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GAMMA IRRADIATION ALTERS BLUETONGUE VIRUS PROTEIN ANTIGEN

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Summary. - Bluetongue virus (BTV) antigen, prepared for a monoclonal antibody (MAb)-based competitive enzyme-linked immunosorbent assay (C-ELISA), was exposed to 1, 2, 3, 4, 5 and 6 Mrad of gamma irradiation. The major group-specific BTV protein (VP7) reactive with the Mab was altered at higher doses of radiation, as revealed by immunoblotting studies. As well, a reduction in immunoreactivity was noted when irradiated antigen was used in the ELISA.

Key words: bluetongue virus; gamma irradiation; BTV inactivation

A variety of chemical and physical treatments including gamma irradiation (GI) are used to inactivate viruses. Inactivated virus preparations are useful as antigens in serological tests because they allow the procedure to be performed without biocontainment. The inactivation of bluetongue virus (BTV), the type species of orbiviruses (Gorman *et al.*, 1983) by GI has been reported (Thomas *et al.*, 1982). Recent studies also suggest that irradiation might be an effective means for the preparation of BTV vaccine (Barber & Campbell, 1984; Campbell *et al.*, 1985). We have used GI to render BTV antigen free of active viruses for the development and application of a group specific monoclonal-based competitive enzyme-linked immunosorbent assay (C-ELISA) to detect antibodies in animal sera (Afshar *et al.*, 1989). In the course of our initial studies to determine the optimal level of GI for inactivating BTV, without compromising the reactivity of the antigen in C-ELISA, we noted a reverse GI dose/antigenic reactivity response (unpublished data). This report presents the results of the effect of GI dosage on BTV antigen, reactive with the monoclonal antibody (MAb) used in the C-ELISA, as revealed by Western immunoblotting analysis.

BTV antigen was prepared according to a method previously described (Anderson, 1984) from

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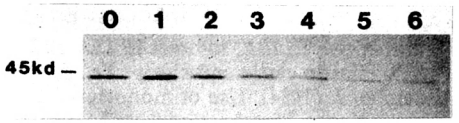

Fig. 1.
Effect of gamma irradation (0, 1, 2, 3, 4, 5 and 6 Mrad) on the major group-specific BTV protein (VP7) reacted with MAb in immunoblotting.

baby hamster kidney cells (BHK-21) infected with plaque-purified BTV type 11 (U.S.A.) and maintained in serum-free medium. Aliquots (0.5 ml) of BTV antigen were lyophilized and while on wet ice were subjected to 1, 2, 3, 4, 5 and 6 Mrad of GI from a ^{60} Co source (Thomas *et al.,* 1982). Following reconstitution with sterile, pyrogen-free, distilled water, samples (25 μl) from each GI treatment and non-irradiated BTV antigen (control, 0 Mrad) were mixed with an equal volume of 0.063 mol/l Tris-HCl buffer, pH 6.8 containing 20 % glycerol, 2 % sodium dodecyl sulfate (SDS) and 5 % 2-mercapto-ethanol. The samples were boiled for 5 min and the proteins were separated by a modified SDS-PAGE (Laemmli, 1970). The resolving gel was prepared by diluting a 30 % acrylamide, 0.8 % BIS stock solution to a final concentration of 7.5 % acrylamide with 0.375 mol/l Tris-HCl buffer, pH 8.8 and 0.1 % SDS. The stacking gel was composed of 5.0 % acrylamide, 0.13 % BIS, 0.1 % SDS in 0.125 mol/l Tris-HCl buffer, pH 6.8. The running buffer was a solution of 0.025 mol/l Tris-HCl pH 8.3, 0.192 mol/l glycine and 0.1 % SDS. Proteins were electrophoretically transferred to a nitrocellulose membrane (0.45 nm, Schleicher and Schuell) and immunoblotting was carried out according to the method described by Towbin *et al.* (1979). The membranes were incubated first with a 1:50 dilution of a group-specific murine MAb to BTV (Anderson, 1984) and then with a commercial horseradish peroxidase conjugated rabbit anti-mouse antibody. The reaction was detected with diaminobenzidine and hydrogen peroxide.

The major group specific BTV protein (VP7) (Gorman, 1985) being approximately 45,000 dalton (45kD) was the only polypeptide band detected by the MAb (Figure 1). The intensity of the reaction in the immunoblot was inversely related to the dosage of GI for BTV antigen. Similarly, we found that the reactivity of the antigen in ELISA decreased at least by eight-fold when irradiation dosage higher than 4 Mrad were used (unpublished data). At a lower dosage of irradiation (1 and 2 Mrad), while residual virus (3–4 log 10) was inactivated in the antigen preparation, no change in immunoreactivity of the group specific protein (VP7) was detected. The immunoblotting result clearly indicates that the reactive protein band is altered after exposure to higher dosage of GI (4–6 Mrad). We have noted a similar effect of GI on another ELISA antigen preparation (e.g. pseudorabies virus) and have successfully used chemicals (e.g. binary ethyleneimine) for rendering this diagnostic reagent free of viable virus without losing antigenic reactivity. Whether alteration of this BTV protein is the result of direct radiation or is due to a secondary effect and free radical formation (Johns & Cunningham, 1983) remains to be studied. Currently, the BTV antigen for ELISA is irradiated with 1 Mrad before being released for use in serological tests in low security laboratories. This level of irradiation is sufficient to inactivate >5 log 10 of BTV (Thomas *et al.,* 1981).

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