Mucosal Tolerance to Aflatoxin B_1[a]

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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.[3,4] The sIgA anti-CT and anti-TG responses of animals immunized

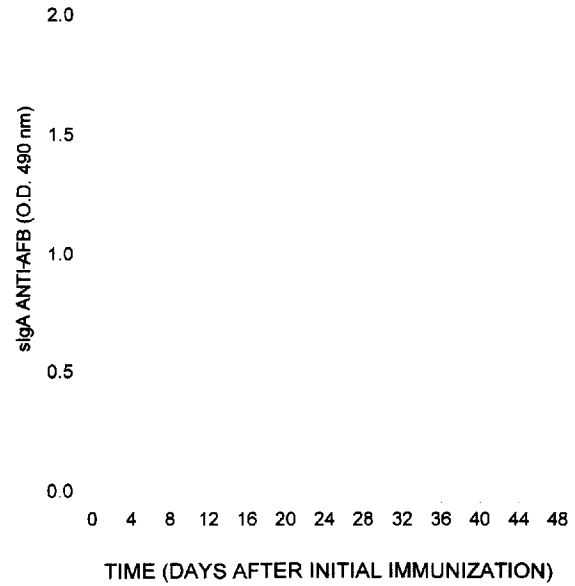

FIGURE 1. sIgA anti-AFB response following mucosal immunization with AFB-TG + CT (circles) or AFB-TG (squares). Intestinal secretions (arithmetic mean, n = 5) were diluted 1 : 8 and assayed by amplified ELISA.

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TABLE 1. Antibody-secreting cells to AFB and TG following Mitogen Stimulation[a]

	ASC Anti-AFB				ASC Anti-TG			
Animal	MLN	LP	PP	SP	MLN	LP	PP	SP
1	10	<1	10	4	3	<1	4	6
2	ND	<1	12	8	ND	<1	15	8

[a] Lymphocytes from mesenteric lymph nodes (MLN), lamina propria (LP), Peyer's patches (PP), and spleen (SP) of unimmunized rabbits were stimulated with lipopolysaccharide (50 μg/mL) and pokeweed mitogen (2.5 μg/mL), and assayed by ELISPOT for ASC to AFB and TG. Numbers represent ASC per 10^6 lymphocytes. ND = not done.

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 immunoadsorbent spot assay (ELISPOT) for the presence of anti-AFB 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*,[5] which is structurally and functionally similar to CT. This phenomenon may thus be a result of prior dietary exposure to aflatoxin B_1.

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