Expression of Surface Antigens during the Cell Cycle in Different Growth Phases of American and African Burkitt's Lymphoma Cell Lines

Hauke Sieverts,1 Oliver Alabaster, Walter Goldschmidt, and Ian Magrath

Pediatric Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland [H. S., W. G., I. M.] and George Washington University, Washington, DC [O. A.]

ABSTRACT

We have studied the expression of five surface antigens in eight Burkitt's lymphoma cell lines during different phases of the cell cycle and in different growth phases (logarithmic and stationary). Cells were stained simultaneously for surface antigens (fluorescein coupled antibodies) and DNA content (propidium iodide), and dual parameter measurements were performed with a flow cytometer. Analysis of cells in specific cell cycle phases during log-phase growth revealed a 1.6-fold increase in surface antigen expression as cells passed from G1 to G2/M. This is almost identical to the measured increase in cell surface area which occurs during passage of cells through the cell cycle and indicates that under optimal conditions surface antigen density is maintained during cell doubling. We also observed a consistent reduction, by about 50%, in the expression of surface IgM (μ), k-light chain, and B1 on the cell lines during a 5-day culture period. Cell lines that only weakly expressed surface IgM were found to have a more rapid decrease, and in such cell lines IgM was ultimately completely lost from the cell surface. In contrast, the expression of β2-microglobulin and HLA-ABC increased in some cell lines, whereas in others a significant decrease of both β2-microglobulin and HLA expression was demonstrated as the cells entered stationary growth phase. Decreased cell volume (and therefore surface area) associated with declining growth rate and fewer late S or G2/M cells could account for 20–30% of the observed reduction in surface IgM, k-light chain, and B1 expression, but the major decrement in fluorescence intensity was due to a reduction in the density of these surface antigens. Thus, the ability to maintain surface antigen densities is frequently lost in suboptimal culture conditions.

INTRODUCTION

An increasing number of surface antigens have been characterized in mammalian cell systems during the past decade, and it has become obvious that these cell surface structures play a key role in cellular functions and interactions. Surface antigens are used extensively to define both phenotypically normal and malignant cells and are used in the diagnosis and characterization of leukemias, lymphomas (1–7) and recently, other solid tumors such as neuroblastoma (8, 9).

To date, most studies of surface markers have focused on qualitative assays, and even flow cytometric data are frequently interpreted by qualitative statements such as "negative" and "positive." It is probable, however, that the expression of some surface antigens is obligate, while others may vary according to the environmental conditions. Knowledge of factors which govern the expression of surface antigens is likely to be important to a greater understanding of their role in the proliferation and differentiation of both normal and neoplastic cells. In addition, the selection of appropriate antigens as targets for monoclonal antibody therapy may be rationalized by such knowledge.

Previous studies have indicated that considerable alterations in surface antigen expression may occur in cell lines under normal culture conditions (10–14). Some investigators have suggested that such changes may be related to the cell cycle (15–20), whereas other studies report variations that relate to alterations of the culture conditions (11, 12, 14, 21–23). In many of these studies indirect measurements of surface antigens have been utilized, e.g., cytotoxicity. Such parameters do not necessarily relate exclusively to the expression of surface antigen. Further, the relative contribution to antigenic variation of changes occurring during the cell cycle and in different growth phases has not been elucidated.

The purpose of the present study was to determine the extent of the phenotypic variations that occur within a uniform tumor cell population during passage through the cell cycle and under optimal and sub-optimal culture conditions (characterized as those resulting in log phase growth and stationary phase growth, respectively). Burkitt's lymphoma cell lines are particularly suitable for such a study, since this lymphoma is of monoclonal origin (21–24) and the cells grow readily in suspension culture (3, 25, 26). Burkitt's lymphoma cell lines probably represent a state of maturation arrest at an early B-lymphocyte level (21, 27, 28).

When fluoresceinated monoclonal or purified polyclonal antibodies are used to detect surface antigens, fluorescence intensity is directly proportional to the amount of antigen expressed by a cell (29, 30). Data from large numbers of cells can be accurately and rapidly acquired by flow cytometry. To study antigens in each cell cycle phase independently, we used fluorescent staining techniques which enabled us to measure DNA content and cell surface antigen expression simultaneously on individual cells. We have thus been able to separate changes relating to the cell cycle phase from those due to the culture conditions.

MATERIALS AND METHODS

Cells and Culture Conditions. Eight cell lines were used in this study. All of them have been kept in stable culture for several years in our laboratory. Table 1 gives their known characteristics, some of which have been published previously (21, 28, 31). Four cell lines (ST486, CA46, EW46, and MC116) were derived from American Burkitt's lymphoma and are Epstein-Barr virus nuclear antigen negative. The other four lines, Daudi, Namalva, AK778, and Raji, originated from African Burkitt's lymphoma and are Epstein-Barr virus nuclear antigen positive. All cell lines were grown in suspension in RPMI 1640 medium (Media 1800).
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Table 1
Characteristics of the cell lines used in the present study

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Original diagnosis</th>
<th>EBNA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>J5 CALLA</th>
<th>B1</th>
<th>B2</th>
<th>Surface immunoglobulin</th>
<th>HLA complex</th>
<th>Modal chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST 486</td>
<td>BL, US</td>
<td>---</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>48</td>
</tr>
<tr>
<td>CA 46</td>
<td>BL, SA</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>45 (43-49)</td>
</tr>
<tr>
<td>EW 36</td>
<td>UL, US</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>47 (46-47)</td>
</tr>
<tr>
<td>MC 116</td>
<td>BL, US</td>
<td>---</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>46 (45-63)</td>
</tr>
<tr>
<td>Namalva</td>
<td>AFR, US</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++++</td>
<td>48</td>
<td>47 (45-47)</td>
</tr>
<tr>
<td>Raji</td>
<td>AFR, US</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>47 (46-48)</td>
<td>+</td>
</tr>
<tr>
<td>AK 778</td>
<td>AFR, US</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>47 (46-48)</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> EBNA, Epstein-Barr virus nuclear antigen; BL, Burkitt’s lymphoma; UL, undifferentiated lymphoma; J5 CALLA, common acute lymphoblastic leukemia antigen; SA, South Africa; AFR, Africa; US, United States; µ, surface IgM (µ-heavy chain); δ, surface IgD (δ-heavy chain); κ, surface Ig (κ-light chain); λ, surface Ig (λ-light chain).

<sup>b</sup> Monoclonal antibody recognizes common HLA region but not β2M.

An indirect immunofluorescent technique using either of two different fluorescein isothiocyanate-coupled second antibodies was used; a rabbit anti-goat IgG (heavy and light chain specific) was used for detection of the polyclonal goat antibody, and a goat anti-mouse IgG (heavy and light chain specific) was used to detect the mouse monoclonal antibodies. Both antibodies were obtained from Cappel Laboratories, Westchester, PA. Neither of these second antibodies showed any cross-reactivity with human surface immunoglobulins.

Appropriate working dilutions for the antibodies were established, and all dilutions were prepared with PBS at pH 7.2.

Staining of Surface Antigens, Total Protein, and DNA. Five x 10<sup>6</sup> cells were used for each measurement. The cells were washed in medium (RPMI 1640 containing 20% fetal calf serum, with penicillin and streptomycin, at 37°C) and then layered under Ficoll-Hypaque lymphocyte separation medium (Litton Bionetics, Kensington, MD) and centrifuged at room temperature at 400 x g for 30 min in 15-ml conical tubes (Falcon). The interphase usually yielded 80 to 90% of the viable cells in the original population and contained consistently less than 5% dead cells. These cells were transferred to 5 ml tubes, washed once more in medium, and resuspended in ice cold antibody solution with a cell concentration of approximately 1 x 10<sup>7</sup> cells/ml. All subsequent procedures were performed at +4°C. After 1 h of incubation the cells were washed twice in PBS and resuspended in the second, fluorescein isothiocyanate-coupled antibody solution and again incubated for 1 h. Cells were then washed twice in PBS and resuspended in 2 ml of normal saline. Cells were fixed by gently mixing the sample on a vortex mixer while slowly adding absolute ethanol to achieve a final concentration of 50% (19, 32). The fixed cells can be stored in a refrigerator for as long as 1 year without alteration in their staining properties (33).

Immediately prior to the measurement of surface fluorescence, pelleted cells were counterstained with propidium iodide solution (propidium iodide (18 µg/ml), Calbiochem, La Jolla, CA; RNase (40 µg/ml), Sigma, St. Louis, MO) (32, 34) in order to measure DNA content simultaneously. After an incubation period of at least 20 min at room temperature the doubly stained cell suspension was used without further processing for flow cytometric analysis.

Analysis of Surface Antigens. Measurements were performed with a flow cytometer (FACS IV; Becton and Dickinson, Mountain View, CA 94043) equipped with a 50-µm nozzle tip. Preliminary experiments showed that by using appropriate filters, the fluorescein and propidium iodide signals could be completely separated. Standardization of the instrument was performed for each experiment with fluorescent beads. All DNA histograms were further standardized by placing the G<sub>0</sub> peak in channel 100 of a 256-channel histogram, and relative changes of the DNA distribution were assessed. Usually, 30,000 cells were measured per sample at a flow rate of approximately 1,000 cells/s.

Cell Size. The Coulter volumes of the cells were analyzed by a Coulter Counter channelizer calibrated previously with microspheres of 5, 10, and 20 µm diameter to convert the Coulter channel numbers to volume.

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<sup>2</sup> The abbreviations used are: SigM, surface immunoglobulin M; β2M, β<sub>2</sub> microglobulin; PBS, phosphate-buffered saline (0.01 M phosphate, 0.15 M sodium chloride, pH 7.2).

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Fig. 1. Growth characteristics of ST 486 cells during the 5-day culture period. Three sequential experiments are shown. Prior to the 5-day period the cells were brought into exponential growth by daily replacement with fresh medium. During the 5-day period there was no further replacement of medium. O, concentration of viable cells; A, concentration of non-viable cells, detected by trypan blue uptake.

Unit, NIH containing 20% fetal calf serum (Grand Island Biological Co.), as well as penicillin (100 units/ml) and streptomycin (100 µg/ml).

For 8 days prior to the experiments the cell lines were maintained in exponential growth and maximal viability by changing the medium and adjusting the cell concentration to 0.5 x 10<sup>6</sup> cells/ml every 48 h. During the following 5 days of the experiment the cultures were left without further changes of medium to create a growth curve that includes an exponential growth and maximal viability by changing the medium and viable cells; S, concentration of non-viable cells, detected by trypan blue uptake.

Growth Curves. The growth of the cell cultures was expressed as the cell concentration in the suspension. Cell counts were performed with a hemocytometer (Neubauer chamber), and viability was determined by trypan blue exclusion.

Antibodies. The cells were tested for their expression of SigM, 2 x immunoglobulin light chains, β2M, and the HLA complex. SigM was detected either with a polyclonal, goat antihuman IgM antibody (µ-chain specific; Cappel Laboratories, Westchester, PA) or with a mouse monoclonal antibody to µ-chains (Becton and Dickinson, Sunnyvale, CA).

Monoclonal antibodies were also used to detect κ-light chain, β2M (both from Becton and Dickinson, Sunnyvale, CA) and the common region of the HLA complex (Bethesda Research Laboratories, Bethesda, MD). A single batch of each antibody was used throughout the course of these experiments.
or surface area. Daily measurements were carried out on viable cells using standardized gain and window settings.

**Data Analysis.** During flow cytometric measurements the FACS IV display and data processing unit was used to follow the data acquisition. The data were stored on a PDP 11/34 computer (Digital Equipment Co.) and processed by the NIH flow cytometry software package.

It is evident that the green fluorescence can only originate from intact cells if the signal is associated with red fluorescence originating from propidium iodide (staining DNA) within the intensity range of the cell cycle (G1 through G2M). In this analysis we therefore excluded all green fluorescence signals outside the propidium iodide fluorescence range of channels 16 to 52 (only 64 channels are available in dual parameter mode). In this way we avoided the majority of potential artifacts caused by fluorescein isothiocyanate binding particles, including subcellular particles and most of the cell aggregates. Because of this and elimination of dead cells by previous Ficoll-Hypaque separation, it was unnecessary to impose light scatter thresholds to exclude dead cells and other artifacts.

**RESULTS**

Table 1 displays some of the characteristics of the cell lines used in the present study. All cell lines have an 8:14 translocation. They all express the common acute lymphoblastic leukemia antigen defined by the monoclonal antibody J5 and a surface antigen defined by the monoclonal antibody B1. All cell lines except Daudi express β₂-microglobulin and the HLA complex. Differences between cell lines of African and cell lines of American origin used in this study include the presence of the Epstein-Barr virus nuclear antigen and their expression of B2 and SlgM.

**Cell Growth during the Culture Period.** The growth pattern of each cell line was reliably reproduced during successive 5-day culture periods, indicating that the culture system established a well-standardized environment for reliable measurements of growth-related events on the cell surface (Fig. 1). The viability of the cell cultures exceeded 95% at the beginning of each culture period. There was no detectable lag phase after initiation of the culture. The viable cell concentration rose exponentially during the first 2 days, remained approximately constant during days 2 and 3, and decreased during days 4 and 5.

The American cell lines accumulated on the average 15% dead cells on day 5 of the culture period with up to 45% dead cells on day 5 in the cell line ST 486 on one occasion. The African cell lines maintained a slightly higher viability with only 5–10% nonviable cells even on day 5. The sum of nonviable and viable cells increased only until day 4 and decreased thereafter, indicating that a fraction of the dead cells disintegrated and could not be detected.

**DNA Histograms during the Culture Period.** Samples for surface marker analysis were taken on each day of the culture period. With the double staining technique the DNA content measurements could be carried out on the same sample. Data for a number of cell lines are summarized in Fig. 2. The relative size of G1 increased, whereas both S and G2/M decreased during the culture period. It can be seen that the cultures initially proliferated rapidly, with up to 60% of the population being in S and G2/M on day 1. As the culture “aged” this fraction decreased to 30%. Representative histograms for one cell line depicting the DNA content distribution on days 1 and 5 are also shown in Fig. 2. A decrease in the proportion of cells in the G2/M peak on day 5 relative to day 1 is clearly demonstrated.

**Cell Size during the Culture Period.** We observed a significant reduction in cell volume during the culture period in all cell lines (Fig. 3). The peak channel number decreased on average by 50%, indicating a volume reduction of approximately 30%. A reduction in the proportion of late S and G2/M cells in the population between days 1 and 5 (from 60 to 30%) can only account for an overall reduction in mean volume of less than 12%. Thus, a true decrease in cell volume must occur during the culture period, although whether this decrease occurs evenly throughout all cell cycle phases or predominantly in one of the cell cycle phases cannot be determined from these data.

**Surface Antigen Profiles during the Culture Period.** Fig. 4 shows the distribution profile of SlgM in the cell line ST 486 on days 1 and 5. There was an obvious shift to the left on day 5 compared to day 1 and a change in the shape of the histogram.
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Fig. 4. The SlgM expression of G, cells (ST 486) on day 1 is compared with G, cells on day 5.

These data were expanded by studying antigen expression in individual cell cycle phases during the culture period.

Surface Antigen Expression during the Cell Cycle. By using the existing NIH software it is possible to generate any "slice" through the 64-channel dual parameter histogram with a thickness from 1 up to 64 channels (Fig. 5, top). These slices can then be analyzed independently. We examined, by means of this technique, the SlgM expression of an entire sample of exponentially growing cells (day 1) and compared the resultant histogram with histograms of slices obtained from each of the cell cycle phases G,, S, and G2/M (Fig. 5, bottom). The entire population was distributed between the DNA fluorescence channels 16 and 52. By using the standardization procedure as explained in methods for the propidium iodide stain, G, peaks always appeared between channels 16 and 27 (see Fig. 2). S-phase cells were distributed from channels 28 to 42, and G2/M was considered to fall into the range of channels 43 to 52. There is a minor overlap of true G, cells and early S-phase cells and a larger overlap between S phase and G2/M cells. Results similar to those shown in Fig. 5, bottom, were obtained for all of the cell lines and surface antigens tested in the present study.

Clearly, the fluorescence distribution of the entire population is the sum of the fluorescence distribution of cells in each of the cell cycle phases. Our data indicate that the surface antigen distribution of cells in different cell cycle phases is not congruent. The mean fluorescence intensity in G2/M cells reached a 1.4-fold higher channel number than the mean fluorescence intensity in Gi cells, and the maximum difference between Gi and G2/M cells was 1.6-fold. This increase in fluorescence intensity was found in all surface antigens tested in exponential growth phase. As shown in Fig. 6, however, this relationship does not necessarily hold when cells are not in log phase growth (see below).

Surface Antigen Expression in Specific Cell Cycle Phases during the 5-day Culture Period. The expression of surface antigens decreased significantly during the culture period. This observation held for µ, κ, and B1 on all cell lines, but the pattern of the decline in β2M and HLA expression differed.

Fig. 6 shows the analysis of SlgM and β2M expression on ST 486 cells throughout the 5-day culture period. The expression of these surface markers is depicted in an "early" (G,) fraction (channels 22, 23) that includes small cells which had just undergone cell division and in a "late" fraction of G2/M (channels 47, 48) which includes large cells just prior to cell division. The mean fluorescence intensity of µ and β2M on ST 486 fell linearly and by the fifth day had reached a point approximately 50% below the day 1 level of the G, fraction. At this time a large proportion of the G, fraction of cells fell within the distribution of the negative control. Lines of regression were calculated to fit the mean values which yielded coefficients of regression (r) over 0.85 in all cases and revealed a difference in the rate of decrease in SlgM compared to β2M in G2/M cells.

For SlgM we observed a convergent course of the mean
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Fig. 6. Mean fluorescence intensities of SlgM and β2M fluorescence are calculated separately for the G1 phase and the G2/M phase during a culture period. Bars, SE. The lines of regression correlate significantly with the mean values (SlgM: r = 0.99; β2M = 0.85). Left, A representative experiment showing SlgM expression during a 5-day culture period. Note the convergence of G1/M and G1 subpopulations, maintaining an approximately constant ratio of G1/M:G1 fluorescence. Each point on these curves is significantly different from the preceding and succeeding points (P < 0.001 by Student’s t-test). Right, β2M expression in the same experiment. There is only a slight decrease of β2M-microglobulin with almost parallel lines of regression. The mean fluorescence values for days 1, 2, and 3 do not differ significantly from each other, but are significantly different from the values for days 4 and 5 (P < 0.001 by Student’s t-test).

Fig. 7. Changes of surface immunofluorescence of five antigens on eight cell lines during a 5-day culture period. X, ST 486; O, CA 46; ●, EW 36; C, MC 116; □, Namalva; △, Akata; ▼, Raji; ●, Daudi. The changes of surface antigen expression on day 5 of the culture period are summarized as a percentage of the day 1 level. Since the five antigens are not expressed as a complete set on each cell line, there are usually less than 8 values given for each antigen. Columns mark the mean percentage of change for all cell lines for each antigen. The μ-heavy chain and κ-light chain expression both show a consistent decrease of more than 50% in all cell lines expressing these antigens apart from CA 46. The expression of the BI antigen decreased by 60 to 100%. However, Daudi and ST 486 showed only a slight decrease or no decrease at all. In contrast to these three antigens, there was no consistent behavior in β2M and HLA-ABC expression among the eight cell lines. ST 486 and EW 36 showed a reproducible decrease of approximately 40 to 50%, whereas three other cell lines never changed, and CA 46 demonstrated a significant increase of immunofluorescence on day 5.

DISCUSSION

The present study shows that the expression of SlgM (μ and κ) and B1 in Burkitt’s lymphoma cell lines decreased considerably during a 5-day culture period. The expression of β2M and HLA ABC also changed during the 5-day culture period, but there was no uniform pattern among the cell lines tested. The observed variations were found to be reversible and highly reproducible during the culture period; i.e., a population with minimal expression of a surface antigen during stationary growth reexpressed the same antigen at full intensity once exponential growth was regained. Our data therefore reveal that there is considerable variability of surface antigen expression within a monoclonal, putatively biologically uniform cell type which relates to both the cell cycle and the growth phase.

The few existing reports concerning variations of surface antigen expression during different growth phases provide rather inconsistent results (13, 14, 17, 18, 35). Cikes and Klein (36), Cikes and Friberg (37), and Cikes et al. (38) studied H-2 and Moloney virus antigen expression on mouse YSAB cells during a 5-day culture period comparable to that used in this study. These authors repeatedly observed a higher level of complement dependent cytotoxicity in populations in stationary growth (day 5) than in exponential growth (day 1). However, a similar study carried out by Lerner et al. (39) on the same cell line did not

expression of the G1 and G2/M fractions, respectively. The slope of the G1 line was found to be half the slope of the G2/M line of regression. The ratio of G2 to G1 mean fluorescence values therefore remained close to 1.6. In contrast, the decline of β2M expression turned out to be consistently parallel for G1 and G2/M. Because of this, the ratio of G2/M to G1 fluorescence increased during the culture period from an initial value of 1.6 to a final value of 3.5 on day 5.

Experiments were repeated twice for each antigen on recultured cells as outlined in "Materials and Methods." The results were very reproducible in recultured cells, which also demonstrated that cell populations with low antigen expression regain their full antigen density once exponential growth has again been achieved.

These experiments were performed on all eight cell lines. The changes of immunofluorescence intensity between days 1 and 5 are summarized in Fig. 7, which shows the shift of the mean immunofluorescence intensity as a percentage of the day 1 value. Since not all of the eight cell lines expressed every surface marker there are usually less than eight values given for each antigen. The expression of the immunoglobulin chains μ and κ decreased consistently, on the average, by 50% in all cell lines. There was an even more pronounced decline of B1 expression in most cell lines, ranging from 60 to 100%. However, in ST486 cells the fluorescence intensity of B1 increased slightly during the culture period. The expression of β2M and HLA ABC decreased in some cell lines but increased significantly in others.
support Cikes’ results but reported a similar degree of sensitivity of the cells in all growth phases.

A study of Thomas (40) on mouse P815Y cells indicated that individual antigens may behave differently during a growth period. These authors found blood group B determinants to be maximally expressed at stationary growth. A similar variability in the pattern of the expression of different antigens during growth in vitro was reported by Guglielmi and Preud’homme (13) for the Burkitt cell line Raji. Guglielmi and Preud’homme observed a change of Raji’s surface marker profile from a pre-B-cell type to a B-cell type which lasted only a few hours during exponential growth. We could not, however, reproduce this phenomenon with the Raji cells available in our laboratory. Our cells failed to express Slg throughout the entire culture period, although we were able to detect cytoplasmic immunoglobulin.

In a recent report, the expression of human breast cancer associated antigens on breast cancer cell lines was studied using flow cytometric measurements (14). A shift of immunofluorescence profiles toward lower intensity levels was observed during a 5-day culture period, implying a decrease in surface antigen expression with declining growth rate.

Thus, existing studies provide contradictory results regarding the influence of growth rate on surface antigen expression (10, 12, 18, 41). Methodological difficulties may in part be responsible for this, especially in the earlier studies in which complement dependent cytotoxicity assays were used to assess surface antigen expression (10, 20, 42). For cell lines in exponential growth, cytotoxicity assays provide reproducible and even quantitative results, but results with cell populations in different growth phases may not readily be comparable to each other, since factors other than surface antigens, e.g., altered membrane repair mechanisms or nonspecific activation of complement, may change with growth and modify the results obtained in a cytotoxicity system. Flow cytometry eliminates most of the potential artifacts of the earlier studies, since the binding of antibody to antigen is the only reaction required on the cell surface, so that other changes in the cell membrane do not interfere with this reaction. In addition, fluorescence intensity is directly proportional to the amount of antibody and hence antigen (29, 30) so that cytofluorimetry provides a truly quantitative measure of surface antigen.

Factors that alter fluorescence intensity include cell size, surface antigen density, and the relative size of subpopulations with differing surface antigen levels within the total cell population. Our results indicate that during a standardized culture period the decrease in surface antigen expression was accompanied by a decline in the proportion of S and G2/M cells and also by a decrease in cell size. In exponential growth the G2/M compartment contained cells with a 1.8-fold higher mean fluorescence intensity than cells in the G1-phase. This corresponds well to the mean fluorescence level of the total sample. The shift of the DNA content distribution during the culture period has, therefore, only a minor effect on surface antigen expression. In fact, the separate analysis of single cell cycle phases during the 5-day culture period revealed that there are major changes of surface antigen expression within specific phases of the cell cycle. Either a decline in antigen density or a decrease in cell surface area with constant antigen density could cause this decrease in fluorescence intensity. Since Burkitt’s lymphoma cells are essentially spherical, surface area and cell volume are strictly related, and changes in cell size without changes in antigen density would directly influence the surface area and hence fluorescence intensity. The loss of mean Coulter volume of 30–40% observed during the 5-day culture period (which included the change consequent upon a reduced proportion of G2/M cells) would result in a decrease of the mean cell surface area by 20–30%.

The decrease in cell size, therefore, can account for a change in fluorescence intensity of not more than 20–30%, if antigen density remains the same. The mean fluorescence intensity of μ- and κ-antigens, however, decreased by approximately 50–70% during the 5-day culture period, and in some experiments carried on for more than 5 days (data not shown), it continued to decrease until it approximated the fluorescence level of the negative control. The major portion of this decrease must therefore be related to a considerable decrease in the density of antigen on the cell surface. This may be a result of either decreased synthesis or failure of insertion into the cell membrane as nutrients are used up or metabolites accumulate in the cells or culture medium. Alternatively, but a less likely explanation, increased loss of antigens from the cell surface would give the same results. It is noteworthy that the decrease in antigen expression was not the same in all cell cycle phases for all antigens. Thus, although for SlgM the ratio of G2/M:G1 fluorescence remained the same during the culture period, for β2M this was not so, and this ratio actually increased during the culture period. The rate of β2M synthesis, or loss from the cell surface, increasingly differed in G2/M cells as compared to G1 cells throughout the culture period.

We conclude that under optimal growth conditions, Burkitt’s lymphoma cells maintain a constant antigen density in spite of cell doubling during progression through the cell cycle. Under suboptimal conditions, however, several surface antigens decline in expression more than can be accounted for by a reduction in the proportion of G2/M cells in the population or the reduction in cell volume which also takes place. The degree of change of an individual antigen is a characteristic of the individual cell line, but some antigens, e.g., μ, κ, and B2, are almost always reduced. Many cells in the population may fail to express such antigens which are present under optimal growth conditions. Other antigens, e.g., HLA, have a more variable change in expression in suboptimal conditions among the cell lines examined, although the direction and degree of change for each individual cell line was reproducible. The relative contributions of changes in antigen synthesis or turnover remain to be determined, although under sub-optimal culture conditions it is probable that decreased synthesis is at least a major factor in producing decreased antigen density. These findings are relevant to targeted cancer therapy or imaging methods utilizing monoclonal antibodies, for many tumor cells, particularly in solid tumors, may survive in suboptimal conditions for growth. This should be taken into account when selecting monoclonal antibodies for in vivo use.
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