

EFFECT OF PRIOR INTRAGASTRIC ANTIGEN
ADMINISTRATION ON PRIMARY AND SECONDARY
ANTI-OVALBUMIN RESPONSES OF
C57BL/6 AND NZB MICE

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Deliberate exposure of animals to antigen via the gastrointestinal tract has long been an established means of producing a state of systemic hyporesponsiveness to the same antigen when subsequently presented in immunogenic form (1-7). Although tolerance induced by enteric antigen exposure has been extensively studied, little attention has been paid to the relationship of this phenomenon to autoimmunity. In particular, the responses of spontaneously autoimmune mice to enteric immunization have not been evaluated. Consequently, a simple method of studying tolerance in autoimmune mice has been overlooked. In this report, we demonstrate that, under appropriate conditions, NZB mice are resistant to the induction of systemic tolerance by prior enteric exposure to ovalbumin (OVA). This finding may provide a means of studying antigen-specific suppressor mechanisms in autoimmunity.

Materials and Methods

Mice. NZB/N mice were from colonies maintained at the NIH. C57BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. Only female mice were used; they were 8 wk old at the start of the experiment. All mice were fed mouse chow that was free of any chicken or egg products.

Gastric Intubation. OVA, five times recrystallized, purchased from Sigma Chemical Company, St. Louis, MO, was dissolved in saline at a concentration of 50 mg/ml. Antigen feeding was carried out under light ether anesthesia by the intragastric administration of 0.4 ml (containing 20 mg) of the OVA solution via 0.58-mm interior diameter polyethylene tubing. Control animals received 0.4 ml of saline.

Measurement of Anti-OVA Response. A modified Farr assay was used to measure antibodies to OVA. Serum was diluted in borate-buffered saline (BBS), and 50 μ l was incubated in 10 \times 75-mm borosilicate glass centrifuge tubes with 1 μ g of ³H-labeled OVA (0.1 μ Ci/ μ g) in 50 μ l of BBS containing 1% bovine serum albumin. The mixture was incubated for 30 min at 37°C and overnight at 4°C; 100 μ l of 85% saturated ammonium sulfate was then added to each sample. After a 1 h incubation in an ice water bath, the samples were centrifuged at 1,500 *g* for 20 min. One-half (100 μ l) of the total volume was removed, and the percent binding of OVA was

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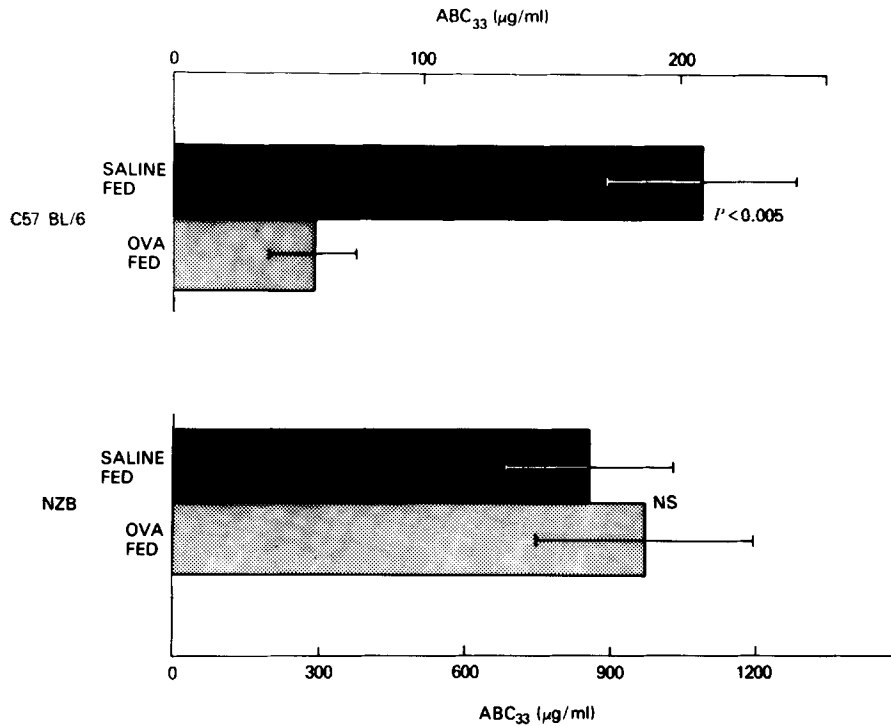


FIG. 1. Groups of eight C57BL/6 or NZB females (8 wk of age) were given either 0.2 ml of saline only or 0.2 ml of saline containing 20 mg of OVA on day 0. On day 7, animals were challenged with 125 µg of OVA in CFA; antigen-binding capacity (ABC₃₃) was determined on day 21 (14 d after challenge). NS, not significant.

determined as follows: percent binding = $([1 - \text{cpm sample}] / [\frac{1}{2} \text{ input cpm}]) \times 100$. Antigen-binding capacities (ABC₃₃) were calculated using a semi-log plot as described previously (8).

Immunizations and Bleeding Schedules. All mice were immunized with OVA 7 d after antigen feeding. For evaluation of primary responses, animals were challenged with a single intraperitoneal injection of 125 µg OVA in complete adjuvant (H37Ra; Difco Laboratories, Detroit, MI) and were bled by orbital sinus puncture 14 d after immunization. Secondary responses were evaluated by immunizing mice with an intraperitoneal injection of 0.2 ml of a mixture containing 25 µg/ml OVA and 25 mg/ml alum (OVA/alum). All animals were challenged 7 and 21 d after OVA feeding and were bled 7 d after the second challenge. The dose of OVA and the timing of serum collection was determined previously by experiments that evaluated both the dose-response relationship and the kinetics of the response (data not shown). OVA/alum-immunized mice were given a third intraperitoneal injection of 0.2 ml OVA/alum 60 d after the second challenge (81 d after OVA feeding); they were bled 7 d later. The specificity of tolerance was determined by immunizing OVA or saline-fed mice intraperitoneally with 5×10^8 sheep erythrocytes (SRBC) or intravenously with 10 µg of TNP₃₅-AECM-Ficoll, (Bio-search, San Rafael, CA). Serum anti-SRBC antibody titers were measured 7 d after immunization by hemagglutination; serum anti-TNP levels were determined 5 d after TNP-Ficoll challenge by a modified Farr assay, as previously described (9).

Results

OVA feeding produced hyporesponsiveness to OVA in C57BL/6 mice but did not diminish the primary NZB anti-OVA response (Fig. 1). A possible explanation of these findings is that NZB mice were primed by intragastric immunization. To test

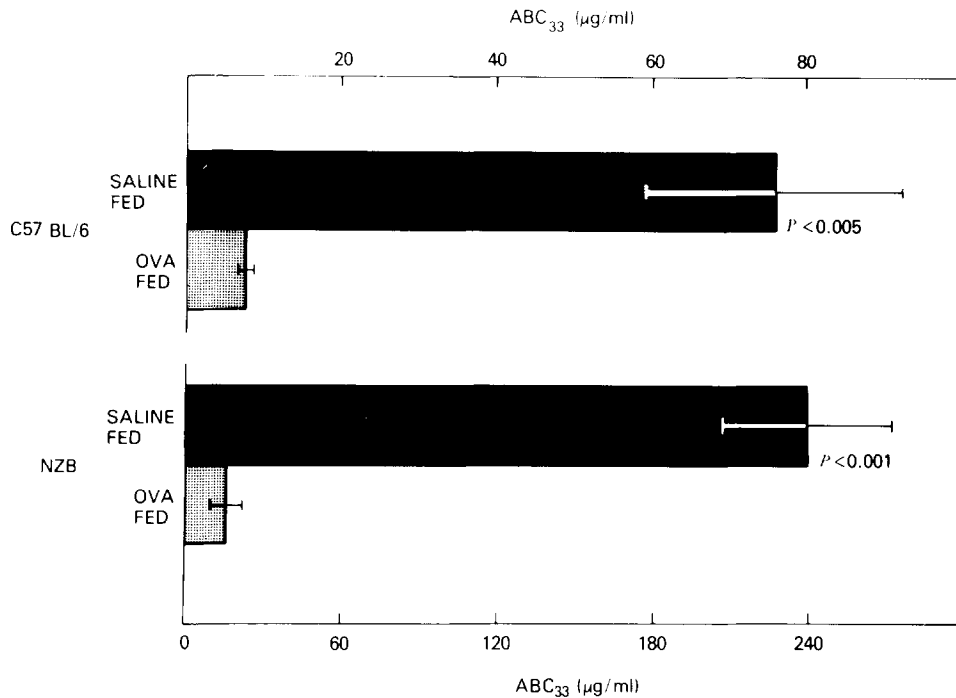


FIG. 2. Groups of eight C57BL/6 or NZB females (8 wk of age) were given either 0.2 ml of saline or 0.2 ml of saline containing 20 mg of OVA on day 0. On days 7 and 21, mice received an intraperitoneal injection of 5 µg of OVA/alum. Antigen-binding capacity (ABC₃₃) was determined on day 28 (7 d after boost).

this hypothesis, we evaluated secondary anti-OVA responses by twice challenging OVA-fed or control animals with 25 µg of OVA adsorbed to 5 mg of alum. This represented a milder form of challenge, as unmanipulated mice of either strain failed to show a significant serum anti-OVA response either 7 or 14 d after a single injection of alum-precipitated OVA; however, a vigorous anti-OVA response could be seen 7 d after a second intraperitoneal injection given 14 d after the first (data not shown). When we evaluated the effect of OVA feeding on this priming-dependent, secondary response, we found that both C57BL/6 and NZB mice were tolerant to a similar degree (Fig. 2); thus, we found no evidence of systemic priming in OVA-fed NZB mice.

Before concluding that NZB mice could be normally tolerized with respect to secondary responses, both C57BL/6 and NZB mice were rechallenged with OVA adsorbed to alum to assess the duration of tolerance in both strains. This procedure represented a third injection of OVA given 60 d after the second. The results, presented in Fig. 3, indicate that the tolerant state seen in OVA-fed animals persisted for at least 81 d after antigen feeding.

To rule out the unlikely possibility that OVA-fed mice were generally hyporesponsive, they were challenged with either SRBC or TNP-Ficoll. The OVA-fed mice made antibody responses to those irrelevant antigens comparable to the responses of controls (Table I). Thus, the hyporesponsiveness of OVA-fed mice was antigen specific.

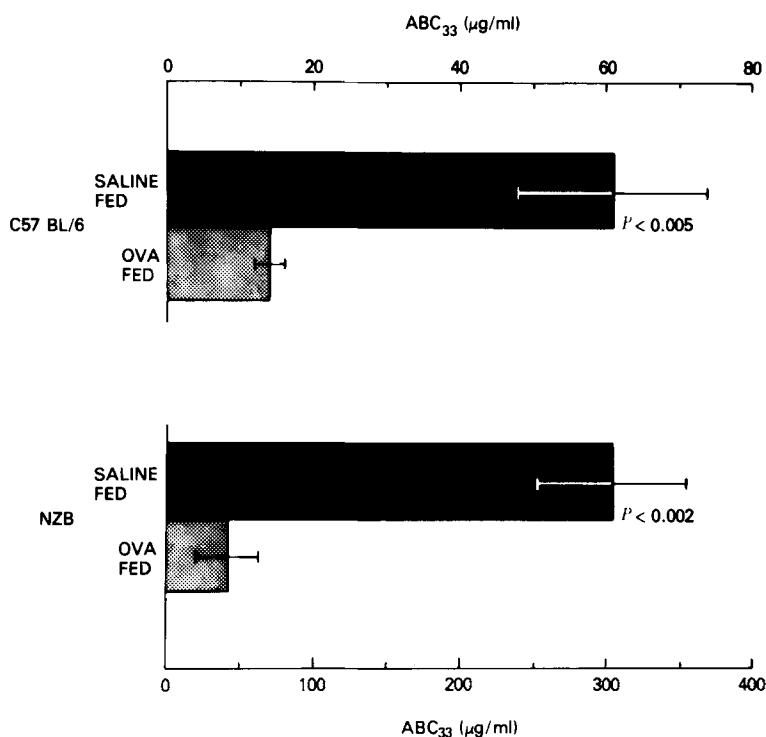


FIG. 3. Animals presented in Fig. 2 were given a third injection of OVA/alum 60 d after secondary immunization (81 d after OVA feeding). Antigen-binding capacity (ABC_{33}) was determined 7 d later.

TABLE I
The Response of OVA-fed and Control Mice to Unrelated Antigens

Strain	Intragastric treatment	Anti-SRBC*	Anti-TNP‡
C57BL/6	Saline	4.4 ± 0.5	53.6 ± 4.1
	OVA	4.6 ± 0.8	48.4 ± 2.2
NZB	Saline	5.4 ± 0.5	63.0 ± 2.0
	OVA	5.6 ± 0.5	58.6 ± 2.1

* Hemagglutination titer ($\bar{X} \log_2 \pm SEM$) 7 d after intraperitoneal injection with 5×10^8 SRBC.

‡ Percent binding of 500 ng of ^{125}I -DNP-BSA by 15 μ l of sera obtained 5 d after immunization with 10 μ g of TNP-Ficoll. Normal mouse serum showed <15% binding.

Discussion

A defect in systemic tolerance after OVA feeding and primary challenge with OVA in complete adjuvant represents a previously undescribed regulatory defect in the autoimmune-prone NZB strain. Their resistance to tolerance differs from the recently reported resistance of C3H/HeJ mice to tolerance induced by SRBC feeding (10, 11). Unlike the C3H/HeJ mice in that study, NZB mice showed no evidence of priming as a result of enteric antigen exposure. Nevertheless, the NZB mouse represents a second strain that, under appropriate study conditions, is resistance to tolerance induction by antigen feeding. Moreover, the NZB mice used in this study were 8 wk

old; therefore, defective tolerance induction cannot be ascribed to the severe clinical manifestations of autoimmunity that such mice develop later in life.

NZB mice are known to be resistant to the induction of tolerance by the injection of deaggregated gamma globulins (12, 13); however, the immune mechanisms responsible for systemic tolerance after antigen feeding contrast with the tolerance mechanisms in recipients of deaggregated protein. A major difference is the larger role of suppressor T cells in the production and maintenance of tolerance after enteric antigen exposure. Although a defect in the T suppressor cell compartment has been ascribed to NZB mice (14), a defect in antigen-specific T suppressor cells has been elusive (15). The study of defective tolerance after antigen feeding may be a means of defining such a defect.

The tolerance resistance of NZB mice reported in this study was observed only at the level of the primary response. This result may be influenced by the dose of antigen involved (or the complete adjuvant) that is required to generate a reasonable primary anti-OVA response. According to such reasoning, defective tolerance in NZB mice may become apparent only when the relative strength of the antigenic challenge is increased; small repetitive doses of antigen may not provide sufficient stimulation to break tolerance. Alternatively, the possibility exists that the mechanisms responsible for regulating primary and secondary responses are different. Consistent with this hypothesis is the observation that NZB mice usually have normal secondary responses at a time when primary responses are abnormal (15, 16). Thus, the abnormality observed in NZB mice could be the result of a specific defect in the regulation of a primary response.

Tolerance to repetitive low-dose antigen challenge was seen in both C57BL/6 and NZB mice after a single intragastric administration of OVA. This represented the initial exposure of these animals to OVA, and the observed systemic tolerance may mimic the natural state of original enteric exposure to a multitude of antigens. This mode of antigen exposure may result in local secretory immunity but systemic tolerance to the small amount of antigen that may be absorbed intact. The present study suggests that NZB mice are probably normal in this regard. This finding is of interest to the experimental treatment of autoimmunity in that repeated feeding of antigen has been shown to reduce the magnitude of an ongoing immune response (7). It is possible that repetitive feeding of relevant self antigens (not usually presented via the gastrointestinal tract) may be able to modulate autoimmune responses.

Summary

We evaluated the effect of antigen feeding on the subsequent primary and secondary anti-ovalbumin (OVA) responses of C57BL/6 and NZB mice. When C57BL/6 mice were given a single 20-mg dose of OVA intragastrically, profound tolerance was observed after challenge, 7 d later, with 125 μ g of OVA in complete adjuvant or after two injections of 5 μ g of OVA adsorbed to alum given 7 and 21 d after antigen feeding. OVA-fed NZB mice failed to become tolerant to a primary challenge with OVA in complete adjuvant, but showed a degree of tolerance similar to that of C57BL/6 mice when challenged two or three times with OVA in alum. These studies demonstrate that NZB mice fail to show tolerance at the level of the primary response after antigen feeding; however, they are normally tolerant when a secondary response to a lower dose of antigen is evaluated. This study suggests that, after antigen feeding,

different mechanisms of tolerance may be involved in the regulation of primary and secondary responses.

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