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Persistence of oral tolerance in mice fed ovalbumin is different for humoral and cell-mediated immune responses

S. STROBEL* & A. FERGUSON† *Department of Immunology, Institute of Child Health, London, and †Gastrointestinal Unit, University of Edinburgh, and Western General Hospital, Edinburgh*

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SUMMARY

The duration of oral tolerance after a single feed of OVA at the age of 6 weeks was studied in BDF_1 mice. Significant suppression of systemic antibody responses was present 3 months later, but not at 6·5 months; in contrast, at all times studied from 2 weeks to 17 months after an OVA feed there was suppression of systemic CMI to OVA as measured by an *in vivo* skin test. This indicates that the two limbs of the immune response differ in the factors responsible for the maintenance of oral tolerance.

A number of immunological and digestive factors are concerned in the phenomenon of oral tolerance (specific local and systemic hyporesponsiveness after the feeding of antigen). The induction phases of immunological tolerance for systemic antibody and CMI responses appear to be controlled by different mechanisms (Chiller & Weigle, 1971; Weigle, 1977). Most work on the duration of oral tolerance has concerned tolerance of the B-cell system, humoral antibodies and antigen-reactive B cells *in vitro* (Waksman, 1977; Chiller, Titus & Etlinger, 1979). Suppression of humoral immunity lasts about 2 months after antigen feeding (Vaz *et al.*, 1977; Ngan & Kind, 1978; Challacombe & Tomasi, 1980). Only for sheep erythrocytes, given as multiple feeds over 2 weeks, has the duration of tolerance for CMI been reported, and it was found that CMI tolerance lasted for at least 6 months (Kagnoff, 1978). We have now documented the presence or absence of oral tolerance for antibody and CMI responses from 2 weeks to 17 months after a single feed of protein antigen to adult mice.

Female BDF_1 [$(C57BL/6J \times DBA2)F_1$] mice, aged 6 weeks at the beginning of the experiment, were bred and held under conventional animal house conditions at the Animal Unit, Western General Hospital, Edinburgh.

Ovalbumin (OVA; Sigma, Poole, Dorset), five times crystallized, dissolved in 0·15 M NaCl (100 mg/ml), was fed once at a dose of 1 mg/g body weight by gastric gavage when mice were aged 6 weeks. Control mice were fed a similar volume of saline. Groups (five to seven mice) of OVA-fed and age-matched animals were immunized with 100 μg OVA in complete Freund's adjuvant (CFA) 0·5, 1, 3, 6·5, 9, 12, and 17 months after the initial feed. Humoral and cell-mediated immunity were tested 21 days after immunization.

Serum antibody levels during the first 6 months of the experiment were measured by an indirect haemagglutination assay (Mowat *et al.*, 1982), and at the later times an ELISA for IgG anti-OVA antibody was used (Strobel & Ferguson, 1984). Results obtained with these two methods of measuring specific antibodies had been shown to correlate closely ($P < 0·001$) (Strobel, 1983). CMI was assessed by measuring the specific increment in footpad thickness 24 hr after intradermal challenge with 100 μg OVA in 0·05 ml 0·15 M saline (Mowat *et al.*, 1982).

Logarithmically transformed haemagglutination titres and DTH responses were compared by Student's *t*-test. Results from ELISA tests were compared by Wilcoxon's rank sum test.

The results are depicted in Fig. 1. Results for mice examined between 2 weeks and 3 months after an OVA feed showed typical oral tolerance but, for antibody responses, there was no significant suppression at 6·5 months. In the mice studied at 12 months after the initial OVA feed, there was 25% suppression of antibody responses when compared with controls. This was statistically significant at the 5% level, but we consider that this probably reflects the random variability in the induction and maintenance of oral tolerance, and probably does not imply that oral tolerance transiently reappeared at this time. In contrast, suppression of CMI responses ranged from 93 to 68% of age-matched controls during the experiment. There was no correlation between the degree of suppression and increasing age of the animals, and CMI responses were still suppressed at 17 months after the initial OVA feed.

In this longitudinal study, we have demonstrated that systemic hyporesponsiveness after feeding OVA to inbred mice is a stable and long-lasting phenomenon, and that it has different effects on both limbs of the immune system. Convincing suppression of humoral immunity was present for only 3 months, whereas CMI was still significantly suppressed 17 months after the initial antigen feed. Although there are no previous reports on the long-term immunological effects of feeding a protein antigen, similar dissociation of antibody and CMI tolerance has been reported in animals given antigen intravenously (Chiller & Weigle, 1971; Weigle, 1977). In these

Correspondence: Dr S. Strobel, Dept. of Immunology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, U.K.

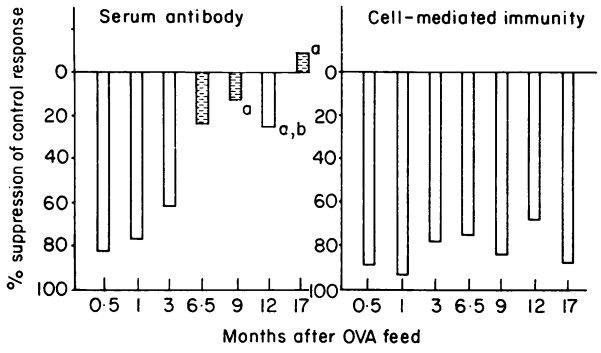

Figure 1. Persistence of orally induced tolerance to ovalbumin. Serum antibody and CMI responses in BDF_1 mice that had been fed OVA or saline at 6 weeks of age, and immunized with OVA in CFA at various times thereafter. Immune responses were measured 3 weeks after immunization. Stippled bars indicate the absence of significant suppression. Significance levels of the results presented are, unless stated otherwise, $P < 0{\cdot}01$: (a) measured with ELISA; (b) significant suppression ($P < 0{\cdot}05$) when compared to controls.

experiments DTH to human gammaglobulin was no longer suppressed after 5–6 months.

As part of a series of experiments on immune responses in neonatal mice, we had found that a feed of OVA on the first day of life primed rather than tolerized. Attempts were made to retolerize these animals by another OVA feed some weeks later. Primed antibody responses were found to be completely suppressible 4 weeks after the initial priming feed, whereas we failed to achieve retolerization of CMI responses even after 14 weeks. We attributed the observed differences between antibody and CMI responses to the fact that memory cells for antibody responses have a shorter life-span than T effector cells or T memory cells for DTH (Strobel & Ferguson, 1984). In the present experiments, we have not attempted to address the possible mechanism whereby oral tolerance for DTH persists, effectively, for the life-span of a mouse. However, the long life-span of DTH memory lymphocytes, when compared with the rapid cell turnover in the B-cell system, is again likely to be relevant, and this would be amenable to investigation by cell transfer studies.

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