resulted from yet another clone or from reemergence of the originally detected malignant cell line.

Three of nine relapses which occurred after 5 mo were discordant with those found in initial tumors, but no discordance was detected among 27 earlier recurrences. Ziegler and associates (24) suggest on the basis of clinical observations, that the mechanisms underlying early and late recurrences may be different. Early relapses appear at previously involved anatomic sites whereas late regrowths often occur in previously uninvolved organs (24). Furthermore, the proportion of late relapses which have favorable therapeutic responses is similar to what is found in primary tumors. In contrast, early relapses almost always have poor responses (24). Ziegler et al. therefore suggest that most early recurrences are due to reemergence of the original malignant clones, while some late recurrences may be due to emergence of new malignant cell lines. The genetic marker (G-6-PD and surface-associated Ig) studies indicate that early recurrences are due to regrowth of the original tumor cell line. One late recurrence was discordant for G-6-PD and two others probably were discordant for IgM possibly reflecting the emergence of new malignant cell lines.

The two most likely origins for the new malignant clones are as follows: One, they could have arisen as reinductions of tumors in hitherto normal host cells. Two, they may have been present, but undetected when the patient was initially studied because, for example, they did not grow sufficiently to form clinically evident tumors. Neither of these possibilities is incompatible with the suggestion that clinically detectable Burkitt lymphoma is generally a clonal disease on its first presentation.

What implications does the probable clonal origin of Burkitt lymphoma have for the disease's putative viral etiology? Although viruses infect many cells, clonal origin of a neoplasm does not exclude its having a viral cause. In fact,

G-6-PD markers suggest a unicellular origin for the one tumor in man with proven viral etiology, *Verruca vulgaris* (common wart) (25). There are at least three circumstances in which a virally-induced neoplasm might have single cell origin. One, if the oncogenic change induced by the virus were relatively rare such as a specific somatic mutation-like event. Two, if the virus were a necessary but not a sufficient etiologic factor and one of the other tumorigenic co-factors affected only a single cell. Three, if many cells are altered by the virus, but once a malignant clone emerges, the growth of other clones is inhibited. In any event, since the disease is rare even in high endemic areas, the underlying biological "accident" must be a rare event. Is this a random phenomenon or do patients who develop Burkitt lymphoma have an underlying genetic and/or environmental predisposition? The suggestion that such patients "cured" of their original disease may sometimes have new occurrences of disease, is compatible with, but does not necessarily imply, the existence of predisposing factors.

SUMMARY

Two independent marker systems, G-6-PD isoenzymes and cell membrane-associated IgM, were used to trace the cellular origin of Burkitt lymphoma. Application of the G-6-PD system is dependent upon the fact that, in accordance with inactivity of one X chromosome in each somatic cell, females heterozygous for the usual B gene (Gd^B) at the X-linked G-6-PD locus and the variant allele Gd^A (or Gd^{A-}) have two types of cells. Gd^B is active in one cell population, which consequently produces B type enzyme; in the other population Gd^A is active, producing the variant A enzyme. Therefore, tumors with a clonal origin in a Gd^B/Gd^A heterozygote should exhibit only one enzyme type (B *or* A) whereas those with multicellular origin may show both A and B enzymes. Utilization of the immunoglobulin system is based upon the supposition that in lymphoid neoplasms with clonal origin either all or none of the tumor cells should have surface-associated IgM and κ-reactivities.

33 of 34 relatively homogeneous (with respect to content of neoplastic cells) individual Burkitt tumors from 19 G-6-PD heterozygotes had single enzyme phenotypes. Similarly, of 95 tumors tested, 92 consisted essentially of IgM(+) or (−) cells. Two neoplasms could not be definitely classified and one tumor had two cell populations. These data suggest a clonal origin for most Burkitt tumors, but the one neoplasm with a double G-6-PD phenotype (A/B) and the one tumor that had two populations of cells with respect to surface IgM, could have originated from multiple cells.

G-6-PD was determined in each of two tumors from seven heterozygotes and in all cases both tumors had the same single enzyme phenotype. Surface-associated IgM was tested in four tumors from one patient, three from another, and in two neoplasms from 11 patients. With one exception, all tumors from the same patient were concordant with respect to IgM. These findings suggest that the entire disease has a clonal origin, i.e., it emerges at one focus and then spreads to other parts of the body.

Cells from 36 recurrent neoplasms were typed for G-6-PD (in heterozygotes) and/or IgM. In one previously reported patient, initial and recurrent tumors were discordant for G-6-PD. Two other patients had IgM phenotypes in recurrences that were discordant with those found in their initial tumors. Phenotypes from three of nine relapses which occurred after 5 mo were discordant for G-6-PD or IgM but no discordance was detected among 27 earlier recurrences. Thus, some "late" recurrences may be due to emergence of "new" maligant cell lines whereas most early relapses are due to reemergence of the original malignant clones.

The probable unicellular origin of Burkitt lymphoma and the findings in tumor recurrences are discussed in terms of the disease's putative viral etiology.

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