

Effects of multiple oral dosing on IgE synthesis in mice: oral sensitization by albumin extracts from seeds of Jack fruit (*Artocarpus integrifolia*) containing lectins

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Summary. The IgE antibody response was studied in DBA/2 mice; the mice were pretreated orally with albumin extracts from seeds of Jack fruit (Jackalbumin) and subsequently immunized subcutaneously with Jackalbumin mixed with ovalbumin (OA) and a synthetic adjuvant, muramyl dipeptide (MDP).

The allergenicity of Jackalbumin was evaluated by its capacity to induce a specific IgE response which was measured by passive cutaneous anaphylaxis (PCA) and by degranulation of washed peritoneal mast cells following antigen challenge (Jackalbumin or OA). Antibody against the crude extracts and anti-lectin (F₁SP) IgE responses were also tested by PCA. After being fed eight doses of 1 mg Jackalbumin, DBA/2 mice became immunized: i.e. specific IgE antibody responses were observed and the peritoneal mast cells became sensitized. An increase in IgE response was verified in mice that were pre-fed and subsequently immunized.

The results indicated that: (i) the albumin extracts from Jack seeds, containing lectins, can be allergenic by the oral route; (ii) multiple oral doses with these extracts can induce an enhancement of the IgE response on subsequent subcutaneous immunization; (iii) antigenically, Jackalbumin does not seem to cross-react with OA; (iv) the lectins contained in the albumin fraction from *Artocarpus* seeds were also

shown to be allergenic; (v) the IgE titres showed an inverse correlation to the degree of purification of the lectin used for PCA challenge.

INTRODUCTION

Concepts of immune responses following oral presentation of immunogens have been established for a long time (Besredka, 1927; Burrows, Elliot & Havens, 1947). These have been well reviewed (Bienenstock, 1974; Tomasi & Bienenstock, 1968). There is ample evidence that orally administered protein can induce a local and systemic immune response (Crabbé *et al.*, 1969; Falchuk *et al.*, 1977; Rothberg, Kraft & Farr, 1967). However, a number of studies have shown that exposure to protein or particulate antigens by the digestive route may result in diminished responsiveness to later immunogenic encounters with specific antigens (André, Bazin & Heremans, 1973; Hanson *et al.*, 1977; Ngan & Kind, 1978; Richman *et al.*, 1978; Stokes & Swarbrick, 1977; Vaz *et al.*, 1977). The relationship between this tolerance and orally-induced antibody remains unclear, although it has been observed that tolerance may occur in some experimental systems after a phase of specific antibody production (Howard & Mitchison, 1975).

The immunoadjuvant activity of MDP has been documented with various antigens *in vivo* (Ellouz *et al.*, 1974; Audibert *et al.*, 1976) and *in vitro* (Leclerc,

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Löwy & Chedid, 1978). Ohkuni *et al.* (1979) have shown the adjuvant activities of MDP and some synthetic analogues in the production of primary and secondary anti-OA IgE responses in mice. Prouvost-Danon & Chedid (1983) have shown a similarity in the adjuvant activities of MDP and $AL(OH)_3$ in primary and secondary anti-OA IgE responses in BALB/c and DBA/2 mice. Based on these findings, the MDP adjuvant was employed in the present study.

Plant lectins can function as antigens and provoke a specific antibody response, e.g. Concanavalin A (Con A) was found to be particularly effective at inducing anti-Con A IgE in certain strains of mice (Mitchell & Clarke, 1979). They can also function as non-specific mitogens, activating a large portion of T cells to either stimulate or suppress the immune response (Schell & Lawrence, 1977; Lawrence & Schell, 1977), depending on experimental conditions.

Protein extracts from different Brazilian seeds and protein fractions submitted to further purification have been reported to induce specific IgE synthesis in mice (Ponte *et al.*, 1980). Silva Lima *et al.* (1980) have shown that peritoneal mast cells from mice degranulate upon addition of albumin extracts and lectins from certain Brazilian seeds. Several lectins were recently demonstrated to modulate the immune response to type III pneumococcal polysaccharide (Taylor *et al.*, 1984).

It is known that *Artocarpus* seeds are consumed as human food in many parts of Brazil. Our special interest in these studies was to investigate the possibility of inducing a state of allergy by oral administration of this soluble food protein. We also studied the effect of prior administration of Jackalbumin by the digestive route on the IgE response to parentally administered antigens (Jackalbumin+OA) in MDP adjuvant. The allergenicity of the albumin fraction containing lectins was evaluated in relation to the purification of the *Artocarpus* lectin used for PCA challenge. A possible mitogenic or immunomodulating effect of Jackalbumin extracts on the IgE responses to the OA antigen is suggested.

MATERIALS AND METHODS

Animals

Adult (8–12-week-old) female DBA/2 mice were obtained from Iffa Credo (Lyon, France). Normal male Wistar rats (250–300 g) were obtained from

Orléans-CNRS and used as recipients for the IgE antibody assay.

Antigens and adjuvant

The crude extract (A), the albumin fraction (B) and purified lectin, F_{II}SP (C), from seeds of Jack fruit (*Artocarpus integrifolia*) were prepared as already described (Moreira & Ainouz, 1981) and obtained from the Department of Biochemistry and Molecular Biology at the Federal University of Ceara, Fortaleza, Ceara, Brazil.

Ovalbumin (five crystallized; Serva, Feinbiochemica, Heidelberg) was dissolved in physiological saline at appropriate concentrations and stored at -20° until used. The synthetic adjuvant, muramyl dipeptide, MDP (N-acetylmuramyl-L-alanyl-D-isoglutamine) was prepared as described elsewhere (Ellouz *et al.*, 1974; Chedid *et al.*, 1976; Parant, 1979), and obtained from Choay Chimie Reactifs (Paris, France). It was added to protein solutions immediately before injection of the mixture into mice.

Oral administration

DBA/2 mice were divided into five groups of six to eight mice. Two groups were fed 1 mg Jackalbumin dissolved in 0.5 ml isotonic saline, and two groups received 0.5 ml saline by gavage for 8 days. A control group of mice without feeding was also included.

There was no evidence of regurgitation of injected fluid or of aspiration of fluid into the lungs. Animals killed immediately after the last feeding showed no evidence of trauma to the mucosa of the mouth, oesophagus or stomach. This was reassuring, in view of the observation that minimal mucosal trauma can result in significantly increased serum antibody levels to food antigens (Taylor, Truelove & Wright, 1964; Thomas *et al.*, 1973).

Parenteral immunization

Four days after finishing the regimen of oral treatment with either Jackalbumin or saline, DBA/2 mice were injected subcutaneously with 100 μ g Jackalbumin mixed with 100 μ g ovalbumin in 100 μ g MDP adjuvant (0.5 ml per animal). Control groups of mice without feeding and/or without s.c. immunization were carried out.

Bleeding

The animals were bled from the retro-orbital venous plexus before starting the oral regimen, at the end of this regimen, and several days after parenteral immunization. The sera were stored at -20° .

Antibody titrations: passive cutaneous anaphylaxis (PCA)

The titres of anti-Jack (crude extracts, albumin, purified lectin, F_{II}SP) and anti-OA IgE antibodies were determined by PCA using 250–300 g male Wistar rats as described elsewhere (Mota & Wong, 1969).

Briefly, the dorsal surface of the body was shaved. Serial dilutions of pooled antisera were injected intradermally in 100 μ l volumes and, 24 hr later, the rats were challenged by intravenous injection of 1 mg total extracts, albumin fraction or lectin (F_{II}SP) from Jack seeds or 2 mg OA in 1 ml of 0.5% Evans blue dye, and reactions were read after 1 hr. A PCA reaction was considered positive if the diameter of the blueing was 5 mm or more. The reciprocal of the highest dilution of serum giving a 5 mm blueing was taken as the titre of IgE antibody. The PCA was set up in duplicate and the assay was repeated. Controls were performed in each rat using a known standard serum with a high anti-Jackalbumin or anti-OA titre: the reaction given by the sera tested were compared to the titre of the standard solution.

Degranulation of peritoneal mast cells

In vitro experiments were performed as previously described (Prouvost-Danon, Silva Lima & Queiroz Javierre, 1966). Peritoneal mast cells were collected from normal or treated DBA/2 mice, washed three times by centrifugation and resuspended in 0.18 ml RPMI medium 1640 (Gibco, Grand Island, NY) buffered at pH 6.9 with 0.005 M HEPES. Normal or sensitized cells were kept in a water-bath at 37° for 10–15 min for temperature equilibration. The specific antigen (10 μ g in a volume of 20 μ l) was added, and incubation was continued at 37° for a further 6 min. Enumeration of degranulated and intact mast cells was done after staining with toluidine blue. The results were expressed as a percentage of degranulated mast cells in relation to the total. About 150–200 mast cells were counted for each measurement. Several countings were performed for each test. Controls in all the experiments were normal or sensitized cells with no antigen. Spontaneous degranulation in the controls was between 1% and 3%.

RESULTS

The results represent a typical experiment out of three identical assays.

Effects of multiple feedings of Jackalbumin on anti-Jack IgE responses

The immunogenic effects of repeated oral dosing of Jackalbumin without subcutaneous (s.c.) immunization are shown in Table 1 (Group IV, A, B, C). Subcutaneous immunization alone elicited only marginal effects, which, however, became highly significant when preceded by oral pretreatment (Groups III and V, A, B, C). Thus, oral feeding of Jackalbumin increased the specific IgE response of subsequent parenteral immunization. Similarly, the anti-Jack IgE response induced by oral feeding appeared to be increased by subsequent s.c. immunization with Jackalbumin (Groups IV and V, A, B, C). Jackalbumin administered only once by the s.c. route in a mixture with OA and MDP adjuvant (compare Group III, A, B, C with Groups I and II, A, B, C) resulted in almost no circulating anti-Jack IgE response, emphasizing the allergenic effects elicited by oral sensitization.

Effect of multiple feedings of Jackalbumin anti-OA IgE synthesis

The results in Table 2 (compare Group V with Groups I, II and III) show that repeated dosing with Jackalbumin also induced stimulatory effects on anti-OA IgE responses, resulting from s.c. immunization with Jackalbumin+OA and MDP adjuvant. A small, but statistically significant, increase in anti-OA IgE responses was observed, beginning on the 11th day after s.c. immunization (Group V), as compared to the control Group III, indicating that Jackalbumin by the oral route can also increase IgE responses to unrelated antigens.

No anti-OA IgE response was detectable in DBA/2 mice treated only with Jackalbumin (Group IV).

Taken together, the pattern of results from Tables 1 and 2 also demonstrates that oral feeding of Jackalbumin exerts a greater immunogenic effect on anti-Jack (Table 1A and B, compare Groups V and III) than on anti-OA IgE responses (Table 2, compare Groups V and III). Mice from group III showed high anti-OA IgE responses (Table 2) when compared with the anti-Jack IgE (Table 1A, B, C), thus, indicating a selective adjuvant action only on the former.

Allergenicity of albumin extracts and lectins from Artocarpus seeds

As observed in Table 1, the allergenicity of Jackalbumin fraction and lectins was evaluated by PCA in rats

Table 1. Effects of oral ingestion of Jackalbumin on anti-Jack IgE responses in DBA/2 mice

				PCA titres*													
				Rats challenged with 1 mg/1 ml of:													
Treatment				Jack crude extract (A)		Jackalbumin (B)		Purified lectin (C)									
				Days after immunization													
Oral administration				S.c. immunization													
				(Day 0)													
Group	Dose	Days		8	11	14	21	8	11	14	21	8	11	14	21		
I	None			<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5		
II	Saline	-12 to -5	None	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5		
III	Saline	-12 to -5	Saline	<10	<10	<10	<10	5	5	5	5	5	5	5	5		
IV	Jackalbumin	-12 to -5	Jackalbumin + OA + MDP†	80	80	80	80	60	60	70	40	20	20	30	30		
	(1 mg)		Saline														
V	Jackalbumin	-12 to -5	Jackalbumin + OA + MDP†	120	160	160	160	80	80	80	80	40	40	40	60		
	(1 mg)																

* Sera from six to eight mice of each group were pooled, and the reciprocal of the highest dilution given PCA reaction was taken as the titre.

† 100 µg Jackalbumin + 100 µg OA + 100 µg MDP.

Table 2. Effects of oral ingestion of Jackalbumin on anti-OA IgE responses in DBA/2 mice

Treatment				PCA titres*: IgE anti-OA			
Oral administration			S.c. immunization (Day 0)	Days after immunization			
Group	Dose	Days		8	11	14	21
I	None		None	< 5	< 5	< 5	< 5
II	Saline	- 12 to - 5	Saline	< 5	< 5	< 5	< 5
III	Saline	- 12 to - 5	Jackalbumin + OA + MDP†	90	40	40	30
IV	Jackalbumin (1 mg)	- 12 to - 5	Saline	< 5	< 5	< 5	< 5
V	Jackalbumin (1 mg)	- 12 to - 5	Jackalbumin + OA + MDP†	90	80	90	80

* Sera from six to eight mice of each group were pooled, and the reciprocal of the highest dilution giving a PCA reaction was recorded as the titre. IgE titres were assayed in duplicate, and rats were challenged with 2 mg/ml OA.

† 100 µg Jackalbumin + 100 µg OA + 100 µg MDP.

challenged with three Jack protein preparations: crude extracts (A), albumin extracts (B) and purified lectin (C). On challenge with crude extracts, the IgE antibody responses were proportionally higher than those detected when challenged with albumin extracts or purified lectin. Intermediate IgE responses were obtained on challenge with albumin extracts, and diminished PCA titres were seen on challenge with purified lectin, indicating that the IgE responses were inversely proportional to the state of purity of the lectins used for PCA challenge. In addition, the results, using purified lectins for PCA challenge,

showed that lectins contained in the albumin fraction from Jack seeds were allergenic.

***In vitro* effects of Jackalbumin on peritoneal mast cells from DBA/2 mice**

Peritoneal mast cells from DBA/2 mice were collected 32 days after s.c. immunization and treated as described in the Materials and Methods.

As can be seen in Table 3, the stimulatory effects of oral ingestion of Jackalbumin on anti-Jackalbumin IgE synthesis were confirmed by *in vitro* degranulation

Table 3. *In vitro* degranulation of sensitized peritoneal mast cells from DBA/2 mice

Treatment				32 days after s.c. immunization	
Oral administration			S.c. immunization (Day 0)	Mast cell degranulation (%)*	
Group†	Dose	Days		Challenge: Jackalbumin (10 µg/ml)	Challenge: OA (10 µg/ml)
I	None		None	1	2
II	Saline	- 12 to - 5	Saline	2	3
III	Saline	- 12 to - 5	Jackalbumin + OA + MDP†	28	ND
IV	Jackalbumin (1 mg)	- 12 to - 5	Saline	15	3
V	Jackalbumin (1 mg)	- 12 to - 5	Jackalbumin + OA + MDP†	70	75

* Detected in pooled peritoneal mast cells from four to five DBA/2 mice of each group (see Materials and Methods).

† Six to eight mice per group.

‡ 100 µg Jackalbumin + 100 µg OA + 100 µg MDP.

of peritoneal mast cells: a low percentage of mast cell degranulation was detected in mice immunized only s.c. (Group III) and in mice treated by the oral route alone (Group IV). In contrast, a high percentage of degranulated mast cells was found in pre-fed and subsequently immunized DBA/2 mice (Group V), thus verifying by the *in vitro* test that anti-Jackalbumin IgE response induced by s.c. immunization was primed by previous oral feeding.

No direct action of Jackalbumin extracts or OA on normal mast cells from DBA/2 mice could be detected, neither was any cross-reactivity between Jackalbumin and OA observed in the *in vitro* assay.

DISCUSSION

Our results demonstrated that repeated oral dosing of Jackalbumin, containing lectins, was capable of inducing IgE antibody formation in DBA/2 mice. Quantitatively, the allergenicity of this albumin fraction seemed to be dependent upon purification of the lectin used for PCA challenge; an inverse correlation between the IgE titres and the degree of purification of lectins was observed, thus demonstrating that the three Jack protein preparations (crude extract, albumin fraction and lectin) are all active in PCA assay and may be (or contain) significant allergens.

Oral pretreatment with Jackalbumin induced an increase of both anti-Jack extracts and anti-OA IgE responses to subsequent parenteral immunization. The enhancing effect was more evident on anti-Jack (crude extract, albumin extract) than on anti-OA IgE synthesis, suggesting a small boosting effect of subsequent s.c. immunization with Jackalbumin on the specific IgE antibody response induced by oral feeding. A mitogenic or immunomodulating effect of orally administered Jackalbumin on anti-OA IgE response is postulated.

In control mice immunized only s.c. with Jackalbumin + OA and MDP adjuvant, almost no circulating IgE against Jack extracts could be detected; however, as discussed previously, this treatment schedule induced a greater formation of circulating anti-OA IgE antibodies, suggesting that Jackalbumin containing lectins might modulate the IgE antibody synthesis to the OA antigen, also when given by the s.c. route. Additional experiments would be necessary to clarify this point. Phytomitogens are generally known to suppress the *in vivo* immune response although, in some circumstances, they may act as stimulants. For

example, Romball & Weigle (1975) showed that Con A enhanced primary humoral immune responses of rabbits to HGG, BSA and SRBC. Con A has also been reported to suppress the *in vivo* primary antibody response to SRBC in the mouse (Barth & Singla, 1973; Egan, Reeder & Ekstedt, 1974). Elves (1967) reported that phytohaemagglutinin (PHA), injected before antigen, suppressed the primary immune response whereas, when given 6 hr after the antigen, it enhanced antibody production. Gollapudi & Kind (1975) have shown that the lectin Con A complexed to ovalbumin increases the allergenicity of ovalbumin.

The pattern of results obtained with mast cells *in vitro* confirmed those obtained in PCA assay, showing an enhancing effect of oral treatment with Jackalbumin on subsequent IgE anti-Jackalbumin responses and a boosting effect of the subsequent s.c. immunization.

There is evidence that some lectins from specific Brazilian seeds are capable of directly activating mouse mast cells to induce their degranulation (Silva Lima *et al.*, 1980). In our studies, no degranulation of normal DBA/2 mast cells could be induced by the albumin fraction from Jack seeds, containing lectins. It was also seen that, antigenically, albumin extracts from *Artocarpus* seeds do not cross-react with OA.

Our data confirm, at least in part, the experiments of other authors, demonstrating that the initial presentation of antigen via feeding can induce the production of circulating IgE antibodies (Bazin & Platteau, 1976; Jarrett *et al.*, 1976). In most of the situations studied, the introduction of antigens by the digestive route favours the appearance of the state of immunological tolerance (André *et al.*, 1973; Hanson *et al.*, 1977; Stokes & Swarbrick, 1977; Vaz *et al.*, 1977; Ngan & Kind, 1978; Richman *et al.*, 1978) due to the appearance of T-suppressor cells (Ngan & Kind, 1978; Richman *et al.*, 1978). Nevertheless, as mentioned above, there is evidence in the literature that antigens introduced orally can also induce the antibody formation (Besredka, 1927; Burrows *et al.*, 1947; Rothberg *et al.*, 1967; Tomasi & Bienenstock, 1968; Crabbé *et al.*, 1969; Bienenstock, 1974; Bazin & Platteau, 1976; Jarrett *et al.*, 1976; Falchuck *et al.*, 1977), reinforcing the concept that the oral route is efficient in providing access to the lymphoid system. The particular conditions of the stimulus and/or of the organism appears to determine whether the contact will result predominantly in immunization or tolerance.

The extreme difficulties in identifying precisely the factors responsible for inducing, potentiating or inhi-

biting the IgE response have been adequately discussed by Tada (1975). However, there is evidence to suggest that oral immunization or priming may occur in rodents under optimum-dose, rather than maximum-dose conditions (Bazin & Platteau, 1976; Jarrett *et al.*, 1976), and that multiple feedings may be required to elevate antibody titres to measurable levels (Falchuck *et al.*, 1977).

In our further studies, the immunogenic effects of repeated feedings with albumin extracts from Jack seeds have been confirmed in other mouse strains (manuscript in preparation). In BDF₁ hybrid mice, we have verified that the oral administration of these extracts can induce IgE synthesis and sensitization of mast cells or inhibit it (manuscript in preparation). The results depend upon experimental conditions. It has been clearly demonstrated that the nature, dose and duration of immunogen challenge are all important in the induction and elicitation of different types of responses. Thus, the possibility remains that varying methodologies of feeding and parenteral challenge may account for some of the differences among these studies.

Since seeds of *Artocarpus integrifolia* are consumed as human food in many parts of Brazil, our findings in mice, showing the allergenicity and the stimulatory effects of the albumin fraction and lectins from these seeds by digestive route, are potentially important for the prophylaxis of human allergies to food.

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REFERENCES

ANDRÉ C., BAZIN H. & HEREMANS J. (1973) Influence of repeated administration of antigen by the oral route on specific antibody producing cells in the mouse spleen. *Digestion*, **9**, 166.

- AUDIBERT F., CHEDID L., LEFRANCIER P. & CHOAY J. (1976) Distinctive adjuvanticity of synthetic analogs of microbacterial water-soluble components. *Cell. Immunol.* **21**, 243.
- BARTH R.F. & SINGLA O. (1973) Differential effects of Concanavalin A on T helper dependent and independent antibody responses. *Cell. Immunol.* **9**, 96.
- BAZIN H. & PLATTEAU B. (1976) Production of circulating reaginic (IgE) antibodies by oral administration of ovalbumin to rats. *Immunology*, **30**, 679.
- BESREDKA A. (1927) *Local Immunization*, p. 181. Williams and Wilkins, Baltimore.
- BIENENSTOCK J. (1974) The physiology of the local immune response and the gastrointestinal tract. *Prog. Immunol.* **4**, 197.
- BURROWS W., ELLIOT M.E. & HAVENS I. (1947) Studies on immunity to asiatic cholera. Excretion of coproantibody in experimental enteric cholera in guinea pigs. *J. infect. Dis.* **81**, 251.
- CHEDID L., AUDIBERT F., LEFRANCIER P. & CHOAY J. (1976) Modulation of the immune response by a synthetic adjuvant and analogs. *Proc. natn. Acad. Sci. U.S.A.* **69**, 855.
- CRABBÉ P.A., NASH D.R., BAZIN H., EYSSEN H. & HEREMANS J.F. (1969) Antibodies of the IgA type in intestinal plasma cells of germ free mice after oral or parenteral immunization with ferritin. *J. exp. Med.* **130**, 723.
- EGAN H.S., REEDER W.J. & EKSTEDT R.D. (1974) Effect of Concanavalin A *in vivo* in suppressing the antibody response in mice. *J. Immunol.* **112**, 63.
- ELLOUZ F., ADAM A., CIORBARU R. & LEDERER E. (1974) Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. *Biochem. biophys. Res. Com.* **59**, 1317.
- ELVES M.W. (1967) Suppression of antibody production by phytohaemagglutinin. *Nature (Lond.)*, **213**, 495.
- FALCHUK K.R., WALKER W.A., PERROTTI J.L. & ISSELBACHER K.J. (1977) Effect of vitamin A on the systemic and local antibody responses to intragastrically administered bovine serum albumin. *Infect. Immun.* **17**, 361.
- GOLLAPUDI V.S.S. & KIND L.S. (1975) Enhancement of reaginic antibody formation in the mouse by Concanavalin A. *Int. Arch. Allergy appl. Immun.* **48**, 94.
- HANSON D.G., VAZ N.M., MAIA L.C.S., HORN BROOK M.M., LYNCH J.M. & ROY C.A. (1977) Inhibition of specific immune response by feeding protein antigens. *Int. Arch. Allergy appl. Immun.* **55**, 526.
- HOWARD J.C. & MITCHISON N.A. (1975) Immunological tolerance. *Progr. Allergy*, **18**, 43.
- JARRETT E., HAIG D., McDUGALL W. & McNULTY E. (1976) Rat IgE production. II. Primary and booster reaginic antibody responses following intradermal or oral immunization. *Immunology*, **30**, 671.
- LAWRENCE D.A. & SCHELL R.F. (1977) Differential effects of Concanavalin A and Phytohemagglutinin on murine immunity. Suppression and enhancement of humoral immunity. *Cell. Immunol.* **31**, 155.
- LECLERC C., LÖWY I. & CHEDID L. (1978) Influence of MDP and of some analogous synthetic glycopeptides on the *in vitro* mouse cell reactivity and immune response to sheep erythrocytes. *Cell. Immunol.* **38**, 286.
- MITCHELL G.F. & CLARKE A.E. (1979) Allergenicity of

- Concanavalin A in mice. *Int. Arch. Allergy appl. Immun.* **58**, 391.
- MOREIRA R.A. & AINOZ I.L. (1981) Lectins from seeds of Jack fruit (*Artocarpus integrifolia* L.): isolation and purification of two isolectins from the albumin fraction. *Biol. Plant. (Praha)*, **23**, 186.
- MOTA I. & WONG D. (1969) Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* **8**, 813.
- NGAN J. & KIND L.S. (1978) Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by oral administration of ovalbumin. *J. Immunol.* **120**, 861.
- OHKUNI H., NOROSE Y., OHTA M., HAYAMA M., KIMURA Y., TSUJIMOTO M., KOTANI S., SHIBA T., KUSUMOTO S., YOKOGAWA K. & KAWATA S. (1979) Adjuvant activities in production of reaginic antibody by bacterial cell wall peptidoglycan or synthetic N-acetylmuramyl dipeptides in mice. *Infect. Immun.* **24**, 313.
- PARANT M. (1979) Biologic properties of a new synthetic adjuvant, muramyl dipeptide (MDP). *Springer Semin. Immunopathol.* **2**, 101.
- PONTE V.A., REINALDO H.M.C., BENEVIDES R.H.P., MOREIRA R.A., SILVA LIMA M. & PROUVOST-DANON A. (1980) Allergenicity of proteins from brazilian seeds. *IVth International Congress of Immunology*, **13**, Abst. 2, 20.
- PROUVOST-DANON A. & CHEDID L. (1983) Influence of muramyl dipeptides (MDP) on IgE synthesis and mast cell degranulation. Proceedings of the XII Congress of the European Academy of Allergology and Clinical Immunology (eds U. Serafini and E. Errigo), p. 389. O.I.C. Medical Press, Firenze.
- PROUVOST-DANON A., SILVA LIMA M. & QUEIROZ JAVIERRE M. (1966) Active anaphylactic reaction in mouse peritoneal mast cell *in vitro*. *Life Sci.* **5**, 289.
- RICHMAN L.K., CHILLER J.M., BROWN W.R., HANSON D.G. & VAZ N.M. (1978) Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins. *J. Immunol.* **121**, 2429.
- ROMBALL C.G. & WEIGLE W.O. (1975) The enhancing effect of mitogens on the *in vivo* immune response of rabbits. *J. Immunol.* **115**, 556.
- ROTHBERG R.M., KRAFT S.C. & FARR R.S. (1967) Similarities between rabbit antibodies produced following ingestion of bovine serum albumin and following parenteral immunization. *J. Immunol.* **98**, 386.
- SCHILL R.F. & LAWRENCE D.A. (1977) Differential effects of Concanavalin A and Phytohemagglutinin on murine immunity. Suppression and enhancement of cell-mediated immunity. *Cell Immunol.* **31**, 142.
- SILVA LIMA M., MOREIRA R.A., PONTE V.A., REINALDO H.C.M., BENEVIDES R.H.P. & PROUVOST-DANON A. (1980) Mast cell degranulation by lectins from brazilian seeds. *IVth International Congress of Immunology*, **13**, Abst. 3, 17.
- STOKES C.R. & SWARBRICK E. (1977) Induction of tolerance after oral feeding of soluble protein antigen. *Biochem. Soc. Trans.* **5**, 1573.
- TADA T. (1975) Regulation of reaginic antibody formation in animals. *Progr. Allergy* **19**, 122.
- TAYLOR C.E., STASHAK F.W., CALDES G., PRESCOTT B., FOWLKES B.J. & BAKER P.J. (1984) Lectin-induced modulation of the antibody response to type III pneumococcal polysaccharide. *Cell. Immunol.* **83**, 26.
- TAYLOR K.B., TRUELOVE S.C. & WRIGHT R. (1964) Serological reaction to gluten and cow's milk protein in gastrointestinal disease. *Gastroenterology*, **46**, 99.
- THOMAS H.C., FERGUSON A., MACLENNAN J.C. & MAZON D.K. (1973) Food antibodies in oral disease: a study of serum antibodies to food protein in aphthous ulceration and other oral diseases. *J. clin. Path.* **26**, 371.
- TOMASI T.B. & BIENENSTOCK J. (1968) Secretory immunoglobulins. In: *Advances in Immunology*. (eds F. J. Dixon Jr. and H. G. Kundel), Vol. 9, p. 1. Academic Press, New York.
- VAZ N.M., MAIA L.C.S., HANSON D.G. & LINCH J.M. (1977) Inhibition of homocytotropic antibody responses in adult inbred mice by previous feeding of the specific antigen. *J. Allergy clin. Immunol.* **60**, 110.