Comparison of Antibody Production Against Aflatoxin B₁ in Goats and Rabbits

P. K. GAUR, O. EL-NAKIB, AND F. S. CHU*

Food Research Institute, Department of Food Science, and Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Wisconsin 53706

Antibody production against aflatoxin B₁ was compared in three rabbits and one goat. Titers obtained were 20 times higher in the rabbits than in the goat. The goat antiserum appeared to have a higher degree of cross-reactivity for other aflatoxins and related metabolites than did the rabbit antiserum.

Aflatoxin B₁ is one of the most potent hepatocarcinogens produced by Aspergillus flavus and A. parasiticus (3). Since aflatoxins are low-molecular-weight secondary fungal metabolites, they are devoid of antigenicity. Studies in our and other laboratories (2, 6, 7) have recently produced specific antibody against aflatoxin B₁ in rabbits by immunizing the animals with aflatoxin-bovine serum albumin conjugate (afla B₁-BSA). Although the antibody so obtained is usable for radioimmunoassay (9), the antibody titers were relatively low when compared with a steroid hormone radioimmunoassay system (8). Efforts to improve antibody production against aflatoxin B₁ were made by immunization of rabbits with different types of afla B₁-BSA conjugates, by injection of different animal species, and by optimization of the immunizing schedule. In this communication, the production of antibody in a goat and an improved schedule for rabbit immunization are discussed.

Aflatoxin B₁-carboxymethylxime was conjugated to BSA as previously described (1, 2), yielding a preparation containing 5 mol of aflatoxin B₁ per mol of BSA. Three New Zealand White female rabbits were immunized by a modification of the method described by Chu and Ueno (2). For initial injections each rabbit received 2 ml of emulsion containing 1 volume of afla B₁-BSA (300 to 1,000 μg) in saline and 3 volumes of Freund complete adjuvant (Difco). Subsequent booster injections in thigh muscles were carried out using 1 to 2 ml of an emulsion prepared by mixing 1 volume of the antigen (250 to 400 μg) with 2 volumes of incomplete adjuvant. The rabbits were bled via the marginal ear vein at weekly intervals. A juvenile female goat (25 lb [ca. 11.3 kg]) was immunized with afla B₁-BSA at the Green Hecter Farm (Oregon, Wis.) and was fed with hay and corn supplements during the experiment. Initial and subsequent booster injections in the goat were carried out as described above for rabbits, using 2 to 4 mg of afla B₁-BSA in 4-ml emulsions.

The immunoglobulin G fraction from the blood of both goat and rabbits was purified by the ammonium sulfate precipitation method of Hebert et al. (5). Antibody titers were determined by a binding assay (4). Titer was defined as the reciprocal of the dilution required for 50% binding of 10,000 cpm of [¹³C]aflatoxin B₁ with a specific activity of 13 Ci/mmol (Moravek Biochemicals, City of Industry, Calif.). The specificity of antibody against aflatoxin B₁ was determined by a competitive binding assay (2).

The antibody titers determined over a period of 96 weeks among the three rabbits are shown in Fig. 1. In the earlier immunization schedule (1 to 28 weeks), rabbits received 300 to 1,000 μg of the antigen in 2 ml for the initial injection, followed by 250 μg of the antigen in 1 ml for booster injections. The highest titers obtained during this period from these rabbits, which received 300, 600, and 1,000 μg of the antigen, were 1,000, 952, and 714, respectively. Two booster injections were made between 28 and 78 weeks, but the antibody production did not show any improvement. However, injection with 400 μg of the antigen in 2 ml of emulsion at weeks 78, 83, 88, and 93 caused a large increase in antibody titer. A titer of 4,762 was obtained at week 96 for the rabbit that was initially immunized with 300 μg of the antigen. These results suggest that continuous, frequent (monthly) booster injections with 400 μg of the antigen in 2 ml of emulsion improved antibody production against aflatoxin B₁ and that the larger amount of the antigen (1,000 μg) did not stimulate production.

Results of antibody production in the goat are shown in Fig. 2. Although a large amount of antiserum could be obtained from the goat in each bleeding, the antibody titers were considerably lower in the goat than those obtained in rabbits. Table 1 summarizes the results of specificity studies of goat and rabbit antiserum against aflatoxin B₁ and related metabolites. The goat antiserum had a higher degree of cross-
reactivity with different aflatoxin analogs than did rabbit antiserum, especially with respect to aflatoxin M₁. In general, the rabbit antiserum was superior to goat antiserum both quantitatively and qualitatively. The results indicated that the modified immunization schedule in rabbits presented here could be used routinely for antibody production against aflatoxin B₁.

This work was supported by the College of Agricultural and Life Sciences (project NC-129), the University of Wisconsin-Madison, and by Public Health Service research grant CA-15064 from the National Cancer Institute.

We thank Daniel and Mary Butz for their assistance in immunizing the goat, Pat Way for her assistance in immunizing the rabbits, and Bruce Steinert for his technical assistance.

**TABLE 1. Activity of goat and rabbit antiseras against aflatoxin B₁**

<table>
<thead>
<tr>
<th>Antibody source</th>
<th>Goat*</th>
<th>Rabbit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin analogs</td>
<td>Amt (ng) for 50% inhibition of binding</td>
<td>Ratio relative to aflatoxin B₁</td>
</tr>
<tr>
<td>B₁</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B₂</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>G₁</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>G₂</td>
<td>220</td>
<td>55</td>
</tr>
<tr>
<td>M₁</td>
<td>500</td>
<td>125</td>
</tr>
<tr>
<td>Aflatoxicol</td>
<td>45</td>
<td>11</td>
</tr>
</tbody>
</table>

* In the inhibition studies, immunoglobulin G, equivalent to 4 μl of the antiserum in 0.1 ml, was inoculated with 0.1 ml of ³H-labeled aflatoxin B₁ (10,000 cpm) and 0.1 ml of unlabeled aflatoxins at different concentrations at 6°C overnight. All the solutions were prepared in 0.1 M sodium phosphate buffer at pH 7.4.

* Data of Chu and Ueno (2).

**LITERATURE CITED**


