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Production of circulating reaginic (IgE) antibodies by oral administration of ovalbumin to rats

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Summary. Reaginic antibody synthesis following parenteral and/or oral administration of ovalbumin and *Bordetella pertussis* organisms as adjuvant has been evaluated in LOU/M/Wsl inbred rats. These rats are able to produce high reaginic antibody serum levels after intraperitoneal injection of this antigen. Primary oral administration of ovalbumin doses between 10 and 100 mg with *Bordetella pertussis* organisms given as adjuvant by the intraperitoneal or the oral route led to characteristic reaginic responses. Secondary reaginic responses were obtained by oral administration of ovalbumin without any adjuvant in animals sensitized by the oral or the intraperitoneal route. A hundred micrograms of ovalbumin was enough to induce reaginic responses but more constant and higher reaginic levels were obtained with a 50 mg dose in the experimental model employed.

INTRODUCTION

Tada and Ishizaka (1970) have demonstrated that, in man and the monkey, the lamina propria of the intestine and its draining lymph nodes possess large numbers of plasma cells containing IgE class immunoglobulins. This location of IgE plasmacytes is similar to that of IgA plasmacytes (Crabbé, Carbonara and Heremans, 1965).

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Previous investigations of the immunological responses evoked by oral immunization and located in the intestine (Crabbé, Nash, Bazin, Eyssen and Hermans, 1969) as well as out of it (Bazin, Lévi and Doria, 1970), have shown that IgA antibodies predominate. In these studies, however, IgE responses were not assessed. Our study investigates the possibility of reaginic immunological responses induced by administering the antigen by the oral route.

MATERIALS AND METHODS

Animals

LOU/M/Wsl inbred strain rats were used in the immunization experiments. They were at least 3 months old. The PCA tests were performed on female Wistar R inbred strain rats. Except where specified, they received water *ad libitum* and were fed with UAR (Villemoisson/orge, France) no. 103 rat food, which contains no ovalbumin.

Antigen preparation

Egg albumin (Koch Light, Colnbrook, England) was prepared in saline solution at the appropriate concentration and was injected immediately. A 10^{10} *Bordetella pertussis* dose was given simultaneously with the primary immunization (Institut Pasteur Production, Paris, 'Perthydral' vaccine containing 0·1 per cent of aluminium hydroxide).

Immunization of the rats

The rats were immunized intraperitoneally or orally. The antigen was given by gastric intubation after a fasting period of 12 h, checking carefully that rats were not injured, because it is known that the passage of protein from the gastrointestinal tract to the blood circulation is significantly increased by (even minimal) mucosal trauma (Bazin, Andre and Heremans, 1973). The antigen or the *Bordetella pertussis* suspension was slowly delivered in order to avoid regurgitation or aspiration of fluid. The quantity was never greater than 1 ml, including the adjuvant solution.

Collection and titration of reaginic sera

Rats were bled from the retro-orbital plexus, under ether anaesthesia. No more than 2 ml of blood was withdrawn when the rats were to be kept. The serum was collected after coagulation and centrifugation and stored at −20°.

The level of circulating reagins was estimated by passive cutaneous anaphylaxis (PCA) (Ovary, 1964). 0·1 ml quantities of appropriate dilutions of test serum were injected intradermally. Seventy-two hours later rats were injected intravenously with 2·5 mg of ovalbumin, and 0·5 ml of 1 percent Evans blue (Gurr, England). The skin reactions were examined after 20 min. The reaginic titre was the greatest dilution that gave a coloured spot with at least a diameter of 5 mm.

RESULTS

Primary and secondary reaginic response following intraperitoneal injection of antigen

In order to test the ability of LOU/M/Wsl rats to produce reaginic antibodies following ovalbumin injection, six females and six males of this strain were given two doses of 1 μg of ovalbumin intraperitoneally with an interval of 30 days between injections. 10^{10} *Bordetella pertussis* organisms were given by the same route with the first injection of antigen.

Table 1 shows the results of the titration of the reaginic response of these rats on days 10 and 30 after the first injection, and on days 5 and 10 after the second injection. The observed results show no difference between the reaginic levels of male or

Table 1. PCA titres in LOU/M/Wsl rats after a primary injection of 1 μg of ovalbumin plus 10^{10} *Bordetella pertussis* and a secondary injection of 1 μg of ovalbumin, both injections being intraperitoneal

	Time after primary injection (days)			
	10	30	35	40
Time after secondary injection (days):			5	10
Rat no.				
1 ♀	32*	4	256	16
2 ♀	64	0	256	32
3 ♀	64	8	512	32
4 ♀	128	16	256	64
5 ♀	256	16	128	16
6 ♀	256	8	512	64
7 ♂	0	0	0	0
8 ♂	32	0	512	64
9 ♂	128	8	512	64
10 ♂	128	16	1024	32
11 ♂	256	2	64	8
12 ♂	512	8	2048	16

* As measured by the 72 h PCA reaction and expressed as the reciprocal of the titre.

Table 2. Effect of a varying first dose of ovalbumin on the level of the reaginic response, both challenges being performed by the oral route; the time of the first challenge is taken as day 0

	Oral challenge		Bleeding on day	PCA titres									
				Primary challenge with 10^{10} *B. pertussis* by:									
	On day	Dose (mg)		Oral route					Intraperitoneal route				
First	0	0	10	0	4	4	8	8*	0	0	0	0	8
Second	30	10	35	0	0	0	0	0	0	0	0	0	0
			40	0	0	0	0	0	0	0	0	0	0
First	0	1	10	0	2	8	8	8	0	0	8	0	16
Second	30	10	35	0	8	0	0	0	0	0	0	0	0
			40	0	0	0	0	0	0	0	0	0	0
First	0	10	10	4	8	8	16	32	0	0	4	128	128
Second	30	10	35	0	0	0	64	0	0	0	64	2	64
			40	0	0	0	0	0	0	0	0	0	0
First	0	50	10	0	4	8	8	16	0	0	0	8	16
Second	30	10	35	0	0	0	2	0	0	2	128	0	128
			40	0	0	0	0	0	0	0	0	0	0
First	0	100	10	0	8	0	32	256	0	0	0	16	256
Second	30	10	35	0	0	128	0	0	0	0	1024	0	16
			40	0	0	0	0	0	0	0	0	0	0

* As measured by the 72 h PCA reaction and expressed as the reciprocal of the titre.

Table 3. Reaginic responses of a group of LOU/M/Wsl rats injected first with 1 μg of ovalbumin plus 10^{10} *B. pertussis* and, 30 days after, with a second challenge of ovalbumin by the oral route

Response	Antigen dose (mg)	Route of administration	Bleeding on days, after the first challenge	PCA titres†									
Primary	0·001	i.p.*	30	0	0	0	2	8					
Secondary	0·0	Oral	35	0	0	0	4	8					
Primary	0·001	i.p.	30	0	0	2	4	4					
Secondary	0·01	Oral	35	0	4	4	16	16					
Primary	0·001	i.p.	30	0	0	0	8	8					
Secondary	0·1	Oral	35	0	0	4	32	64					
Primary	0·001	i.p.	30	0	0	2	4	8	0	0	2	2	0
Secondary	1	Oral	35	0	0	2	8	8	64	64	64	64	256
Primary	0·001	i.p.	30	0	8	4	2	4					
Secondary	10	Oral	35	32	32	64	128	256					
Primary	0·001	i.p.	30	0	0	0	2	4					
Secondary	50	Oral	35	256	512	512	512	1024					
Primary	0·001	i.p.	30	0	0	0	2	4					
Secondary	100	Oral	35	0	4	64	64	64					

* i.p. = Intraperitoneal injection.

† As measured by the 72 h PCA reaction and expressed as the reciprocal of the titre.

female rats. All the rats used in the experiments described hereafter were females.

Primary and secondary immunization with ovalbumin administered orally

Fifty rats were given 0, 1, 10, 50 or 100 mg of ovalbumin by the oral route. Half of them received 10^{10} *Bordetella pertussis* organisms by the oral route and the others by intraperitoneal injection at the same time. Ten days after immunization, all the rats were bled and the reaginic titrations performed. Rats given 10, 50 and 100 mg of antigen responded erratically, but some of them with high reaginic antibody levels. On day 30 after the first immunization, all the rats were given (orally) a single 10 mg dose of ovalbumin without adjuvant. The reaginic serum levels on day 5 and on day 10 after the second antigenic administration were determined. The secondary response was also very erratic although some rats were clearly immunized. No difference was observed between the two routes of administering the *B. pertussis* adjuvant, either in the primary or in the secondary response (Table 2). Sometimes, weak PCA titres were obtained with sera from unimmunized rats. PCA reactions were considered as positive only for rat sera giving titres higher than 1/8.

Primary administration of ovalbumin by the intraperitoneal route followed by secondary oral dosage

Forty female rats were injected with 1 μg of ovalbumin plus *Bordetella pertussis* and their serum was tested on day 30 for reaginic antibody levels. Groups of five or ten rats were challenged with 0, 0·01, 0·1, 1, 10, 50 and 100 mg of ovalbumin by the oral route without adjuvant. Table 3 shows the results of titration performed on the sera obtained on day 5 after the oral challenge. Consistent with the results obtained in the first experiment (Table 1), only a few of the animals were producing reaginic antibodies on day 30 after primary injection. On day 5 after the oral challenge, two rats out of five showed a slight increase in reaginic antibody levels following challenge with 10 μg dose. 100 μg to 50 mg doses resulted in positive responses in most of the rats. Maximum reaginic antibody levels were obtained with a 50 mg dose, to which the five rats of the group responded. Challenge with 100 mg resulted in poorer reagin response than with the 50 mg dose.

DISCUSSION

The reaginic response of an individual to certain antigens depends on various genetic loci (Levine, 1973; Kunz, Gill and Borland, 1974). Induction of reaginic antibodies requires very small doses of antigen (Vaz, Vaz and Levine, 1971; Jarrett and Stewart, 1974) and represents a good example of an immune response that can be studied only with high or middle responder animals. As shown in Table 1, the inbred LOU/M/Wsl rats seem to respond well to ovalbumin as antigen plus *Bordetella pertussis* organisms given as adjuvant. The primary response was similar to that of the Hooded Lister strain, a very high responder strain for ovalbumin (Jarrett and Stewart, 1974). The LOU/M/Wsl male rats seem to respond as well as the female, as already demonstrated in some other strains of rat (Murphey, Brown, Milkos and Fireman, 1974).

Administration of the antigen by the oral route was performed after a 12 h fasting period since previous experiments proved that such a fasting led to more constant results (Pomeranz, 1970; André, Bazin & Heremans, 1973).

Previously, the doses of antigen given by the oral route in order to obtain classical immunological responses of the IgA, IgM and IgG classes were very large (Crabbé *et al.*, 1969; Bazin *et al.*, 1970, 1973; Dolezel and Bienenstock, 1971). These massive doses were used with the idea that the intestinal mucosa represented a barrier to the absorption of antigens from the lumen. In fact, it has been demonstrated that the impermeability of the intestinal mucosa is relative (Bazin, 1976).

A 250 g rat consumes about 20 g of food a day. The greatest dose of antigen used in this study corresponds to 1/200 of the daily intake and seems physiological.

It has been demonstrated in many experimental systems with various species including man, that it is possible to immunize by the oral route. According to the models employed, the results obtained were different. In most of the cases, however, IgA, IgM and IgG with IgA class antibodies predominating have been demonstrated in the serum (Heremans and Bazin, 1971).

Table 2 shows that it is also possible to obtain reaginic immunological responses after oral immunization. In the primary response, an antigenic dose of 10 mg seems to be sufficient to obtain high reaginic titres on day 10. In the secondary response,

positive results are characterized by the great variability of the reaginic titres obtained among the experimental groups. The titres of the secondary responses do not seem to go higher than those of the primary responses. The secondary responses appear to be transient. Finally, the administration of *B. pertussis* organisms by the oral or the parenteral route seems to lead to a similar adjuvant effect.

Table 3 gives the results obtained by administering antigens by the oral route to animals sensitized by the intraperitoneal route. If the titres obtained with 10 μg doses are not clearly significant, those obtained with higher doses are, especially with 50 mg doses. In particular, oral administration of ovalbumin doses between 10 and 50 mg to primary immunized LOU/M/Wsl rats led to serum reaginic levels as high and constant as 1 μg of this same antigen by intraperitoneal injection. A 100 mg dose seems to be inhibitory to the production of high reaginic titres. This could be the result of the absorption into the circulation of quantities of antigen sufficiently large to have an inhibitory effect on the reaginic response (Jarrett and Stewart, 1974). It is also possible that the response induced by a large dose of antigen might not be evident because reaginic antibody could be rapidly removed from the circulation by binding to excess antigen.

It is clear, from these experimental results and from those by Jarrett, Haig, McDougall and McNulty (1976), that it is possible to obtain reaginic immunological responses in rats after oral ingestion of antigens. We have recently found (unpublished results) that similar oral immunization can be achieved in Gif TB mice.

In order to stimulate IgE responses by the oral route or other routes in laboratory rodents, it is necessary to fulfil certain conditions. It is, however, entirely possible that similar conditions could occur naturally (see discussion by Jarrett *et al.*, 1976). Our results are therefore probably of direct relevance to the clinical situation.

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