Enhanced Content of IgG in Burkitt’s Lymphoma Cells after Treatment with the Topoisomerase II Inhibitor, Lucanthone

Robert Bases*, 1 Rukmani Lekhraj, 2 Xudong Tang, 2 Jinghang Zhang, 2.3 Zhi Duan, 4 Jennifer T Aguilan, 5 and Edward Nieves 6

1Departments of Radiology and Radiation Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, 111 East 210th Street, Moses Research Building, 301, Bronx, NY 10467, USA
2Departments of Neurosurgery, Montefiore Medical Center, Bronx, NY 10467, USA
3Departments of Microbiology and Immunology, Albert Einstein College of Medicine, NY 10461, USA
4Departments of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
5Departments of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
6Departments of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Abstract

Burkitt’s lymphoma cells (CRL-1647) which had survived treatment with lucanthone contained 3.6 fold more IgG than untreated cells, although most of the cellular immunoglobulins were still IgM. DNA activation induced cytidine deaminase (AID) was increased 5 fold in these surviving cells, consistent with active Class Switch Recombination (CSR).

Progeny of the small fraction of cells which had survived 20 h exposure to 8 µM lucanthone before rescue were cloned. 1.5 × 10⁸ cloned cells contained ~1 µg of cytidine DNA deaminase, as determined from affinity column isolation of the enzyme, assayed by digestion of a 30 nt 32P labeled specific DNA substrate. Before lucanthone treatment, little AID could be detected. After the second treatment, a six fold increase in AID was found. In confirmation, Western blot determinations of AID from lysates of lucanthone treated cells showed 5 fold increased AID content. These results suggest that lucanthone led to increased IgG content of surviving cells, consistent with their increased AID activity. The surviving cells were also more resistant to the standard lucanthone treatment, as determined in clonogenic assays.

IgG could not be detected in the cell membranes of CRL cells before or after lucanthone by immunostaining and flow cytometry, but both cell types secreted 80 kDa and 25 kDa immunorelated protein.

Lucanthone, formerly used to safely treat hundreds of thousands of schistosomiasis patients, might be considered as a means to promote IgG synthesis in macroglobulinemia patients.

Keywords: Cytidine deaminases; Class Switch Recombination (CSR); Lucanthone; Clonogenic assay; Activation Induced Deoxycytidine deaminase (AID)

Introduction

The role of DNA Activation Induced Deoxycytidine deaminase (AID) in class switch recombination (CSR), i.e., switching from synthesis of IgM to IgG lymphocytes, has been well established [1,2]. Furthermore, AID deficiency has been implicated in the hyper-IgM syndrome [3]. The proto oncogene, c-myc, which regulates antibody secretion, is significantly involved in CSR [4].

We considered ways to enhance CSR in Burkitt lymphoma cells, as a model to learn how to enhance CSR in clinical situations. AID deaminates DNA cydridines on single stranded DNA [5] leaving DNA uridines that are then removed by ubiquitous cellular uracil DNA glycosylases. Abasic sites and double strand breaks result, which may be essential for CSR. We sought to mimic the deamination step by treating Burkitt lymphoma cells with lucanthone, an inhibitor of topoisomerase II [6], whose ability to create Abasic sites and double strand breaks in DNA of HeLa cells had been demonstrated [7,8]. As a caveat, we note that DNA deamination activity of AID might not alone represent its physiological function [9,10].

Burkitt lymphoma cells CRL-1647 synthesize IgM but little IgG. Here we describe experiments to learn if IgG synthesis was stably enhanced many generations after the lucanthone treatment of Burkitt lymphoma cells.

Continuous exposure to concentrations of 8 µM lucanthone beyond 2-4 h progressively sterilized HeLa cells. This lethal effect must be taken into account in the results to be described here i.e., could the increase in IgG synthesis result from selection of a special cell population?

Lucanthone's effects on HeLa cells were readily reversible [11]. Furthermore, it has an unblemished clinical safety record [12]. It was given to hundreds of thousands of patients who were treated for schistosomiasis in the past. Currently, lucanthone is used clinically as an adjuvant to radiation therapy [13]. We encountered no hematogetic toxicity or other toxicity when care was taken to avoid interference from concurrent medications. Lucanthone was well tolerated when administered to patients daily for several weeks, maintaining serum concentrations of 8 µM lucanthone. The in vitro studies described here employed 8 µM lucanthone. Lucanthone is promptly eliminated by the liver and kidneys in experimental animals. HeLa cells suffered no loss of clonogenic survival for 2 h in 8 µM lucanthone when growth medium was then changed. But a thousand fold loss of survival was found if lucanthone removal was delayed 20 h [11]. Serum lucanthone

*Corresponding author: Robert Bases, Professor of Radiology and Radiation Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, 111 East 210th Street, Moses Research Building, 301, Bronx, NY 10467, USA, Tel: 914-380-2757; Fax: 914-632-3056; E-mail: rbases@verizon.net

Received June 19, 2017; Accepted July 12, 2017; Published July 19, 2017

Citation: Bases R, Lekhraj R, Tang X, Zhang J, Duan Z, et al. (2017) Enhanced Content of IgG in Burkitt’s Lymphoma Cells after Treatment with the Topoisomerase II Inhibitor, Lucanthone. J Bioanal Biomed 9: 186-193. doi:10.4172/1948-593X.1000176

Copyright: © 2017 Bases R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
concentrations of 8 μM are readily achieved, and are well tolerated. The 8 μM lucanthone in cell culture increased the frequency of DNA abasic sites and double strand DNA breaks [7,8]. A study of lucanthone concentration dependence would be of interest but is beyond the scope of this initial report. The Burkitt lymphoma cell line was chosen because it was a B cell line which contained IgM but little or no IgG, so that it was a good target for study.

Materials and Methods

Cells

CRL-1647 Burkitt human lymphoma cells (0L) were purchased from American Type Culture Collection (ATCC), Manassas, VA 20108. They were grown in suspension at 37°C in Roswell Park Memorial Institute 1640 medium with 10% fetal bovine serum in 8% CO₂ in a humidified atmosphere. The cell culture doubling time was 24 h. Media and sera were from ATCC. After two serial treatments with lucanthone, the lymphoma cells are designated: 2L.

Cell lysates

Cells were sedimented from phosphate buffered saline without Ca⁺⁺ or Mg⁺⁺, resuspended in lysis buffer [14] with 10 μM Aprotinin and sonicated with 20 one-second strokes, leaving 1-2% unbroken cells. Lysates of 10⁷ to 10⁸ cells were clarified by centrifugation at 15,500 g for 12 min contained approximately 1 μg/μl of protein [15].

Western blots

For most experiments, 7 cm mini gels, purchased from BioRad Laboratories, Los Angeles, CA were used. Buffer without SDS or methanol, containing 25 mM Tris, pH 8.3 and 192 mM glycine were used for gel electrophoresis and Western blot transfer.

Detection of IgM and IgG in cell lysates was made in Western blots, using goat anti-human IgG pre-coupled to horseradish peroxidase (SC Laboratories, Los Angeles, CA were used. Buffer without SDS or methanol, containing 25 mM Tris, pH 8.3 and 192 mM glycine were used for gel electrophoresis and Western blot transfer.

Enzyme activity was measured by cleavage at the single cytidine, which released 32P labeled from unlabeled 30 nt substrate. Usually, 10 μL reaction mixtures containing 0.5 pmol of 32P labeled substrate in 10 mM Tris-HCl pH 8.0, 1 mM EDTA and 1 mM DTT were incubated with 5 μL of sample at 37°C for 15 min. Cell lysates were pretreated by digestion with 10 μg/ml of pancreatic RNase for 30 min at 37°C.

Enzyme DNA glycosylase, 1 μL (1 unit/μL), Invitrogen, was added, followed by 15 min of incubation at 37°C to remove uracil products of AID digestion. The resulting abasic sites were cleaved by adding 1 μL of 2 N NaOH to the reaction mixture and heating for 5 min at 95°C. Then 10 μL of formamide were added.

For most experiments, 7 cm mini gels, purchased from BioRad Laboratories, Los Angeles, CA were used. Buffer without SDS or methanol, containing 25 mM Tris, pH 8.3 and 192 mM glycine were used for gel electrophoresis and Western blot transfer.

Detection of IgM and IgG in cell lysates was made in Western blots, using goat anti-human IgG pre-coupled to horseradish peroxidase (SC Laboratories, Los Angeles, CA were used. Buffer without SDS or methanol, containing 25 mM Tris, pH 8.3 and 192 mM glycine were used for gel electrophoresis and Western blot transfer.

Enzyme activity was measured by cleavage at the single cytidine, which released 32P labeled from unlabeled 30 nt substrate. Usually, 10 μL reaction mixtures containing 0.5 pmol of 32P labeled substrate in 10 mM Tris-HCl pH 8.0, 1 mM EDTA and 1 mM DTT were incubated with 5 μL of sample at 37°C for 15 min. Cell lysates were pretreated by digestion with 10 μg/ml of pancreatic RNase for 30 min at 37°C.

Enzyme DNA glycosylase, 1 μL (1 unit/μL), Invitrogen, was added, followed by 15 min of incubation at 37°C to remove uracil products of AID digestion. The resulting abasic sites were cleaved by adding 1 μL of 2 N NaOH to the reaction mixture and heating for 5 min at 95°C. Then 10 μL of formamide were added.

After these additions, 10-15 μL of TBE sample buffer (BioRad, 161-0768), were added and the samples were placed in the 30 μL sample wells of 15% acrylamide TBE urea gels (BioRad 161_1117). For electrophoresis for 60-75 min, the gels were exposed to x ray film for 60 min or more. The radioautograms were scanned by quantitative densitometry. Radiographic densities of faster migrating 16 nt DNA enzyme lysis products were quantitated and expressed as fractions of the sum of residual 30 nt substrate and 16 nt product densities.

Oligodeoxycytidylid affinity column purification of AID

1 ml HiTrap affinity columns (GE Health Care, Biosciences, Uppsala, Sweden SE 75184) were first prepared by binding oligodeoxycytidine (American Biosciences, Piscataway, NJ 08855) according to the manufacturer’s directions. We expected that few of the primary amino groups of the polydeoxycytidylidic would be eliminated by the binding step at 4°C; 600 μg of oligo dC were bound to the column. Cell lysates from 1.5 × 10⁷ cells were introduced for binding at 4°C and after 125 min, the column was washed at 4°C to eliminate unbound AID and other proteins. The column was kept in Phosphate Buffered Saline (PBS) at 4°C to prevent cytidine deaminase digestion of the column bound cytidylidic amino groups.

To release cytidine deaminases from the column, elutions were made with 0.5 ml of 0.2N NaCl in 0.04M Glycine at pH 3.0 and then 0.5 ml of 0.5N NaCl in 0.1 M Glycine at pH 3.0, followed by PBS. 5.0 μl aliquots of each 500 μL column fraction were assayed for cytidine deaminases.
Results

Lucanthone treated cells contain more IgG

Goat antihuman IgG reacts with both IgG and IgM. IgG was much more reactive, as noted in the legend for Figure 1A. For ease of comparison we scored both their immunoreactivities. Estimates were also made of protein content in gel bands, by reference to standard IgM and IgG gel band protein contents. Figure 1A and Figure 1B clearly show that lymphoma cells which survived lucanthone 1L contained 3.6 times more IgG (by relative immunofluorescence) than untreated cells (0L). Furthermore, when estimated from protein content without lucanthone treatment, IgM was 398 times more abundant; after lucanthone treatment, IgM was 53 times more abundant indicating increase in relative IgG abundance.

Figure 1B shows results of determinations made in another experiment with a CRL-1647 cell lysates. Enhancement of IgG was again found in the cells from this culture, which also had grown from cells which survived treatment with 8 μM lucanthone. From these 2 experiments we found that lucanthone survivors of one lucanthone exposure contained 3.6 fold enrichment of IgG, determined from Western blots of Figure 1A and 1B; p ≤ 0.01. Survivors of a second lucanthone exposure exhibited a small increase in IgG/(IgG+IgM).

In an experiment (not shown) the ratio for 1 × lucanthone survivors was 0.75 ± 0.0067 SE; for the 2 × lucanthone survivors it was 0.85 ± 0.0110 SE, the difference was significant, p<0.001. In 5 separate experiments with survivors of one lucanthone treatment, the IgG/IgG+IgM was 0.57 ± 0.009SE, significantly different from results with 4 separate experiments with untreated cells (0.20±0.05SE) at p>0.01.

Determination of enhanced content of IgG in cell lysates in individual lanes did not depend upon comparisons between individual lanes.

The actin content of lysates of Figure 1B as well as lysate from cells which had survived two lucanthone treatments (2L cells) was compared in Figure 1C. Lucanthone survivor's actin content was two to three
folds greater than obtained with untreated cells, over a wide range of molecular sizes. Determination of their post lucanthone enhanced IgG was not related to actin content (Figure 1C); both IgG and IgM in each lane were normalized by the same factor.

ELISA assays for the four IgG isotypes in 0L and 2L cells’ lysates show enrichment of the four isotypes in 2L cells, confirming in 4 separate assays, the determinations made by Western blots previously (Figure 2).

We eliminated the possibility that IgG isotypes had been absorbed to 2L cells from the fetal bovine serum in growth medium. Testing the serum by ELISA assays failed to detect such antigenicity (not shown). Because all four IgG isotypes were involved, RT PCR studies would probably not be helpful.

**Aid is enhanced in lucanthone survivors**

Single cell survivors of 8 µM lucanthone were cloned by terminal dilution in a 96 well plate. One clone, A-7, was grown and tested for IgM and IgG by relative fluorescence. As expected, IgG cell content was enhanced, as measured by immunofluorescence, to 0.49 and 0.36 in the uncloned and cloned cells (not shown).

To learn if cytidine DNA deaminase was present in A-7 cells, cell lysates were purified by binding to polydeoxycytidine containing column and eluting, as described in Materials and Methods. Three major fractions contained enzyme activity (Figure 3).

Previously, two major fractions were found when bone fide AID was analyzed on a similar column [16]. AID activity of these 2L cells was increased (Figure 4). AID from 3/8 as many cells assayed in the experiment of Figure 3 yielded 2.5 fold more AID in the experiment of Figure 4, comparing the areas under the elution curves of Figures 3 and 4. Therefore, an overall six fold increase in AID activity was achieved. Untreated lymphoma cells contained little detectable AID activity (Figure 4).

Western blot analyses in Figure 5 showed that AID in 2L cells was 5.4 fold more abundant than in 0L cells, as expected from previous results shown in Figures 3 and 4.

**Figure 1C:** The aggregate actin related densities from cell lysates were scored. Cell lysates had not been treated with heat and dithiothreitol before electrophoresis, resulting in several actin positive bands over a range of sizes. The range of sizes from 2 × 10^5 to 8.3 × 10^4 Actin content of the cells in the study of Figure 1B was compared by immunofluorescence, as described in the Methods section. Lysates from 2 × 10^5 cells of each of the three groups were compared. Lanes 1, 2, 3—No lucanthone; relative immunodensities: 955, 859, 1117, mean: 977. Lanes 4, 5, 6—Lucanthone survivors; relative immunodensities: 2776, 2442, 2777, mean: 2665. Lanes 7, 8, 9—2 times lucanthone treatments; relative immunodensities: 2326, 2001, 2487, mean: 2272.

**Figure 2:** Legend for ELISA assay of OL/2L cells. Lysates were prepared by sonication of 1.5 × 10^8 OL cells and 2.0 × 10^8 2L cells; supernatants were harvested after centrifugation at 12,365 g for 16 min. Equal concentrations of the four isotypes of IgG were compared by ELISA assays, as shown in Figure 2, using reagents from Life Technologies ref 991000.

**Figure 3:** Binding and release of cellular deoxycytidine deaminase from an affinity column containing ~600 µg of bound 100 nt long polydeoxy cytidine oligomers. 1.5 × 10^8 CRL-1647 (A-7) cells cloned after 18 h treatment with 8 µM lucanthone were sonicated and then sedimented at 12,365 g for 16 min. Supernatants were treated with 10 µg/ml of DNAase free pancreatic ribonuclease and then bound to the column for 125 min at 0ºC. Serial elutions were made with phosphate buffered saline; 0.2 N NaCl in 0.04 M glycine pH 3.0; 0.5 N NaCl in 0.1 M glycine, pH 3.0 and finally with phosphate buffered saline. 0.5 ml fractions were collected. Enzyme activity in the fractions was determined by measuring release of 16 nt 32P labeled DNA fragments from 32P labeled 30 nt DNA substrate. Release is expressed as the fraction of 30 nt DNA released as 16 nt DNA product, determined from autoradiographs of each gel lane (●●●).

**Figure 4:** Aid is enhanced in lucanthone survivors. Single cell survivors of 8 µM lucanthone were cloned by terminal dilution in a 96 well plate. One clone, A-7, was grown and tested for IgM and IgG by relative fluorescence. As expected, IgG cell content was enhanced, as measured by immunofluorescence, to 0.49 and 0.36 in the uncloned and cloned cells (not shown).

To learn if cytidine DNA deaminase was present in A-7 cells, cell lysates were purified by binding to polydeoxycytidine containing column and eluting, as described in Materials and Methods. Three major fractions contained enzyme activity (Figure 3).

Previously, two major fractions were found when bone fide AID was analyzed on a similar column [16]. AID activity of these 2L cells was increased (Figure 4). AID from 3/8 as many cells assayed in the experiment of Figure 3 yielded 2.5 fold more AID in the experiment of Figure 4, comparing the areas under the elution curves of Figures 3 and 4. Therefore, an overall six fold increase in AID activity was achieved. Untreated lymphoma cells contained little detectable AID activity (Figure 4).

Western blot analyses in Figure 5 showed that AID in 2L cells was 5.4 fold more abundant than in 0L cells, as expected from previous results shown in Figures 3 and 4.

Western blot determination of AID from 0L and 2L cells (Figure 5) showed that from 2.4 × 10^6 0L cells 0.025 ± 0.011 micrograms were found; from 2.4 × 10^6 2L cells 0.13 ± 0.025 µg were found, indicating a 5.4 fold increase in AID in the lucanthone treated cells. A confirming experiment (Figure 6) showed that normalizing for actin content in 0L and 2L lysates indicated 7.7 fold increases in AID.
Actin content of 0L and 2L cells was much greater than in Ramos cells. Ramos cells’ AID content was four fold greater than the AID content of 2L cells, but elevated IgG was not found in Ramos cell survivors of lucanthone treatment and little or none.

IgG was found in untreated Ramos cells (not shown). Despite the AID activity in Ramos cells, IgG enhancement was not observed. So far, we have not been able to induce (or select) for cells with enhanced IgG content in Ramos 6.4 human Burkitt lymphoma cells or in a transplantable mouse lymphoma MOPC 460d (a kind gift of Dr. Michael Potter, National Cancer Institute, USA).

Resistance of lucanthone survivors to retreatment, determined by clonogenic assays in polyornithine coated plastic dishes

Normally CRL-1647 lymphoma cells grow in suspension and do not attach to plastic surfaces. To evaluate their clonogenic fraction, plastic dishes were first coated with polyornithine [17]. Macroscopic colonies were counted in single-cell assays after 12 days of incubation. Parallel studies of growth in uncoated 96 well plastic plates, after terminal dilution confirmed the clonogenic fractions were the same. The clonogenic fraction of 0L cells was 0.097 ± 0.03 SE. After recovery, the clonogenic fraction of log phase survivors of one or two lucanthone exposures [20 h in 8 µM lucanthone], were 0.18 and 0.11, respectively. By contrast, when these cell cultures were challenged by 8 µM lucanthone for 20 h and immediately tested, the clonogenic fraction of naive cells was 1.5 × 10^{-4} ± 0.7 × 10^{-4}, but for the survivors of the one or two serial exposures to lucanthone the clonogenic fractions were 1.3 × 10^{-3} and 2.9 × 10^{-3}. Lucanthone survivors were therefore tenfold more resistant to the standard challenge. They also exhibited enhanced AID content (Figures 5 and 6). These findings are compatible with selection of lucanthone resistant cells with enhanced IgG content and AID content.
Despite much effort, we failed to detect IgG on or in the cell membranes of 0L cells and 2L cells by immuno-flow cytometry. Nevertheless, we found that both cell types continuously released IgG and IgM immunorelated 80 and 25 kDa species into their cell culture fluids (Figures 7 and 8). Secreted Ig related proteins were characterized by LC-ESI MS/MS.

Quantitation of the ~80 kDa and ~25 kDa bands from 0L and 2L supernatants

0L and 2L cells in the log phase growth were re-suspended in medium RPMI 1640 without serum at 2.4 × 10^6/ml. After incubation at 37°C in 5% CO2 for 6 h, 200 ml of cell free supernatant were concentrated 2,000-fold by membrane filtration (10 kDa MWCO). Two aliquots of 15 μl each of the concentrated 0L (293 μg) and 2L (296 μg) were subjected to 1D-SDS-PAGE. One replicate gel was used for Western blot analysis while the second replicate gel was stained with Coomassie and used for mass spectrometry analysis. Western blot analysis showed 80 and 25 kDa protein bands were immunoreactive with goat anti-human IgG/IgM antibody conjugated to horseradish peroxidase. Protein identification of the 80 and 25 kDa gel bands was performed by in-gel trypsin digestion and peptide digests were analyzed by LC-ESI MS/MS. Western blot 80 kDa Ig from the 2L cells were scanned; 9.0 density units were determined after 30 minutes exposure. With 0L cells 17.3 density units were found. So far, absolute amounts of Ig gel proteins cannot be determined because structures and specific immunoreactivity of the 80 and 25 kDa proteins have not yet been determined. Nevertheless, results here are in agreement with previous results from mass spectrometry noted for the Ig mu chain C region. A 30 second exposure of the Western blot of Figure 8A was made to reduce the intensity of the 25 kDa immunoproteins. For the 25 kDa proteins from 2L cells 25.2 density units were found; for 0L cells 70.9 density units were found. Therefore, the 25 kDa immunoproteins from 0L cells were nearly three times as abundant secreted immunoproteins. Nevertheless, 2L Ig kappa chains secreted were 1.4 times more abundant than 0L kappa chains, previously noted.

Obtained based on the 30 s exposure. 0L is 3 × higher compared to 2L for the ~25 kDa kappa light chains.

Protein identification by LC-ESI-MS/MS

Based on 99% protein and 95% peptide probabilities and a minimum of 2 unique peptides, a total 122 proteins were identified from both the 80 and 25 kDa bands. Three immunorelated proteins were identified for both 0L and 2L cells. The Ig mu chain C region was identified from the 80 kDa gel band while both the Ig kappa chain C region and Ig kappa chain V-II region were identified for the 25 kDa gel band. Label free quantitation based on normalized emPAI values (exponentially modified Protein Abundance Index) was used to estimate the ratios of immunorelated proteins identified for 0L and 2L cells. The Ig mu chain C region from 0L cells was 0.69 while Ig mu chain C region from 2L cells was 0.31. Hence, 0L was 2 × higher compared to 2L cells for the Ig mu chain C region. The normalized emPAI values for both Ig kappa chain C and V-II for the 0L cells was 0.84 while Ig kappa chain C and V-II for the 2L cells was 1.16. Hence, the Ig kappa chains for 2L cells were 1.4 × higher than 0L cells.

Discussion and Conclusion

Here we describe four novel results on the effects of lucanthone on
human Burkitt lymphoma cells. Surviving cultures after two consecutive 20 h treatments with 8 μM lucanthone (2L cells) exhibited enhanced cell content of IgG relative to IgM content. The 2L survivors contained enhanced levels of AID. They were much more resistant to challenge by further lucanthone treatments, as determined by clonogenic assays. Mass spectrometry determinations on secreted IgG suggest reduced IgM related proteins and increased IgG related proteins, consistent with these results.

These results show that CRL-1647 Burkitt lymphoma cells which survived the exposure to 8 μM lucanthone contained relatively more IgG than unexposed cells. Moreover, this was a stable feature, expressed in progeny of a single cell which had been cultured over many generations.

Was the enhancement because of selection and proliferation of a pre-existing minor population or induction of a new character in the survivors?

Lucanthone survivors expressed deoxycytidine deaminase (Figures 5 and 6). This enzyme is an essential feature of CSR; its presence in the lucanthone survivors is consistent with their enhanced elaboration of IgG. The relatively small amount of IgG contained in CRL-1647 cells prior to lucanthone exposure (0L cells) suggests small amounts of IgG activity. If AID provided survival value to some cells, then this population would be expanded, possibly accounting for the results described here.

Studies on the elaboration of IgG messenger RNA and on the schedule of DNA rearrangements in lucanthone treated cells are of interest but are beyond the scope of this initial report and would involve 4 different mRNAs. Recent studies have implicated hnrRNPs as cofactors for AID [18]. Their role, if any, in lucanthone's effects need further consideration. Finally, the roles of selection or induction of IgG enhancement warrants further study, perhaps by fluctuation tests.

Overall, results here demonstrated that lucanthone treatments of Burkitt's lymphoma cells (0L) produced progeny cells (2L) which contained 5 fold more IgG and AID than the parent cell line. IgM was still the predominant Ig species. So far, we have not found enhanced release of IgG related 80 kDa species from 2L cells (Figure 8). Both 0L cells and 2L cells continuously release this material over many hours.

We cannot yet account for the large differences in the content of 80 kDa Ig's in 2000 fold concentrated supernatants from 0L and 2L cell cultures (Figure 7), in contrast to little or no difference in 80 kDa Ig's from freshly harvested un-concentrated 0L and 2L cell supernatants (Figure 8). The isolation from gels and the concentration process, which were needed for mass spectrometry analyses, might be responsible for differences in amounts of 80 kDa and 25 kDa Ig's after preparation for mass spectrometry. We have found a small increase in IgG related 26 kDa Ig from the 2L cells, but this must be confirmed and is not yet certain. Secretion of IgG has been noted in CHO cells and human leukemic cells [19-21].

The full identity of the secreted IgG species remains to be established. Do they attach specifically to antigens? What might be the effect of these secreted immunospecies on the immune response of Burkitt lymphoma patients if their lymphoma cells also secrete them?

Overall, two mechanisms which might account for the increased IgG in these Burkitt's lymphoma cells after treatment with lucanthone. Increased cell content of AID, by a mechanism presently unknown, might have stimulated class switching. But interference with orientation specific joining [22,23] by inhibition of topoisomerase II [6] should also be considered. Perhaps one orientation was favored by inhibition of DNA strand orientation [22,23].

Recently Kanu, et al. found increased APOBEC 3 cytidine deaminases (related to AID) in cancer cells after treatment with several chemotherapeutic agents [24], in general agreement with results of increased AID cytidine deaminase described in this report.

Grant Support

This work was supported by the Rome Sisters Foundation for Cancer Research, by Grants to R. Bases.

Author Contributions

Conception and design: R. Bases, Rukmani Lekhraj; IgG and IgM and AID assays: R. Bases; Isotype determinations: Xudong Tang; Flow cytomtery measurements of cell surface Ig: Jingheng Zhang; AID measurements: Zhi Duan; Mass Spectrometry: Jennifer Aguilan, Edward Nieves

References

1. Ramiro R, San-Martin BR, McBride K, Jankovic M, Barreto V, et al. (2007) The role of activation-induced deaminase in antibody diversification and chromosome translocations. Adv Immunol 94: 75-107.
2. Muramatsu M, Kinoshita K, Fagarasan S, Yamada I, Shinkai Y, et al. (2000) Cell switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102: 553-563.
3. Revy P, Muto T, Levy V, Geissmann F, Piebani A, et al. (2000) Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). Cell 102: 565-575.
4. Fernandez D, Ortiz M, Rodriguez L, Garcia A, Martinez D, et al. (2013) The proto-oxitogene c-myc regulates antibody secretion and Ig class switch recombination. J Immunol 190: 6135-6144.
5. Brar S, Sacho E, Tessmer I, Croceu E, Erle D, et al. (2008) Activation-Induced deaminase, AID, is catalytically active as a monomer on single stranded DNA. DNA Repair 7: 77-87.
6. Bases RE, Mendez F (1997) Topoisomerase inhibition by lucanthone, an adjuvant in radiation therapy. Int J Radiat Oncol Biol Phys 37: 1133-1137.
7. Mendez F, Goldman J, Bases R (2002) Abscis sites in DNA of HuLa cells induced by lucanthone. Cancer Invest 20: 983-991.
8. Bases R, Mendez F, Elequin F, Liebeskind D, Kozin A, et al. (1978) Early steps in mutagenesis by hycanthone. Cell Stress and DNA Repair 7: 77-87.
9. Sabouri S, Kobayashi M, Begum NA, Xu J, Hirota K, et al. (2014) C-terminal region of activation-induced cytidine deaminase (AID) is required for efficient class switch recombination and gene conversion. Proc Natl Acad Sci USA 111: 2253-2258.
10. Shivarov V, Shinkura R, Hoonjo T (2008) Dissociation of in vitro DNA deamination activity and physiological functions of AID mutants. Proc Natl Acad Sci USA 105: 15866-15871.
11. Bases R (1970) Enhancement of x-ray damage in HeLa cells by exposure to lucanthone (Mireclol D) following radiation. Cancer Res 30: 2007-2011.
12. Blair DM (1958) Lucanthone hydrochloride; a review. Bull World Health Organ 18: 989-1010.
13. Del Rowe JD, Bello J, Mitnick R, Sood B, Filipp C, et al. (1999) Accelerated regression of brain metastases in patients receiving whole brain radiation and the topoisomerase II inhibitor, lucanthone. Int J Radiat Oncol Biol Phys 43: 89-93.
14. Bases R (2006) Heat shock protein 70 enhanced deoxyribonucleic acid base excision repair in human leukemic cells after ionizing radiation. Cell Stress Chaperoners 11: 240-249.
15. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254.
16. Bases R (2011) Heat shock protein 70 (Hsp70)-stimulated deoxycytidine deaminase from a human lymphoma cell but not the activation-induced cytidine deaminase (AID) from Ramos 6.4 human Burkitt's lymphoma cells. Cell Stress Chaperoners 16: 91-96.
17. Letourneau PC (1975) Possible Roles for cell-to-substratum adhesion in neural morphogenesis. Dev Biol 44: 71-91.
18. Hu W, Begum NA, Mondal S, Stanile A, Honjo T. (2015) Identification of DNA cleavage- and recombination-specific hnRNP cofactors for activation-induced cytidine deaminase. Proc Natl Acad Sci U S A 112: 5791-5796.
19. Tait AS, Hogwood CE, Smales CM, Bracewell DG (2012) Host cell protein dynamics in the supernatant of amAb producing cell line. Biotechnol Bioeng 109: 971-982.
20. Premkumar E, Singer PA, Williamson AR (1975) A human lymphoid cell line secreting immunoglobulin G and retaining immunoglobulin M in the plasma membrane. Cell 5: 87-92.
21. Nilsson K, Ghetie V, Sjöquist J (1975) A human lymphoid cell line with an IgG-like membrane component. Eur J Immunol 5: 518-526.
22. Dong J, Panchakshari RA, Zhang T, Zhang Y, Hu J, et al. (2015) Orientation-Specific joining of AID initiated DNA breaks promotes antibody class switching. Nature 525: 134-139.
23. Di Noia JM (2015). Molecular biology: Unequal opportunity during class switching. Nature 525: 44-45.
24. Kanu N, Cerone MA, Goh G, Zalmas, LP, Dietzen M, et al. (2016) DNA replication stress mediates APOBEC 3 family mutagenesis in breast cancer. Genome Biol 17: 185-200.