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 µM 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 thiaxanthenone, 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 37ºC 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 Ca²⁺ or Mg²⁺, resuspended in lysis buffer with 10 µM 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 µg/µl 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 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 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 immunodensities were determined in the gel lanes. Their sizes were from ~10^5 MW to ~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 Biolegend, 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 Biolegend.
- 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, Dmax 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 lucanthone (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/(IgG+IgM). Lucanthone 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 lucanthone may be supplementary to the radiation induced activity. Radiation might be inducing a more primitive APOBEC cytidine deaminase [2].

Lucanthone's action in inducing abasic cites 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 lucanthone and APOBEC cytidine deaminases after radiation are as yet unknown.

Lucanthone was formerly used to safely treat schistosomiasis in hundreds of thousands of patients [7]. More recently, we found that lucanthone 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.

**Author Contributions**

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


