

The Non-Specific Enhancement of Allergy

I. *In Vivo* Effects of *Bordetella pertussis* Vaccine on IgE Synthesis

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Bordetella pertussis organisms, with or without a small dose of alumhydroxide, enhance in rats the production of IgE antibodies to an unrelated antigen, even if this antigen has been administered 6 weeks beforehand. This non-specific enhancement of IgE antibodies is accompanied by a substantial rise in total serum IgE and by the production of IgE antibodies to *B. pertussis*. The effect of *B. pertussis* on IgE synthesis is dose-dependent. No effect of *B. pertussis* on IgE production can be observed in nude rats.

Key words: adjuvants, immunologic; aluminium hydroxyde; allergy; IgE; Pertussis vaccine; rats.

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CLINICAL ASPECTS

The present experimental work shows two points which may be clinically relevant:

1. Bacterial organisms (*Bordetella pertussis*) can non-specifically enhance the production of IgE antibodies to an unrelated and previously administered antigen. This non-specific effect is probably mediated via T lymphocytes.
2. Bacterial organisms can induce the production of IgE antibodies to bacterial antigen without the need of another adjuvant. This animal model may prove useful in the study of the role of bacteria-specific IgE that has been demonstrated in asthma.

Bordetella pertussis (Bp) has long been used as adjuvant for the induction of reaginic antibodies (12). Although Bp enhances the formation of antibodies of the IgG and IgD class, it preferentially stimulates the production of IgE antibodies (15, 18). Bp has some B cell mitogen activity but its adjuvant effect is thought to be due to the stimulation of carrier-specific helper T cells (18). Bp stimulates the formation of inducers of IgE-binding and IgE-potentiating factors from T cells (7, 8). Immunization with Bp is accompanied by a substantial rise in total serum IgE (15). The increase in total serum IgE cannot be explained solely by the production of IgE antibodies specific to the employed antigen. Similar levels of specific IgE antibodies, using a low amount of alumhydroxide as adjuvant, are not accompanied by a

significant rise in total serum IgE, and increase of total serum IgE after Bp administration is observed even when only very small quantities of specific IgE antibodies can be demonstrated in the serum. The present study was planned to investigate the non-specific stimulating effect of Bp vaccine on IgE production.

MATERIAL AND METHODS

Animals

Outbred Wistar rats, inbred BN rats, nude rats rnu/rnu and heterozygotes rnu/+ were purchased from Proefdierencentrum, Louvain, Belgium. All animals were aged 2–3 months when used for study.

Adjuvants

Al(OH)₃ was prepared according to the method of Levine & Vaz (11). Equal volumes of 2 N Al₂(SO₄)₃ and 2N NaOH were mixed together. The gel was then washed repeatedly with water and homogenized with a mixer. Solid NaCl and Tris were added to a final concentration of 0.15 M NaCl and 0.01 M Tris. The pH was adjusted to 7.5 with 1M NaOH. The final Al(OH)₃ concentration was measured by dry weight determination. The gel was divided into small aliquots and stored at -80°C until use.

Two batches of Bp vaccine were used. Perthyrilal (Institute Pasteur Production, Paris) contained 10¹⁰ Bp organisms and 2.5 mg Al(OH)₃ per 2 ml. Bp organisms without Al(OH)₃ were obtained from the same source as a gift. Another Pertussis vaccine containing 2.10¹⁰ Bp/ml without Al(OH)₃ was obtained from the Swedish State Bacteriology Laboratory (by courtesy of H. Bergstrand). These two strains will hereafter be referred to as Bp Pasteur and Bp SBL, respectively.

Immunoglobulin E levels

Total serum IgE levels were measured using a direct solid phase radioimmunoassay method, the monoclonal IgE being purified by the technique of Bazin & Beckers (2, 16). Specific IgE antibodies against ovalbumin and Bp were measured with a solid phase radioimmunoassay method (17). The paper discs with Bp were prepared by incubating 1 g activated paper discs with 2.10¹¹ Bp SBL organisms in suspension. IgE antibody levels were expressed in arbitrary PRAST-units, using a laboratory standard as reference. One PRAST unit of specific IgE antibody to ovalbumin equals the amount present in a 1/64 dilution of rat serum with a PCA (passive cutaneous anaphylaxis) titer of 64. One PRAST unit of specific IgE antibody to Bp equals the amount present in a 1/100 dilution of rat serum with a PCA titer of 512. The PCA titer was determined using the technique of Bazin & Platteau (3).

Immunization

All immunizations were performed by intraperitoneal injection according to schedules discussed in the results section. Blood was taken by puncturing the orbital sinus under light ether anaesthesia at the indicated times.

Statistical methods

Non-parametric tests were used for statistical analysis (paired and unpaired Wilcoxon) as the IgE values were not normally distributed. A difference was regarded as significant if *P* values were less than 0.05. Median values are used for the graphical illustrations.

RESULTS

Influence of Bp vaccine on total serum IgE

BN rats were injected intraperitoneally on day 0 with either 10¹⁰ Bp Pasteur, 10¹⁰ Bp SBL, 10¹⁰ Bp Pasteur plus 2.5 mg Al(OH)₃, 10¹⁰ Bp SBL plus 2.5 mg Al(OH)₃ or 2.5 mg Al(OH)₃ (Fig. 1). A significant increase of total IgE was observed after Bp administration, not after the injection of Al(OH)₃ alone. The total serum IgE level was already significantly increased 7 days after administration of Bp except in the animals given Bp Pasteur without Al(OH)₃, where the IgE level rose after 2 weeks. The total serum IgE level remained significantly elevated up to day 28 in the four groups treated with Bp. The group of animals treated with Bp Pasteur plus Al(OH)₃ had a basal IgE value that was significantly higher than the other four groups. The IgE levels after Bp Pasteur plus 2.5 mg Al(OH)₃ were also significantly elevated compared with the other groups. The addition of Al(OH)₃ to Bp SBL did not enhance the IgE stimulating effect. In contrast, the preparation containing 10¹⁰ Bp Pasteur and 2.5 mg Al(OH)₃ had a significantly higher stimulating effect than 10¹⁰ Bp Pasteur alone. The serum levels of IgE antibodies specific to Bp are given in Table 1. Specific IgE antibodies to Bp were demonstrable in all four groups of animals given Bp, the highest levels being observed after injection of Bp SBL with or without

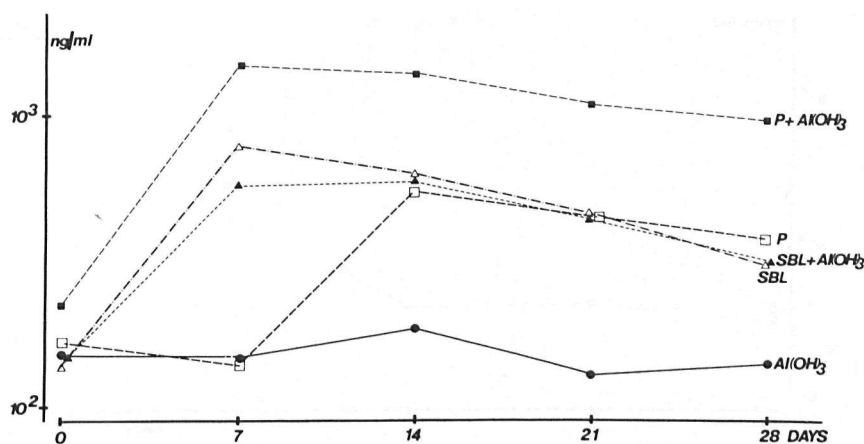


Fig. 1. Median total serum IgE levels in BN rats (10 animals/group) injected on day 0 with 2.5 mg Al(OH)₃ (●), 10¹⁰ Bp SBL (△), 10¹⁰ Bp SBL plus 2.5 mg Al(OH)₃ (▲), 10¹⁰ Bp Pasteur (□), or 10¹⁰ Bp Pasteur plus 2.5 mg Al(OH)₃ (■).

Table 1

Levels of IgE antibodies specific to Bp in BN rats injected intraperitoneally on day 0 with different combinations of Bp and Al(OH)₃.

Treatment	n		Day 0	Day 7	Day 14	Day 21	Day 28
Al(OH) ₃	10	median	0	0	0	0	0
		mean	0	0	0	0	0
		SD	0	0	0	0	0
10 ¹⁰ Bp SBL	10	median	0	40	28	28	28
		mean	0	46.5	33.5	32.4	32.8
		SD	0	37.2	21.7	17.9	11.0
10 ¹⁰ Bp SBL plus 2.5 mg Al(OH) ₃	10	median	0	36	28	28	28
		mean	0	33.1	32	36.6	32.2
		SD	0	16.1	16.9	20.2	12.4
10 ¹⁰ Bp Pasteur	10	median	0	0	7	1	0
		mean	0	6.6	12.5	6.9	3.5
		SD	0	13.9	17.4	12.9	6.8
10 ¹⁰ Bp Pasteur plus 2.5 mg Al(OH) ₃	10	median	0	6	23	28	8
		mean	0	15.7	37.6	8.6	3.4
		SD	0	30.2	34.4	5.7	1.7

Bp = *Bordetella pertussis*.

Al(OH)₃. The addition of Al(OH)₃ had no enhancing effect when Bp SBL was used but significantly increased the synthesis of Bp-specific IgE antibodies when Bp Pasteur was administered.

Effect of the dose of Bp vaccine

Three groups of Wistar rats received on day 0 respectively 5.10⁸ Bp Pasteur plus 0.125 mg Al(OH)₃ (n = 9), 5.10⁹ Bp Pasteur plus 1.25 mg

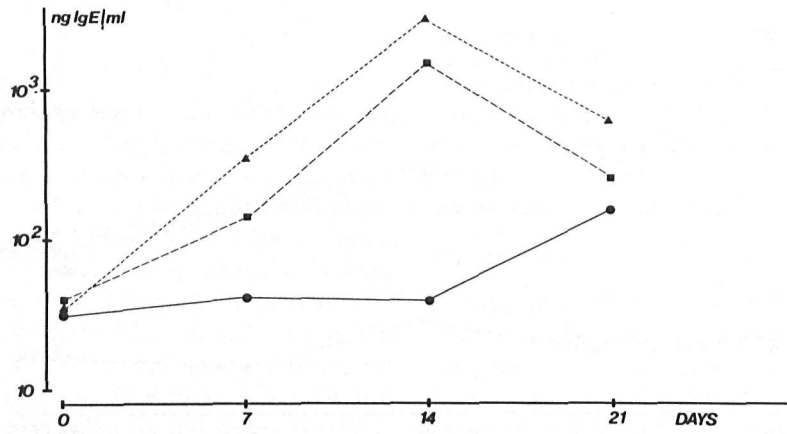


Fig. 2. Median total serum IgE levels in Wistar rats, receiving intraperitoneally on day 0 respectively $5 \cdot 10^8$ Bp Pasteur plus 0.125 mg Al(OH)_3 (●), $5 \cdot 10^9$ Bp Pasteur plus 1.25 mg Al(OH)_3 (■), or 10^{10} Bp Pasteur plus 2.5 mg Al(OH)_3 (▲).

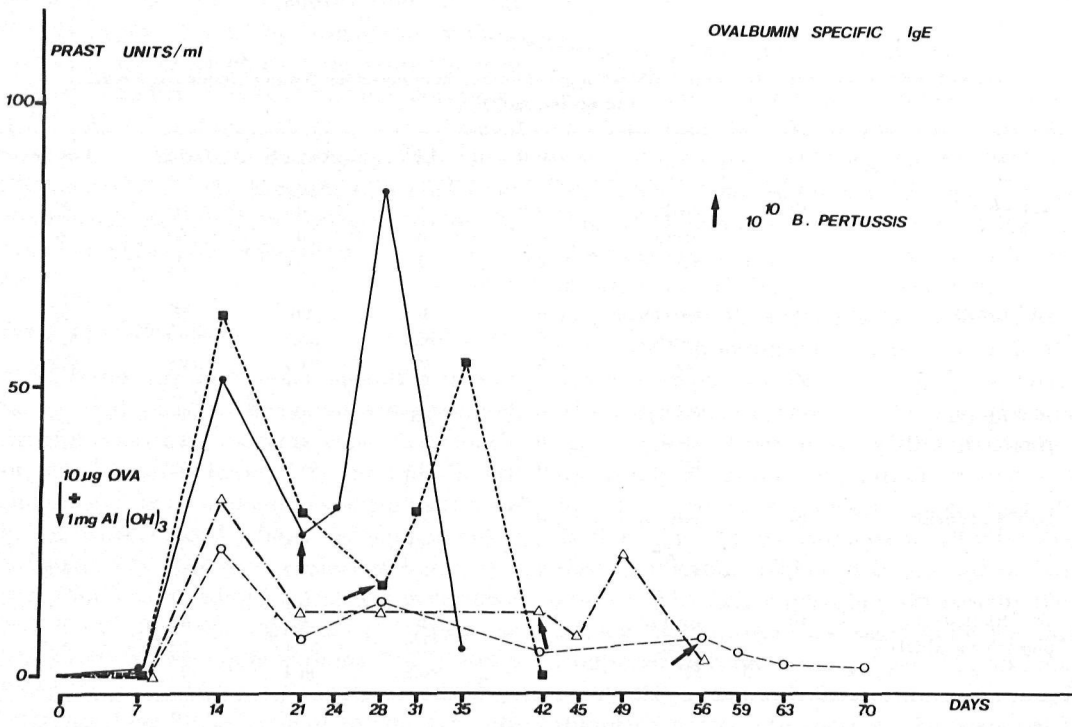


Fig. 3. Median serum levels of IgE antibodies specific to ovalbumin in four groups of 10 BN rats, who were immunized on day 0 with 10 μg ovalbumin and 1 mg Al(OH)_3 and who received at different times (indicated with $\downarrow$) 10^{10} Bp Pasteur plus 2.5 mg Al(OH)_3 .

Al(OH)_3 ($n = 8$), and 10^{10} Bp Pasteur plus 2.5 mg Al(OH)_3 ($n = 15$) by intraperitoneal injection. The total serum IgE levels are shown in Fig. 2. $5 \cdot 10^8$ Bp Pasteur organisms did not sig-

nificantly increase the total serum IgE level. However, $5 \cdot 10^9$ or 10^{10} Bp organisms in Wistar rats also caused a substantial increase of total serum IgE, demonstrable already 7 days after

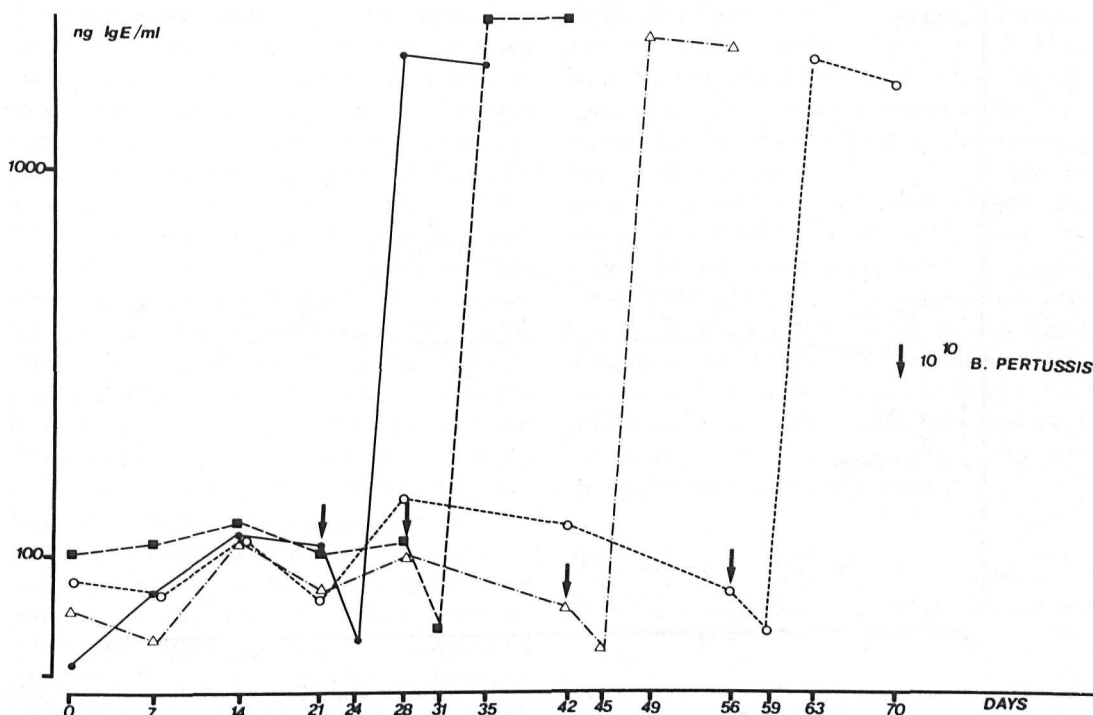


Fig. 4. Median total serum IgE levels in four groups of 10 BN rats, who were immunized on day 0 with 10 μ g ovalbumin and 1 mg $\text{Al}(\text{OH})_3$ and who received at different times (indicated with $\downarrow$) 10^{10} Bp Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$.

administration and reaching maximal values at day 14. The increase in total serum IgE was clearly dose-related, the highest dose of Bp organisms causing the steepest increase in total serum IgE. Small amounts of Bp specific IgE antibodies were detectable in the serum of the three groups of rats from day 7 on. The specific IgE levels were highest in the rats injected with 10^{10} Bp Pasteur organisms (results not shown).

Effect of Bp vaccine on the ongoing IgE response

Two groups of 20 BN rats each were immunized on day 0 with 10 μ g ovalbumin and 1 mg $\text{Al}(\text{OH})_3$ intraperitoneally and received respectively 10^{10} Bp Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$ or 10^{10} Bp SBL on day 21. Table 2 gives the serum levels of IgE antibodies specific to ovalbumin. The rats injected with 10^{10} Bp Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$ showed a booster response of their IgE antibody synthesis. In both groups a

substantial increase of the total serum IgE level and IgE antibodies specific to Bp were demonstrable at day 28 and day 35 (results not shown). In a further experiment four groups of 10 BN rats were immunized on day 0 in the same way with 10 μ g ovalbumin and 1 mg $\text{Al}(\text{OH})_3$ intraperitoneally and received respectively at day 21, day 28, day 42 and day 56 an intraperitoneal injection of 10^{10} Bp Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$. Fig. 3 illustrates the levels of IgE specific to ovalbumin and shows that the longer the time lag between the immunization and the administration of Bp the lower the enhancement of the IgE synthesis. However, the response of the total serum IgE level remained the same (Fig. 4).

Effect of Bp vaccine in nude rats

Twenty nude rats (rnu/rnu) and 15 heterozygotic (rnu/+) controls were injected intraperitoneally with 5 μ g ovalbumin and 10^{10} Bp

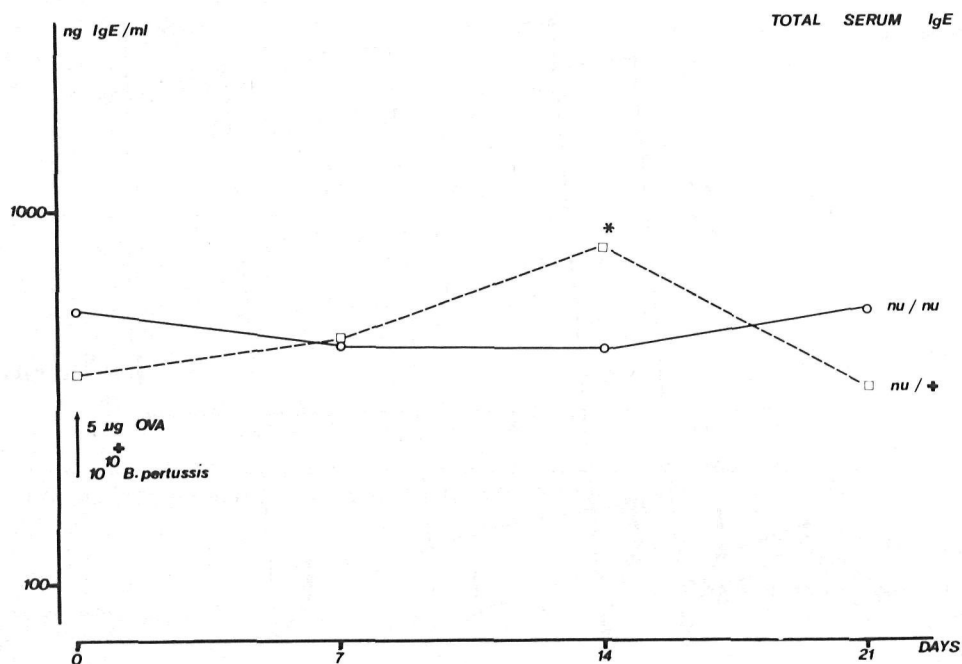


Fig. 5. Median total serum IgE level in nude (nu/nu) and heterozygotic (nu/+) rats immunized on day 0 with 5 µg ovalbumin and 10^{10} Bp Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$.

(* means that there is a significant difference between the two groups at that moment).

Table 2

IgE antibodies specific to ovalbumin in BN rats immunized on day 0 with 10 µg ovalbumin and injected at day 21 with 10^{10} *B. pertussis* organisms

Treatment			Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
A. Day 0: 10 µg OVA + 1 mg $\text{Al}(\text{OH})_3$	median		0	0	15	13	144	6.7
	mean		0	0.36	19.8	16.2	199.8	11.8
	SD		0	1.6	19.7	16.7	223.8	16.7
Day 21: 10^{10} Bp Pasteur + 2.5 mg $\text{Al}(\text{OH})_3$ (n = 20)								
B. Day 0: 10 µg OVA + 1 mg $\text{Al}(\text{OH})_3$	median		0	0	36	14	0	0
	mean		0	1.1	28.0	17.8	3.5	1.0
	SD		0	2.4	23.8	19.4	6.8	2.8
Day 21: 10^{10} Bp SBL (n = 20)								

Pasteur plus 2.5 mg $\text{Al}(\text{OH})_3$ on day 0. As illustrated in Fig. 5, the nude rats showed no increase in their total serum IgE level, in contrast to the heterozygotic control group, nor did they produce specific IgE to Bp or ovalbumin.

DISCUSSION

The adjuvant effect of Bp on the formation of IgE antibodies was demonstrated for the first

time by Mota (12). This observation has since then been confirmed many times (9, 18). Other biological activities of Bp, including promotion of leukocytosis, sensitization to histamine and other mediators and activation of the pancreatic islets, have also been shown (13).

Our results show that Bp has an enhancing effect on IgE synthesis even if it is not administered together with an antigen. The increase in total serum IgE, observed after the administration of Bp, can probably be explained by

the enhancement of IgE antibodies with many different specificities. This hypothesis is based on the observation that Bp enhances the synthesis of IgE antibodies specific to an antigen that has been administered several weeks before. The enhancement, however, decreases when the time period between antigen administration and the injection of Bp is increased. The potentiation of a preexisting IgE antibody response and the stimulation of the total serum IgE level has already been shown with parasitic infestations (10, 14). The potentiation by parasitic infestation is much more marked than the enhancement by Bp and although we could demonstrate a dose response effect with Bp vaccine we could not obtain a further enhancement by repeated administration of 10^{10} Bp organisms (results not shown).

Alumhydroxide, at the dose used, did not potentiate the IgE response. Higher doses are needed for that purpose (4). However, $\text{Al}(\text{OH})_3$ appears to have an additive or synergistic effect with Bp Pasteur in the enhancement of IgE synthesis and in the boosting of the synthesis of ovalbumin specific IgE. Only the Bp Pasteur preparation containing $\text{Al}(\text{OH})_3$ had a stimulating effect. Bp SBL did not have a similar stimulatory capacity. It is therefore impossible, with the present data, to conclude or exclude that alumhydroxide at a low dose acts synergistically with Bp on the IgE synthesis.

The formation of IgE antibodies to Bp was demonstrable both in Wistar and BN rats. BN rats, known as good IgE responders, showed the highest levels. The formation of IgE antibodies to Bp has already been shown (13) and IgE antibodies to bacteria other than Bp were also produced in experimental animals (5, 6, 19). In contrast with other bacteria, Bp does not need an additional adjuvant for the induction of specific IgE antibody. Bp contains both the antigenic and adjuvant material. The present data do not allow a conclusion as to whether the molecules responsible for the antigenic properties and the adjuvant activities are the same or different.

The non-specific effect of Bp on the ongoing IgE synthesis could be explained by the production of inducers of IgE potentiating factors

(7, 8). The time of the *in vivo* effect is comparable to the time period during which these inducers and IgE potentiating factors are demonstrable. The *in vitro* experiments have shown that the inducers and IgE potentiating factors are produced by helper T cells and act upon IgE-producing B cells in an antigen non-specific way (7, 8). Our results in the nude rats, where the IgE potentiating effect of Bp was not demonstrable, are in accordance with this hypothesis. The IgE in the serum of nude rats is thought to be derived from circulating IgE-bearing B cells by shedding (1). Further experiments are needed to prove our hypothesis but this *in vivo* model may be useful in studying the factors regulating IgE synthesis.

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