The booster injection of antigen during active sensitization of guinea-pig modifies the anti-anaphylactic activity of the PAF antagonist WEB 2086

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1 Serum IgG levels of sensitized guinea-pigs bled at various times after the booster injection were evaluated and its capacity to sensitize passively lung strips from normal guinea-pigs assessed. Following the booster injection, both serum IgG and the ability to sensitize passively lung strips increased during the first week and decreased slowly thereafter.

2 The PAF antagonist WEB 2086 (3 mg kg⁻¹, i.v.) blocked the anaphylactic bronchoconstriction induced by intravenous administration of ovalbumin (1 mg kg⁻¹) when guinea-pigs were challenged 2 and 4 days after the booster injection, but became ineffective when tested in guinea-pigs challenged 7, 28 and 56 days after the booster injection.

3 The ability of WEB 2086 to reduce anaphylactic bronchoconstriction of guinea-pigs challenged 2 and 4 days after the booster injection was unrelated to either the selective involvement of one type of immunoglobulin, low IgG titres in sera or a reduced sensitizing capacity.

4 The booster injection, which accounts for the loss of efficacy of WEB 2086 from the fourth day thereafter, probably operates as a PAF-independent inflammatory challenge.

5 The protocol for immunisation and the day of experiment after the booster injection determines the sensitivity of the anaphylactic bronchoconstriction to inhibition by PAF antagonists.

Introduction

It has been suggested that platelet-activating factor (PAF) is involved in the pathogenesis of anaphylaxis in several species, including the guinea-pig (Vargaftig *et al.*, 1980; Basran *et al.*, 1984). In the past, we have used passive sensitization to study the ability of PAF antagonists to interfere with anaphylactic shock, because we thought that the transfer of antibodies to a non-sensitized animal, which is shocked within 10 days, favours IgE-dependent mechanisms, involved in human asthma. We later demonstrated that in fact serum from actively sensitized animals transfers a mixture of IgE and IgG antibodies, which are relevant for anaphylactic bronchoconstriction (S. Desquand, B. Rothhut & B.B. Vargaftig, unpublished). Since asthma involves active human sensitization and multiple rather than single exposures to antigen, we became interested in the potential antagonism by a standard PAF antagonist of anaphylactic bronchoconstriction in this model.

The effectiveness of the triazolothienodiazepine WEB 2086 (Casals-Stenzel, 1986) against active anaphylaxis was previously reported but in fact, different experimental procedures of sensitization may lead to differences in the effectiveness of this PAF antagonist against active anaphylaxis. Thus, Casals-Stenzel (1987) found that WEB 2086 blocks anaphylactic bronchoconstriction, whereas Pretolani *et al.*, (1987) reported that the same antagonist was ineffective. Since these differences might be pertinent to the sensitization regimen or to other factors, we have now studied the correlation between the time-course of the anaphylactic responses, the titres of circulating IgG and the ability of serum collected from actively sensitized guinea-pigs at different intervals after the sensitizing injection to transfer antigen responsiveness to isolated tissues. The possible relationship between the presence of different subclasses of immunoglobulins involved and particularly the role of the booster injection were correlated with the ability of WEB 2086 to interfere with anaphylaxis.

Methods

Procedures of active sensitization

Guinea-pigs (300–400 g) were actively sensitized at day 0 by the s.c. injection of 0.5 ml of 0.9% w/v NaCl solution (saline) containing ovalbumin (OA) 10 µg dispersed in 1 mg Al(OH)₃ (modified from Andersson, 1980; 1981). When appropriate, guinea-pigs were boosted with a similar injection of antigen at day 14. They were used 2, 4, 7, 28, 56 or 84 days after the booster injection (groups called respectively 14+2, 14+4, 14+7, 14+28, 14+56 or 14+84). In some experiments, the booster injection was omitted and the animals were used 14, 21, 42, 70 or 98 days after the first injection (groups called respectively 0–14, 0–21, 0–42, 0–70 or 0–98).

Determination of ovalbumin-specific IgG by ELISA

Blood (0.5 ml) was collected from the carotid artery of pentobarbitone-anaesthetized sensitized guinea-pigs of the different groups described below, for the determination of IgG titres by enzyme-linked immunosorbent assay (ELISA). Flat-bottomed polystyrene microtitre plates (Nunc) were coated overnight at 4°C with 100 µl of OA 1 µg ml⁻¹ in 0.05 M sodium carbonate buffer, pH 9.5. The plates were washed three times in 0.15 M PBS (phosphate buffer solution), pH 7.5 containing 0.1% Tween 20 (PBS-Tween) and drained. All washes were performed in this way, and subsequent incubations were for 2 h at 37°C. All dilutions of sera and antisera were prepared in PBS-Tween. Each well was incubated successively with the following solutions with washes between each step: 200 µl of 1 µg ml⁻¹ of bovine serum albumin (BSA); 100 µl of labelled goat anti-guinea-pig IgG antiserum diluted 1 to 2000; 100 µl of the substrate solution at pH 4.0 containing 0.1 M citrate, 3% hydrogen peroxide (30% w/v) and 250 µg ml⁻¹ of 2,2'-azino-bis(3-ethyl benzothiazoline sulphonic acid) di-ammonium salt, 0.5H₂O (ABTS). The enzyme-substrate reaction was stopped after 30 min by adding to each well 50 µl of a 10% sodium dodecyl sulphate (SDS) solution in distilled water. Absorbances were measured at 450 nm with an automated

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microplate-reader (MR 700, Dynatech Laboratories Inc., Marnes La Coquette, France). Titres of OA-specific IgG were expressed as the maximal dilution giving 50% of the maximum net absorbance after subtraction of the values obtained with control sera from non-immunized guinea-pigs.

Preparation of the isolated lung strips

Non-immunized guinea-pigs were anaesthetized and ventilated. A mid-thoracotomy was performed, the lungs were then removed and rinsed with 20 ml of Krebs solution injected through the pulmonary artery. As described by Lulich *et al.*, (1976), peripheral sub-pleural strips (2.5–3 cm long; 3–4 mm width) were dissected and mounted under 2 g tension in an isolated organ bath containing 16 ml Krebs solution at 37°C and aerated with 95% O₂ plus 5% CO₂. Each strip was connected to a Gould-Statham force displacement transducer. After approximately 1 h for equilibration, histamine was added cumulatively (1–10–100 µM) at 3 min intervals. After three rinses, 30 min later, the same cycle was repeated; the maximal contraction induced by cumulative concentrations of histamine was taken as a reference.

Lung strips were incubated with 0.1, 0.3, 1, 3 or 30 µl ml⁻¹ of serum provided from actively sensitized guinea-pigs of the group 14+7 and with 30 µl ml⁻¹ of serum prepared with guinea-pigs from the other groups described below.

After 1 h of incubation, the Krebs solution was removed and a series of three rinses was performed. Twenty minutes later, the lung strips were stimulated with OA 10 µg ml⁻¹, added to the organ bath. The intensities of the contractions were expressed as a percentage of the maximal contraction induced by histamine. Aliquots of 0.2 and 1 ml of the Krebs solution were removed from the organ bath before and 20 min after stimulation by OA, to evaluate thromboxane B₂ (TxB₂) formation and histamine release respectively.

In vivo preparation

Guinea-pigs of groups 0–14, 14+2, 14+4, 14+28 or 14+56 were anaesthetized with sodium pentobarbitone (40 mg kg⁻¹, i.p.) and prepared for the recording of bronchial resistance to inflation on a Beckman Dynograph R 511 (Schiller Park, IL, U.S.A.) as described by Lefort & Vargaftig (1978).

Spontaneous breathing was suppressed with pancuronium (2 mg, i.v.). A jugular vein was catheterized for drug injection and both carotid arteries were cannulated, one to measure arterial blood pressure with a Statham-Gould transducer P23Db and the other one for blood sampling. The bronchial reactivity was assessed with 5-hydroxytryptamine (5-HT, 3 µg, i.v.) and experiments were initiated when reproducible responses were obtained. According to Casals-Stenzel (1987) and Pretolani *et al.* (1987), guinea-pigs were treated 5 min before challenge with 5 µg kg⁻¹ of the histamine H₁-receptor blocker, mepyramine, to prevent the sudden death following antigen challenge but neither bronchoconstriction nor the arterial hypotension of acute anaphylaxis (Brocklehurst, 1960). Guinea-pigs were then challenged with a bolus injection of OA 1 mg kg⁻¹, i.v. Treated animals were injected with WEB 2086 3 mg kg⁻¹, i.v., 10 min before OA, control animals being injected with the solvent as previously described (Pretolani *et al.*, 1987). The bronchopulmonary resistance to inflation following administration of the antigen was monitored and bronchoconstriction was expressed in cm² of the area of the tracing over the base line for 20 min.

Blood cell counts

Blood samples (200 µl) were collected from the carotid artery for the assessment of total leucocyte and platelet counts with a Coulter Counter ZBI. Blood samples were obtained before then 1, 3 and 6 min after the injection of OA.

Radioimmunoassay of thromboxane B₂

TxB₂ was assayed according to the method of Sors *et al.*

(1978). Briefly, aliquots (200 µl from the organ bath) were left at room temperature for 60 min (to allow conversion of TxA₂ into its stable metabolite TxB₂) and were stored at –20°C. For the radioimmunoassay, the samples were thawed and incubated overnight at 4°C with ¹²⁵I-iodine-labelled TxB₂ and anti-TxB₂ antiserum in a phosphate buffer (10 mM, pH 7.4) containing bovine gamma globulin (0.3% w/w). The next day, bound and free fractions were separated by the addition of a solution of polyethyleneglycol 6000 (30% in distilled water) followed by centrifugation at 2000 g and 4°C for 10 min. The supernatant was decanted and the radioactivity present in the pellet, corresponding to the bound fraction was counted for 1 min in a Kontron Analytical gamma counter (model MDA 312).

Spectrofluorimetric assay of histamine

One ml of the collected fractions was mixed with 1 ml of perchloric acid (0.8 N). After centrifugation for 30 min at 3500 r.p.m. and at 4°C, the supernatants were stored at 4°C. An automatic fluorimetric assay for histamine was performed according to Shore *et al.* (1959), as modified by Lebel (1983).

Data analysis

The results are expressed as the means ± s.e.mean. Statistical differences between two means of data were evaluated by the unpaired Student's *t* test.

Materials

The Krebs solution was composed of mM: NaCl 118, KCl 4.7, CaCl₂ · 2H₂O 2.5, MgSO₄ · 7H₂O 1.2, KH₂PO₄ 1.2, NaHCO₃ 25 and glucose 5.6.

The PAF antagonist WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6H-thieno (3,2-f) (1,2,4)-triazolo-(4,3-a)-(1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone; Boehringer-Ingelheim, F.R.G.) was initially dissolved in HCl 50 mM in distilled water and further diluted to the appropriate concentrations in distilled water. The other drugs were dissolved in saline and were obtained as indicated: sodium pentobarbitone (Nembutal, Sanofi, France); pancuronium bromide (Pavulon, Organon, France); mepyramine maleate (Rhône-Poulenc Santé, Vitry/Seine, France); 5-hydroxytryptamine (serotonin, 5-HT), histamine, ABTS, SDS (Sigma Chemical Co., St. Louis, MO, U.S.A.); chicken ovalbumin (OA, Miles, Naperville, IL, U.S.A.); Al(OH)₃, perchloric acid, polyethyleneglycol 6000 (Merck, Darmstadt, FRG); peroxidase-labelled goat anti-guinea-pig IgG (Biosis, Compiègne, France). The antibody radiolabelled ligands for the radioimmunoassay of TxB₂ were from the UR1A (Unité Associée Institut Pasteur-INSERM n° 207).

Results

Time-course of the IgG production and of the ability of sera from actively sensitized guinea-pigs to sensitize lung strips in vitro

Sera of guinea-pigs collected up to 14 days after the sensitizing injection of OA contained neither IgG (Figure 1) nor transferable sensitizing activities as measured by parenchyma lung strip contraction and release of TxB₂ and histamine following OA challenge (Figures 2, 3, 4). When the animals were not boosted and were bled 21, 42, 70 or 96 days after the sensitizing injection of OA, their sera contained very low amounts of IgG (Figure 1) and also failed to sensitize passively lung strips from normal guinea-pigs (Figures 2, 3, 4).

In contrast, when a booster injection of OA was administered at day 14, the IgG titres as well as the intensity of the contractions of passively sensitized lung strips, accompanying

Figure 1 Titres of IgG in sera of guinea-pigs bled at different times during sensitization. The titres of IgG of the sera of guinea-pigs bled 2, 4, 7, 28, 56 or 84 days after the booster injection at day 14 (○) were compared to those of sera of non-boosted guinea pigs bled at days 14, 21, 70 or 98 (●). Titres are expressed according to the reciprocal of the maximal dilution giving 50% of the absorbance by ELISA. Bars = s.e.mean. Experiments with sera of guinea-pigs of group 14 + 2: $n = 10$; group 14 + 4: $n = 15$; group 14 + 7: $n = 34$; group 14 + 28: $n = 20$; group 14 + 56: $n = 10$; group 14 + 84: $n = 3$; group 0-14: $n = 7$; group 0-21: $n = 15$; group 0-42: $n = 7$; group 0-70: $n = 8$; group 0-98: $n = 6$.

Figure 2 Ovalbumin-induced contractions of lung strips sensitized *in vitro* for 1 h with sera from actively sensitized guinea-pigs and bled as described in legend to Figure 1. The vertical scale shows the intensity (in % of the maximal effect of histamine) of the contractions induced by ovalbumin $10 \mu\text{g ml}^{-1}$ added to the organ bath after a 1 h tissue sensitization with $30 \mu\text{g ml}^{-1}$ of sera from guinea-pigs sensitized and boosted at day 14 and bled at the indicated intervals (□) or non-boosted (■). Bars = s.e.mean. Experiments with sera of guinea-pigs of group 14 + 2: $n = 10$; group 14 + 4: $n = 12$; group 14 + 7: $n = 4$; group 14 + 28: $n = 14$; group 14 + 56: $n = 13$; group 14 + 84: $n = 3$; group 0-14: $n = 9$; group 0-21: $n = 14$; group 0-42: $n = 4$; group 0-70: $n = 4$; group 0-98: $n = 4$.

formation of TxB_2 and release of histamine rapidly increased to a maximum at day 21, to decrease slowly thereafter (Figures 1, 2, 3, 4).

It is noteworthy that the IgG titres followed the same time-course as the sensitizing capacity of the sera, with the exception that sera from guinea-pigs of group 14 + 4 showed a remarkable ability to sensitize isolated lung strips even though they contained low IgG titres (Figures 1, 2, 3, 4). To verify if the increase of the sensitizing activity involved antibodies of the IgE sub-class, strips were sensitized with serum from guinea-pigs of group 14 + 4, which had been previously heated at 56°C for 4 h to inactivate IgE (Parish, 1970). The OA-induced tissue contractions, the TxB_2 formation and histamine release from lung strips sensitized with heated sera were reduced, as compared to experiments with paired un-heated sera, by $41.4 \pm 17.6\%$, $22.4 \pm 13.3\%$ and $25.7 \pm 14.9\%$ ($n = 4$), respectively. These reductions were not statistically significant.

Figure 3 Ovalbumin-induced formation of thromboxane B_2 (TxB_2) by lung strips sensitized *in vitro* for 1 h with sera from actively sensitized guinea-pigs and bled as described in legend to Figure 1. The vertical scale shows the amounts of TxB_2 (ng ml^{-1}) formed following the addition of ovalbumin $10 \mu\text{g ml}^{-1}$ to the organ bath after a 1 h tissue sensitization with $30 \mu\text{g ml}^{-1}$ of sera from guinea-pigs sensitized and boosted at day 14 and bled at the indicated intervals (□) or non-boosted (■). Bars = s.e.mean. Experiments with sera of guinea-pigs of group 14 + 2: $n = 10$; group 14 + 4: $n = 12$; group 14 + 7: $n = 4$; group 14 + 28: $n = 14$; group 14 + 56: $n = 13$; group 14 + 84: $n = 3$; group 0-14: $n = 9$; group 0-21: $n = 14$; group 0-42: $n = 4$; group 0-70: $n = 4$; group 0-98: $n = 4$.

Figure 4 Ovalbumin-induced release of histamine by lung strips sensitized *in vitro* for 1 h with sera from actively sensitized guinea-pigs and bled as described in legend to Figure 1. The vertical scale shows the amounts of histamine (ng ml^{-1}) released following the addition of ovalbumin $10 \mu\text{g ml}^{-1}$ to the organ bath after a 1 h tissue sensitization with $30 \mu\text{g ml}^{-1}$ of sera from guinea-pigs sensitized and boosted at day 14 and bled at the indicated intervals (□) or non-boosted (■). Bars = s.e.mean. Experiments with sera of guinea-pigs of group 14 + 2: $n = 10$; group 14 + 4: $n = 12$; group 14 + 7: $n = 4$; group 14 + 28: $n = 14$; group 14 + 56: $n = 13$; group 14 + 84: $n = 3$; group 0-14: $n = 9$; group 0-21: $n = 14$; group 0-42: $n = 4$; group 0-70: $n = 4$; group 0-98: $n = 4$.

Dose-dependence of the contraction, formation of thromboxane B_2 and release of histamine of lung strips sensitized *in vitro*

Lung strips were incubated with 30, 3, 1, 0.3, $0.1 \mu\text{l ml}^{-1}$ of serum from actively sensitized guinea-pigs of group 14 + 7. The OA-induced responses were proportional to the concentration of sera used to sensitize the lung strips (Figure 5). Similar experiments were performed with sera from the other groups of sensitized guinea-pigs (boosted or non-boosted) and displayed the same patterns of response (data not shown).

Effect of WEB 2086 on bronchoconstriction induced by the injection of ovalbumin to guinea-pigs actively sensitized with a booster injection (groups 14 + 2, 14 + 4, 14 + 28 and 14 + 56)

Administration of OA 1 mg kg^{-1} to non-boosted guinea-pigs

Figure 5 Intensity of contractions (in % of the maximal effect of histamine, a) and release of thromboxane B_2 (TxB₂ ng ml⁻¹, b) and histamine (ng ml⁻¹, c) following the stimulation with ovalbumin ($10 \mu\text{g ml}^{-1}$) of lung strips sensitized by different concentrations of serum provided from guinea-pigs of group 14+7. Bars = s.e.mean. Experiments with $0.1 \mu\text{l ml}^{-1}$: $n = 8$; $0.3 \mu\text{l ml}^{-1}$: $n = 10$; $1 \mu\text{l ml}^{-1}$: $n = 9$; $3 \mu\text{l ml}^{-1}$: $n = 12$; $30 \mu\text{l ml}^{-1}$: $n = 4$.

7 days after the initial sensitizing injection failed to induce bronchoconstriction. In addition, erratic responses following provocation with OA were observed at day 14. In contrast, the administration of OA, 2 days after the booster injection, induced bronchoconstriction. The intensity of the response observed in guinea-pigs used 2, 4, 28 and 56 days after the booster increased gradually, although this was not significant. As shown in Figure 6, intravenous administration of WEB 2086, 3 mg kg^{-1} , blocked significantly the anaphylactic bronchoconstriction when OA was administered 2 and 4 days after the booster injection. In contrast, WEB 2086 failed to modify the anaphylactic bronchoconstriction when OA was administered 28 and 56 days after the booster injection (Figure 6). It is noteworthy that sera of both groups of guinea-pigs had a capacity to passively sensitize the isolated lung strips from non-immunized guinea-pigs similar to that from actively sensitized guinea-pigs of group 14+4 (Figures 2, 3, 4).

Failure of WEB 2086 to prevent the decrease of blood cell counts induced by ovalbumin

Anaphylactic bronchoconstriction in the different groups of guinea-pigs was accompanied by thrombocytopenia and leucopenia during the 6 min following the challenge (Table 1). These parameters were not modified by pretreatment with WEB 2086, even when bronchoconstriction was blocked (Table 1).

Figure 6 Interference of WEB 2086 (3 mg kg^{-1}) with the intensity of bronchoconstriction (cm²) induced by an i.v. injection of ovalbumin 1 mg kg^{-1} to actively sensitized and mepyramine-treated ($5 \mu\text{g kg}^{-1}$) guinea-pigs, challenged 2, 4, 28 or 56 days after the booster injection at day 14 (groups 14+2, 14+4, 14+28 or 14+56 days respectively). Open columns represent the responses of control guinea-pigs and closed columns those of treated animals. Bars = s.e.mean. * $P < 0.05$. Groups 14+2 and 14+4, control guinea-pigs: $n = 5$ for each; treated guinea-pigs: $n = 6$ for each. Group 14+28, control guinea-pigs: $n = 6$; treated guinea-pigs: $n = 6$. Group 14+56, control guinea-pigs: $n = 6$; treated guinea-pigs: $n = 5$.

Discussion

In this study, the temporal development of guinea-pig sensitization, as evaluated by the IgG titres and by the appearance of homocytotropic antibodies in serum, i.e., antibodies which sensitize passively isolated tissues from the same animal species, was investigated by a procedure modified from that of Andersson (1980; 1981), which induces the formation of a mixture of IgE and IgG (S. Desquand, B. Rothhut & B.B. Vargaftig, unpublished).

Neither IgG nor transferable sensitizing activities were detected in serum up to 14 days after the first injection of antigen. In addition, sera from non-boostered animals bled 21, 42, 70 or 96 days later contained very low amounts of IgG and failed to sensitize passively lung strips from control guinea-pigs. In contrast, when a booster injection of antigen was administered at day 14, the IgG titres and the capacity of serum to sensitize lung strips increased rapidly, peaked at day 21, decreasing very slowly thereafter.

It is noteworthy that the IgG titres and the capacity of serum to sensitize lung tissues passively and to induce passive cutaneous anaphylaxis follow a similar time-course (Pretolani *et al.* 1989). We recently demonstrated that IgG of guinea-pigs of group 14+7 account more for the transferable activity of sera than does IgE, since they are thermo-resistant, whereas IgE are thermo-labile (S. Desquand, B. Rothhut & B.B. Vargaftig, unpublished). However, it should be noted that the serum of guinea-pigs of group 14+4, which contained low IgG titres, displayed a high *in vitro* sensitizing capacity, which was also not accounted for by IgE, since it was thermo-resistant (Parish, 1970).

The time-dependent profile of immunisation was used to understand the differential effectiveness of PAF antagonists. Indeed, Casals-Stenzel (1987) showed that WEB 2086 blocks the antigen-induced anaphylactic bronchoconstriction in guinea-pigs actively sensitized by a single injection of OA and used 28 days later in the presence of mepyramine. In contrast, Pretolani *et al.* (1987) reported that WEB 2086 fails to inhibit the anaphylactic bronchoconstriction when OA is administered to mepyramine-treated actively sensitized guinea-pigs of group 14+7. The differences in the ability of WEB 2086 to block antigen-induced bronchoconstriction reported by Casals-Stenzel (1987) and Pretolani *et al.* (1987), might have resulted from the different types and amounts of immunoglobulins involved or from a role of the booster injection. We have recently shown that anaphylactic bronchoconstriction induced by the intratracheal administration of antigen to

Table 1 Interference of WEB 2086 with the decrease of blood cell counts induced by ovalbumin (OA)

Cell count decrease (%)	min	14 + 2		14 + 4	
		Control	WEB 2086	Control	WEB 2086
Platelets	1	29.0 ± 16.7	28.7 ± 8.9	68.6 ± 12.9	71.8 ± 13.2
	3	46.2 ± 16.9	39.0 ± 11.4	88.8 ± 4.2	84.0 ± 8.2
	6	56.7 ± 20.5	52.7 ± 11.1	93.6 ± 1.6	9.11 ± 3.4
Leucocytes	1	18.5 ± 9.2	12.8 ± 3.7	31.2 ± 15.2	34.2 ± 8.4
	3	33.5 ± 8.6	36.5 ± 10.6	41.6 ± 20.4	22.0 ± 10.9
	6	38.7 ± 7.2	40.8 ± 11.1	33.2 ± 17.5	23.8 ± 11.9
	min	14 + 28		14 + 56	
		Control	WEB 2086	Control	WEB 2086
Platelets	1	39.8 ± 13.5	51.8 ± 16.3	2.7 ± 1.7	6.8 ± 2.2
	3	64.5 ± 18.4	77.0 ± 10.8	12.0 ± 6.5	10.6 ± 3.8
	6	67.5 ± 16.9	85.7 ± 4.7	35.8 ± 10.5	36.6 ± 12.3
Leucocytes	1	16.3 ± 10.4	24.3 ± 9.3	21.3 ± 7.3	26.6 ± 3.3
	3	34.8 ± 6.7	51.7 ± 6.1	33.8 ± 9.1	35.2 ± 12.5
	6	59.2 ± 10.0	51.4 ± 9.7	35.7 ± 12.2	28.8 ± 14.8

Platelet and leucocyte count decreases were evaluated 1, 3 and 6 min after the challenge by an i.v. injection of OA to 1 mg kg⁻¹, to mepyramine-treated guinea-pigs actively sensitized and used 2, 4, 28 or 56 days after the booster injection (groups 14 + 2, 14 + 4, 14 + 28, or 14 + 56 respectively). Treated animals were injected with WEB 2086 3 mg kg⁻¹, 10 min before OA. Figures are the % reduction in counts.

Groups 14 + 2 and 14 + 4, control guinea-pigs: *n* = 5 for each; treated guinea-pigs: *n* = 6 for each.

Group 14 + 28, control guinea-pigs: *n* = 6; treated guinea-pigs: *n* = 6.

Group 14 + 56, control guinea-pigs: *n* = 6; treated guinea-pigs: *n* = 5.

actively sensitized guinea-pigs without a booster injection is suppressed by another PAF antagonist, BN 52021, whereas bronchoconstriction triggered in boosted guinea-pigs becomes refractory to this PAF antagonist (S. Desquand, J. Lefort, C. Dumarey & B.B. Vargaftig, unpublished). For this reason, we studied the effects of WEB 2086 against the anaphylactic bronchoconstriction induced by the i.v. administration of OA to actively sensitized guinea-pigs at different time intervals after the booster injection. Since anaphylactic bronchoconstriction in actively sensitized and boosted guinea-pigs (group 14 + 7) is largely mediated by histamine (Detsouli *et al.*, 1985), the animals were pretreated with mepyramine to avoid sudden death (Brocklehurst, 1960), and thus allow the study of the supposedly PAF-dependent component of the anaphylactic reaction. Bronchoconstriction was always present, irrespective of mepyramine and of the group of boosted guinea-pigs concerned. These responses were of comparable intensity, even if a slight increase was noted with the increasing intervals between the booster injection and the day of the experiment.

It should be noted that guinea-pigs of group 14 + 2, which have low amounts of circulating immunoglobulins in the serum, also developed anaphylactic bronchoconstriction. This indicates that immunoglobulins are associated with the targeted inflammatory cells before their levels become detectable in the serum. Surprisingly, although anaphylactic bronchoconstrictions were of the same magnitude, WEB 2086 only antagonized bronchoconstriction when it was administered 2 and 4 days after the booster, but not later. This pattern of activity may result from a shift from a PAF-dependent early phase of sensitization to a less PAF-dependent delayed phase. The booster is the essential factor determining this shift, which accounts for the effectiveness of WEB 2086 in non-boosted animals (Casals-Stenzel, 1987) and for its failure 7 days after the booster (Pretolani *et al.*, 1987). In addition, since the titres of homocytotropic antibodies in the serum of the animals of groups 14 + 2, 14 + 28 and 14 + 56 had a time-course similar to that of IgG, the sensitivity of anaphylactic bronchoconstriction to WEB 2086 does not result from a selective involvement of one type of immunoglobulin. Furthermore, interference of WEB 2086 with anaphylactic bronchoconstriction in guinea-pigs of group 14 + 2 and 14 + 4 is probably not due to reduced IgG titres and/or capacity to induced passive sensitization, since sera of animals of group 14 + 4 were as able to cause passive sensitization of isolated

tissues as those from guinea-pigs of groups 14 + 28 and 14 + 56. A final argument in favour of the dissociation between the sub-class and amount of immunoglobulins formed on one hand, and the failure of PAF antagonists to block bronchoconstriction on the other, is provided by our results (S. Desquand, J. Lefort, C. Dumarey & B.B. Vargaftig, unpublished) showing that guinea-pigs of the group 14 + 84, with low titres of circulating immunoglobulins were still at this late stage, as refractory to inhibition by BN 52021 as those of groups 14 + 7, 14 + 28, 14 + 56, which had elevated IgG titres and which were effective in passively sensitizing lung parenchyma strips. The failure of WEB 2086 to inhibit anaphylactic bronchoconstriction is thus accounted for by the same booster which triggers the development of lung hyper-responsiveness (Pretolani *et al.*, 1988). In addition, it is clear that the subtle differences in the effectiveness of PAF antagonists against anaphylaxis are neither specific for WEB 2086 nor result from different sub-classes or amounts of immunoglobulins.

Our claim that the booster injection determines whether the anaphylactic bronchoconstriction will become refractory to WEB 2086 four days later explains the initial disappointing results obtained with PAF antagonists used against active anaphylactic bronchoconstriction and negates previous conclusions that PAF is not important for acute anaphylaxis in actively sensitized guinea-pigs (Detsouli *et al.*, 1985; Lagente *et al.*, 1985; Danko *et al.*, 1988). The pharmacological modulation of the bronchopulmonary and secretory effect of PAF changes between the fourth and the seventh day following the booster injection, since the latter triggers important modifications of the lung reactivity to mediators including PAF probably due to its invasion by inflammatory cells (Pretolani *et al.*, 1988).

In conclusion, our results suggest that the booster injection to a pre-sensitized animal will determine the relative efficacy of a PAF antagonist against bronchoconstriction. As a corollary, potential allergic drugs should be tested in guinea-pigs sensitized according to very precise protocols, including boosted guinea-pigs, particularly since in clinical situations, atopic patients are repeatedly exposed to the allergen.

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