Mucosal Tolerance to Aflatoxin B$_1$

A. R. OLIVER, L. K. SILBART, D. F. KEREN, B. MILLER, AND R. A. MCDONALD

Center for Environmental Health
Department of Animal Science
University of Connecticut
CANR, Box U-39
Storrs, Connecticut 06269

Department of Pathology
University of Michigan
Ann Arbor, Michigan 48109

In an attempt to generate a secretory IgA (sIgA) immune response to the dietary carcinogen aflatoxin B$_1$ (AFB), we coimmunized New Zealand white rabbits through chronically isolated ileal loops [1] with AFB coupled to porcine thyroglobulin (TG) and cholera toxin (CT), a potent mucosal adjuvant [2]. This protocol resulted in a negligible sIgA anti-AFB response (Fig. 1), in contrast to prior studies with conjugates made with the carcinogen 2-acetylaminofluorene [3]. The sIgA anti-CT and anti-TG responses of animals immunized

![Figure 1](image)

**FIGURE 1.** sIgA anti-AFB response following mucosal immunization with AFB-TG + CT (circles) or AFB-TG (squares). Intestinal secretions (arithmetic mean, n = 5) were diluted 1:8 and assayed by amplified ELISA.

This work was supported in part by USPHS (NCI) Grant CA47132. This is scientific contribution #1623, Storrs Agricultural Experiment Station, University of Connecticut.
with AFB-TG plus CT were, however, comparable to those of control animals immunized with TG plus cholera toxin.

Parenteral immunization with AFB-TG emulsified in complete Freund's adjuvant generated a strong serum IgG anti-AFB response, demonstrating the immunogenicity of the conjugate preparation and the presence of appropriate systemic B- and T-cell repertoires to respond to AFB. Mucosal immunization followed by parenteral immunization generated a serum IgG anti-AFB response comparable to that of animals immunized parenterally without prior mucosal immunization. This demonstrates that the tolerance to AFB is specific to the mucosa and that it is not accompanied by systemic suppression (i.e., oral tolerance).

In order to test for the presence of AFB-reactive B-cell precursors in the mucosa, we stimulated lymphocytes from Peyer’s patches (PP), spleen (SP), mesenteric lymph nodes (MLN), and lamina propria (LP) of naive animals with lipopolysaccharide and pokeweed mitogen. The stimulated cells were assayed by enzyme-linked immunosorbent spot assay (ELISPOT) for the presence of anti-- and anti-TG antibody-secreting cells (ASC). As anticipated, ASC were found in SP but also in PP and MLN (TABLE 1), demonstrating that mucosal tolerance to AFB is not a result of B-cell deletion in the gut mucosa.

Taken together, our results demonstrate that rabbits exhibit mucosal but not systemic tolerance to AFB. This tolerance is specific to AFB, is not accompanied by systemic suppression, is not due to deletion of AFB-reactive B cells in the gut, and is not surmountable by CT. This is consistent with the observation that an established mucosal tolerance cannot be broken by coadministration with the heat-labile enterotoxin of *E. coli*, which is structurally and functionally similar to CT. This phenomenon may thus be a result of prior dietary exposure to aflatoxin B₁.

**REFERENCES**
