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Ionizing Radiation and Lucanthone Enhance the IgG Content of Burkitt's Lymphoma Cells

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Abstract

Ionizing radiation or lucanthone treatment of CRL-1647, Burkitt's lymphoma cells, increased their content of IgG three fold. Radiation induced increases in IgG cell content relative to IgM persisted for several cell generations. IgM remained the predominant immunoglobulin after either treatment. Elevated AID, activation induced cytidine deaminase, was not found after 5 Gy.

However, 8 μ M lucanthone for 48 h increased cellular AID cell content three fold and, as previous results showed, progeny of lucanthone survivors exhibited increased levels of AID many generations later.

Keywords: Lucanthone; Ionizing radiation; Class switch recombination (CSR); Activation induced deoxycytidine deaminase (AID)

Introduction

Lucanthone, a thioxanthone, once widely used to treat schistosomiasis, induced 3.6 fold increases in immune globulin G (IgG) relative to immune globulin M (IgM) in CRL-1647 Burkitt's lymphoma cells [1]. This increase persisted for many generations and was accompanied by 7 fold increases in cellular activation induced cytidine deaminase (AID) [1]. AID is a key factor in Ig class switching from IgM to IgG.

Here we examined the effects of ionizing radiation on the ratio of IgG/(IgG+IgM) (Figure 1) and the content of AID in CRL-1647 cells. Lucanthone increased both by 48 h (Figures 1 and 2).

The novel results with ionizing radiation described later raise general questions about the mechanisms responsible for changes in IgG/(IgG+IgM). AID seems not alone in altering it. While class switch recombination by AID is being very actively pursued, other mechanisms, induced by clinically available tools, deserve attention.

Materials and Methods

Cells

CRL-1647 Burkitt human lymphoma cells 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 hours. Media and sera were from ATCC.

Cell lysates

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

Western blots

For most experiments, 7 cm minigels, 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

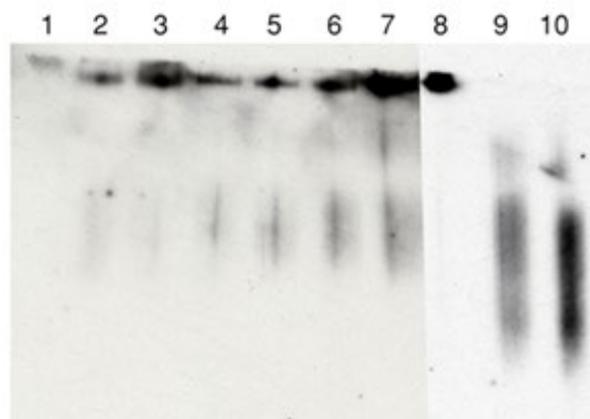


Figure 1: Radiation enhanced ratio of IgG/(IgG+IgM) in Burkitt's lymphoma cells CRL-1647. Lane 1: No cell lysate. Lanes 2 and 3: Lysates from 5.3 X 10⁵ untreated cells; ratios 0.13, 0.10. Lanes 4 and 5: Lysates from 7.5 X 10⁵ cells 22 h after 10 Gy; ratios 0.35, 0.38. Lanes 6 and 7: Lysates from 6.0 X 10⁵ cells growing 4 days after 5 Gy, ratios 0.38, 0.38. Lane 8: IgM 0.46 μ g. Lane 9: IgG 10-3 μ g. Lane 10: IgG 10-2 μ g. Lanes 1-7: 20 min exposure. Lanes 8, 9, 10: 1 min exposure. Lysates from 5.3 X 10⁵ untreated CRL-1647 cells released 10-5 μ g of IgG and 0.12 μ g of IgM into the gel. The ratios IgG/(IgM+IgG) obtained from untreated cells compared with those of radiation survivors were different at $p \leq 0.00006$.

used for gel electrophoresis and Western blot transfer.

Detection of IgM and IgG in cell lysates was made in Western blots,

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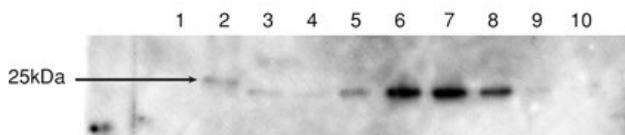


Figure 2A: Lucanthon treated CRL-1647 Burkitt's lymphoma cells contain more AID than untreated cells or the survivors of 5 Gy. Lane 1: No cell lysates. Lane 2: AID 0.25 µg 25 kDa. Lanes 3, 4, 5: Lysates from 1.4×10^6 untreated cells AID 0.26 µg. Lanes 6, 7, 8: Lysates from 3.3×10^5 cells 48 h after 8 µM lucanthon AID 0.98 µg. Lanes 9, 10: Lysates from 1.5×10^6 cells 35 generations after 5 Gy AID 0.14 µg. Normalization of AID values by reference to cellular actin content (Figure 2B) indicated that the elevated AID obtained after lucanthon treatment were significant at $p \leq 0.024$. Radiation treatment did not increase cellular AID. Lane 2: 0.25 µg of standard AID: 392 density units (arrow). Lanes 3, 4, 5: 251, 306, 628 density units for lysates from untreated cells. Lanes 6, 7, 8: 1,982, 1,533, 969 density units for lysates from lucanthon treated cells. Lanes 9, 10: 338, 99 density units for lysates from irradiated cells.

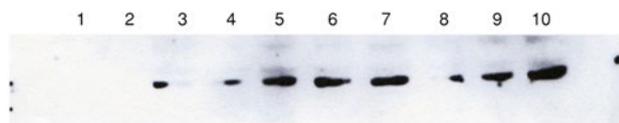


Figure 2B: Actin content of cell lysate fractions of Figure 2A. Antibodies were stripped from the nitrocellulose membrane of Figure 2A and it was restained with mouse anti actin, followed by horseradish peroxidase goat anti-mouse antibodies. 42 kDa bands are shown. Lane numbers correspond to lanes in Figure 2A. Lanes 3, 4, 5: 17,828; 23,664; 67, 739 density units, untreated cells. Lanes 6, 7, 8: 60,527; 62,402; 22, 814 density units, lucanthon treated cells. Lanes 9, 10: 55,084, 89216 density units, irradiated cells.

using goat anti human IgG precoupled to horseradish peroxidase (SC 2453) (Santa Cruz Biotechnology, Inc, Santa Cruz California, 95060).

This antibody reacts with IgM and more strongly with IgG. An advantage is that both IgG and IgM can be determined in the same cell lysate aliquot in the same gel lane. Quantitation is made by reference to immunodensities of reference standards in the gel. To characterize the relative abundance of IgG and IgM in an aliquot, relative immunodensities were determined: $IgG/(IgG+IgM)$. For untreated Burkitt lymphoma cells the ratio was 0.20 ± 0.05 SE. After transfer of immunoglobulins to nitrocellulose membranes and blocking with 5% milk proteins, membranes were stained with antibody, rinsed, and immunofluorescence was induced with ECL reagent RPN2209 (GE Health Care, Amersham, Buckinghamshire, HP7 9NA, UK). After exposure to x-ray film, gel band IgG and IgM were scored by densitometry. Complete transfer of proteins from gels to nitrocellulose membranes was verified by loading one lane of each gel with a mix of multicolored purified proteins encompassing the sizes of IgM and IgG (Kaleidoscope-BioRad). After transfer, bands were found in the membranes, none in the gels.

Actin was determined in cell lysates by electrophoresis in tris glycine minigels and transfer to nitrocellulose membranes. The actin bands were stained first with mouse anti actin (Cal Biochem mAb, JLA 20 CAT #CP01) at 1:5000 dilution for 2 hours followed by goat anti mouse IgM precoupled to horse radish peroxidase (JA 1200) at 1:2000 dilution for 2 hours. Immunofluorescence then was induced by the ECL reagent and bands detected by exposure to X-ray film were scored by densitometry. Cell lysates had been prepared without mercaptoethanol, to prevent interference with IgM. Because of this, actin aggregates

were encountered in the gel lanes. Their sizes were from $\sim 10^5$ MW to $\sim 10^6$ MW. Therefore, the densities of all the actin reactive species in each lane were determined together.

- Human IgG 1 purified Ultraleaf Isotype CTRL was purchased from Biologend, 9727 Pacific Heights Blvd, San Diego, CA 92121.
- Human IgM was purchased from Fisher ThermoFisher.com.
- Horseradish peroxidase linked donkey anti human IgG was from Biologend.
- AID was purchased from Enzymax, Lexington, KY 40503.
- Anti AID: Rat monoclonal antibody was purchased from Cell Signaling (AIDEK2569 - Item 4959S) 3 Trask Lane, Danvers, MA 01923.
- Goat anti rat IgG coupled to horseradish peroxidase was from Cell Signaling (Item 7077S).
- Irradiations of cells in growth medium were made in plastic flasks at 4 Gy/minute, D_{max} 1.5 cm, by a Varian linear accelerator.

Results and Discussion

Figure 1 shows increased IgG to IgM cell content ratios 22 h after 10 Gy and 4 days after 5 Gy. However, increased cellular AID was not found after radiation (Figures 2A). By contrast, increased cellular AID was significantly elevated 48 h after 8 µM lucanthon (Figures 2A and 2B) and the $IgG/(IgG+IgM)$ ratios were also elevated (Figure 1).

These results suggest two or more enzyme activities which elevate $IgG/(IgM+IgG)$. Lucanthon increased cellular AID content as well. Ionizing radiation induced the IgG increase with little or no participation by AID, suggesting a role for other enzyme activities. AID due to lucanthon may be supplementary to the radiation induced activity. Radiation might be inducing a more primitive APOBEC cytidine deaminase [2].

Lucanthon's action in inducing abasic sites in cell DNA [3] and DNA strand breaks [4] likely caused a different spectrum of strand break species than encountered after radiation. DNA nicks separated by 250 nucleotides on opposite strands can strongly mediate class switch recombination by AID [5]. Double strand break response factors influence end joining features of IgH class switch [6].

DNA strand species determinants of the AID response by lucanthon and APOBEC cytidine deaminases after radiation are as yet unknown.

Lucanthon was formerly used to safely treat schistosomiasis in hundreds of thousands of patients [7]. More recently, we found that lucanthon was a clinically useful adjuvant to radiation therapy in the treatment of brain metastases [8]. Patient serum levels of 8 µM, the concentration used in the present study, were maintained for several weeks without incident, when care was taken to avoid interference from other medications.

Roles for lucanthon and radiation in clinical macroglobulinemias are worth considering. Such treatment might restore enhanced levels of IgG, consistent with *in vitro* results described here.

Author Contributions

- Conception and design: Bases R.
- AID, IgG and IgM assays: Bases R and Lekhraj R.

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