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# Effects of gamma rays on the immunogenicity (IgG types) of ovalbumin

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**Abstract**

Ionizing radiation has been successfully employed to modify the immunological properties of biomolecules. Very promising results were obtained when crude animal venoms, as well as isolated toxins, were treated with gamma rays, yielding toxoids with good immunogenicity. However, little is known about the modifications that irradiated molecules undergo and even less about the immunological response that such antigens elicit. In the present work, we used ovalbumin as a model to investigate possible immunogenic differences between native and irradiated proteins.

Native ovalbumin (2 mg/ml in 150 mM NaCl) was irradiated with 2 kGy of $^{60}$Co gamma rays with a 570 Gy/h dose rate. B10.PL mice ($n = 5$) were then immunized with either the native or the irradiated protein. After three immunizations, serum samples were collected and the antibody titers and isotypes were determined by enzyme-linked immunoadsorbant assay. Our data indicate that no difference could be noticed when the antibody titers of the two groups were compared. However, the isotyping assays indicates that the native protein induced high levels of IgG1, while its irradiated counterpart displayed mostly IgG2b antibodies. These data suggest that after irradiation, an antigen known to induce a Th2 response, is able to switch the immune system towards a Th1 pattern.
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*Keywords:* Ionizing radiation; Ovalbumin; Immunological; Isotypes

## 1. Introduction

Ionizing radiation consists of electromagnetic waves resulting from nuclear transitions. It can interact with biomolecules in two ways: directly, when the radiation hits the molecule, or indirectly when free radicals are generated and these react with the target molecule. With proteins, radiation promotes changes in their enzymatic, pharmacological and immunological properties; the two latter being more radioresistent (Grosh and Hoopywood, 1979; Butler et al., 1987; Garrison, 1987). Radiation has been successfully employed to modify biomolecules, reducing or abolishing their biological activity without affecting their immunogenic properties (Nascimento et al., 1996). This methodology could be used to produce toxoids and vaccines. However, in order to develop such methodology, a good comprehension on the immunological behavior of irradiated antigens would be necessary. In the present work, we used ovalbumin as a model to further characterize the immune response against irradiated proteins.

Ovalbumin is a thermally and chemically stable protein and is considered a classical allergen (Kume and Matsuda, 1995). These same authors showed that, following irradiation, the protein showed discrete conformational changes, reducing its ability to react with antibodies raised against the native form of ovalbumin. Our goal was to further characterize ovalbumin from the immunological point of view.

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## 2. Materials and methods

### 2.1. Reagents

All reagents were commercially obtained and were of analytical grade.

### 2.2. Animals

B10.PL isogenic mice were obtained from the animal housing facility of IPEN/CNEN/SP and maintained in sterilized isolators and absorbent media, with food and water ad libitum. The manipulation of these animals before or during the experiments were according to the ''Principles of Laboratory Animal Care'' (NIH publ. No 86-23, revised em 1985) and to the ''Principles of Ethics in Animal Experimentation'' (COBEA—Colégio Brasileiro de Experimentação Animal).

### 2.3. Proteins irradiation

Ovalbumin was dissolved in 0.15 M NaCl to a final concentration of 2 mg/ml. This solution was irradiated with a 2000 Gy dose using gamma rays derived from a $^{60}$Co source (GammaCell, Atomic Agency of Canada Ltd) at room temperature and in the presence of atmospheric $O_2$, with a 5170 Gy/h dose rate.

### 2.4. Production of antibodies

Specific anti-native or anti-irradiated ovalbumin antibodies were obtained by immunizing B10.PL mice, with the protein in its native or irradiated form, following a classical immunization protocol (Harlow and Lane, 1988). Blood samples were collected and after centrifugation, the plasma was separated and frozen.

### 2.5. Enzyme linked immunosorbent assay (Elisa)

Ninety six well microplates were coated with native ovalbumin (1.0 μg/well/100 μl) overnight. The plates were then blocked with 5% skim milk in phosphate buffered saline (PBS). The plasma samples were then incubated for 1 h after a 1/20,000 or 1/40,000 dilution in PBS. Peroxydase-labelled antibodies specific against mouse IgG1, IgG2a or IgG2b were then allowed to react individually with the bound antibodies. Finally, the reaction was developed adding a chromogenic solution containing 0.5 mg/ml orto phenylene diamine in 50 mM citrate buffer pH 5 in the presence of 1 μl/ml hydrogen peroxyde. After 20 min incubation, the reaction was interrupted by the addition of 50 μl 2 M citric acid and the plates were analyzed on a microplate reader at 450 nm. As negative controls, plasma samples collected before immunization were used.

## 3. Results and discussion

Our results indicate that both forms of the protein induced detectable amounts of antibodies with the two dilutions used in our assay (Fig. 1). We could also observe that the plasma of the animals immunized with native ovalbumin had higher IgG1 titers, indicating the predominance of a Th2-type response. Brewer et al. (1994), observed this behavior in macrophage-depleted animals. These authors observed that after depletion, the animals presented an increased IgG1 level, which is under control of Th2 cells, a cell type involved in the humoral immune response, modulating the production of antibodies by B lymphocytes (Sprent and Surh, 2002).

Also, our data indicate that the irradiated protein induced higher titers of IgG2a and IgG2b (Fig. 1), suggesting that Th1 cells were predominantly involved in the immune response. This population is involved in the upregulation of cellular response, specifically macrophage activation (Delves and Roitt, 2000).

The differential activation of T cells (Th1 or Th2) could be explained by the fact that these subpopulations respond selectively to antigens presented by different antigen-presenting cells (APC): Th2 cells proliferate intensely when stimulated by antigens presented by B cells, while Th1 cells respond to antigens presented by macrophages (Chang, et al., 1990). It has been shown that the uptake of proteins by macrophages is enhanced when the protein is irradiated, and that this increase is associated with a scavenger receptor of these cells which is Involved in the removal of oxydized biomolecules (Cardi et al., 1998). Thus, irradiated ovalbumin would be preferentially presented by macrophages, explaining

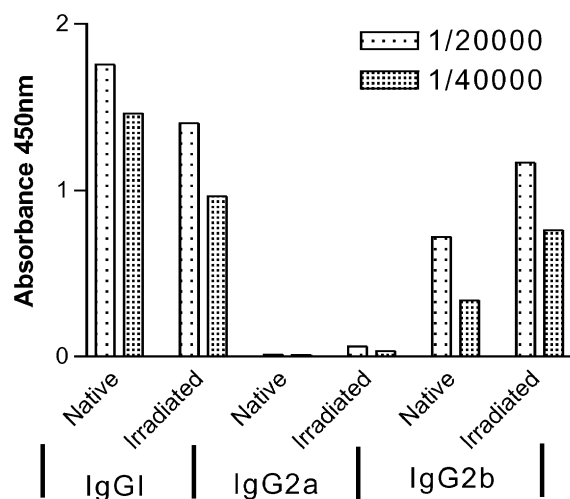


Fig. 1. Enzyme Linked Immunosorbent assay isotyping of the antibodies raised against native and irradiated ovalbumin samples.

the switch towards Th1 response in the animals immunized with the irradiated sample.

## 4. Conclusions

The preferential activation of Th1 and Th2 cells depends, amongst other things, on whether the antigen is in its native or irradiated form, the latter inducing a predominantly Th1-type response.

The native and irradiated forms of ovalbumin were able to stimulate the immune system and the resulting antibodies were able to react with the native form of the protein.

## References

Brewer, J.M., Richmond, J., Alexander, J., 1994. The demonstration of an essential role for macrophages in the in vivo generation of IgG2a antibodies. Clin. Exp. Immunol. 97, 164–171.

Butler, J., Hoey, B.M., Swallow, A.J., 1987. Radiation chemistry. Annu.Rep.Prog.Chem. 83, 129–175.

Cardi, B.A., Nascimento, N., Andrade Jr., H., 1998. Irradiation of *Crotalus durissus terrificus* crotoxin with $^{60}$Co gama rays induces its uptake by macrophages through scavenger receptors. Int. J. Radiat. Biol. 73 (5), 557–564.

Chang, T., Shea, C.M., Urioste, S., Thompson, R.C., Boom, W.R., Abbas, A.K., 1990. Heterogeneity of helper/inducer T lymphocytes-Responses of IL-2 and IL-4-producing (Th1 and Th2) clones to antigens presented by different accessory cells. J. Immunol. 145 (9), 2803–2808.

Delves, P.J., Roitt, I.M., 2000. The immune System—first of two parts. Adv. Immunol. 343, 37–49.

Garrison, W.M., 1987. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. Chem.Rev. 87, 381–398.

Grosh, D.S., Hoopywood, L.E., 1979. Biological Effects of Radiation 2nd ed.. Academic Press, New York.

Harlow, E., Lane, D., 1988. Antibodies. A Laboratory Manual. Lab, Cold Spring Harbor, NY.

Kume, T., Matsuda, T., 1995. Changes in structural and antigenic properties of proteins by radiation. Radiat. Phys. Chem. 46 (2), 225–231.

Nascimento, N., Seebart, C.S., Francis, B., Rogero, J.R., Kaiser, 1996. Influence of ionizing radiation on crotoxin: Biochemical and immunological aspects. Toxicon 34 (1), 123–131.

Sprent, J., Surh, C.D., 2002. T Cell Memory. Annu. Rev. Immunol. 20, 551–579.