

Selective Suppression of Antibody Production with the Aid of Radiolabelled Birch Pollen Allergen

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In accordance with the clonal selection theory we intended to prevent the development of artificially induced birch pollen allergy in rabbits with the aid of the radiolabelled pollen allergen (75–1000 $\mu\text{Ci}^{125}\text{I}$ -pollen/animal) intravenously administered prior to pollen sensitization. The birch pollen allergen, in accordance with Burnet's working hypothesis, reacts only with a genetically determining B cell subpopulation. The fixation of the radiolabelled birch pollen allergen to the receptors of the competent B cell clone causes the lesion of the latter. Compared with the control group, this group of rabbits showed an extensive suppression of anaphylactic reagin-like PCA-antibodies, and haemagglutinating antibodies in the blood as well as in nasal secretion. In addition, we tried to influence the already ongoing synthesis of the antibodies with the aid of a subsequent intravenously administered radiolabelled birch pollen allergen (750–1000 $\mu\text{Ci}^{125}\text{I}$ -pollen/animal). An intensive suppression of the synthesis of antibodies could also be proved in this case. The simultaneous immunization of the control rabbits with birch pollen and egg albumin resulted in the production of antibodies against both antigens, as expected. The hot-labelled birch pollen antigen intravenously injected before or after immunization with egg albumin and birch pollen led selectively to suppression of anti-birch-pollen PCA antibodies. The synthesis of anti-egg albumin PCA antibodies was unaffected.

Key words: hot labelled allergen; ^{125}I -labelled birch pollen allergen; radioactive damage of B-cell clone; selective immunosuppression.

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The working clonal selection theory implies that the allergen reacts only with receptors of a genetically determined immunocompetent cell subpopulation. Thus, by using allergen which is labelled with ^{125}I of high specific activity, radiation damage can be selectively induced in the competent cell clone. In this study, induced birch pollen allergy in rabbits was used as an experimental model. Rabbits can be easily sensitized by birch pollen administered intravenously and pernasally. This combined sensitization results in the formation of skin sensitizing PCA antibodies and haemagglutinating antibodies in the blood as well as in nasal secretion (1).

Our first question was whether it is possible to disturb the production of anti-birch pollen antibodies by previous intravenous administration of hot labelled birch pollen antigen, and second, whether it is possible to suppress the already induced immunological process, i.e. the already ongoing production of anti-birch pollen antibody by the intravenous application of the hot labelled birch pollen. The last question concerned the selectivity of the immunosuppression. The animals were immunized parallelly with two antigens: birch pollen allergen and egg albumin. We tried to suppress selectively the production of anti-birch pollen antibodies by intravenous application of hot label-

led birch pollen allergen in one group of rabbits by a previous, and in another group by subsequent application of the hot radio-labelled birch pollen antigen.

MATERIAL AND METHODS

Group 1 (11 control rabbits)

Dry pure birch pollen was purchased from ALLERGON, Sweden. Birch pollen contains approx. 10 antigenic components (2). The allergenically most active fractions were composed mainly of proteins with a molecular weight range of 10,000–50,000 daltons. These active fractions represented only 3% of the total protein and carbohydrate content of the crude extract. It was possible to purify birch pollen allergen extract from the bulk of non-allergenic contaminants (low molecular weight carbohydrates) by a single step of gel filtration procedure (4).

Sensitization was induced by eight applications of a suspension of 10 mg raw birch pollen in 1 ml saline. Every 3–4 days, 1 ml of the suspension was injected intravenously and at the same time cotton swabs soaked with 0.5 ml of the birch pollen suspension were inserted into each lumen of the nose. Fourteen days after the last combined sensitization, PCA and haemagglutinating antibodies were determined in blood and in nasal secretion. Blood samples were taken by cardiac puncture, nasal secretion was collected by means of a soft plastic tube inserted into the nasal cavity. Secretion was stimulated by blowing fine soap powder into both nostrils about 10 min before the collection. In this way, 1–2.5 ml of mucous secretion could be obtained from each animal. The secretion was then homogenized.

Homologous passive cutaneous anaphylaxis (PCA) tests were carried out by a modified method of Zwaifler & Becker (6) using a 72-h sensitization period and 0.2 ml serum or serum dilution for the intracutaneous injection. The antigen, i.e. 2 ml crude extract of birch pollen, which had been dialysed against physiological

saline + 2 ml of 1% Evans blue dye were then injected intravenously according to the method of Belin & Strannegard (1).

The passive haemagglutination was carried out according to Stavitsky (5). Tanned red sheep cells were coated with allergenic antigens by incubation with crude, 10 times diluted unheated birch pollen extract. The sera and nasal secretions were absorbed with sheep red cells prior to testing in the haemagglutination assay.

Group 2 (9 rabbits)

On the 1st and 5th day the rabbits received radioactive ^{125}I (in form of 750–1000 $\mu\text{CiNa}^{125}\text{I}$ /animal) intravenously.

Combined sensitization was started 3 days after the second injection of ^{125}I , that is on the 8th day, and performed in the same manner as in group 1. Bleeding by heart puncture and collection of the nasal secretion was carried out 14 days after the last sensitization.

Group 3 (12 rabbits)

Hot-labelled birch pollen allergen was injected on days 1 and 5. The total activity of each injection was 750–1000 μCi per injection and animal. Combined sensitization (intravenous and pernasal) was started 3 days after the second hot-labelled allergen injection. Fourteen days after the last sensitization samples of blood and nasal secretion were collected.

The iodination procedure of birch pollen was the following: 2–3 mCi (0.0740–0.148 GBq NaJ^{125}) (Amersham, carrier free in NaOH solution, pH 7–11, free from reducing agent, contains less than 1% J^{125}) in 20 μl solution + birch pollen suspension (20 μg in 50 μl phosphate buffer/0.04 molar, pH 7.4/).

During shaking, 300 μg Chloramin-T dissolved in the above mentioned phosphate buffer (freshly prepared solution) is added in 30 μl . After incubation time (3–4 min), the reduction of excess iodine is achieved by further addition of 500 μg $\text{Na}_2\text{S}_2\text{O}_5$ in 100 μl (in the above mentioned phosphate buffer). After labelling, the separation of the labelled pollen-antigen from the remaining free radioactive

iodine has been done by dialysis against aqua dest.

The most obvious method for increasing the specific activity is to increase the amount of iodine substitution. This over-iodination (to achieve more "decay" effect) was performed by the addition of the pollen-antigen suspension to previously oxidized iodine¹²⁵ (with Chloramine-T). The average specific activity (120 $\mu\text{Ci}/\mu\text{g}$ pollen) increased here up to about 900 $\mu\text{Ci}/\mu\text{g}$ pollen. The average yield of iodination was 57 %.

The iodination degree, i.e. I atom/mol pollen antigen, was calculated after the formula (3):

$$\text{Iod. degree} = \text{SA} \times T_{1/2} \times M \times 8.862 \times 10^{-14}$$

in which SA = specific activity mCi/mg substance

M = mol. wt. of the substance

$T_{1/2}$ = half-life of the labelled isotope.

In this equation the change in the substance's molecular weight as a consequence of labelling has been omitted.

Group 4 (12 rabbits)

Combined sensitization was carried out, as in the foregoing groups. Hot-labelled birch pollen allergen was intracardially applied on days 3 and 6 after the last sensitization. Blood and nasal secretion sampling was carried out 14 days after the second injection of the hot-labelled birch pollen.

Group 5 (10 rabbits)

The rabbits were simultaneously sensitized with birch pollen allergen and egg albumin. The sensitization with pollen was carried out as in group 1. The egg albumin in phosphate buffered saline, emulsified with an equal amount of complete Freund's adjuvant (Miles Laboratories), was injected into all four footpads. This mixture contained 1.0 mg/ml of egg albumin and approximately 0.3 ml was injected into each footpad. 30 days later a booster of 1.0 mg/ml egg albumin was given intramuscularly. Moreover, on days 1 and 30, all animals received, intraperitoneally, a mixture containing 5 mg egg albumin and 2.0 mg pertussis vaccine (25

billions of germs) (Neo Dm tys "Specia"). Blood sampling was carried out 14 days after the last combined sensitization. Determination of PCA-antibodies was performed in the blood only.

Group 6 (11 rabbits)

On days 1 and 5 intravenous injection of hot-labelled birch pollen allergen was carried out; the total activity was 750–1000 μCi per injection and animal. Combined sensitization with birch pollen allergen and egg albumin was performed in the same manner as in group 5, beginning 3 days after the last injection of labelled pollen. Fourteen days after the last sensitization blood sampling and determination of PCA antibodies in the blood was carried out.

Group 7 (13 rabbits)

Sensitization with birch pollen and egg albumin was carried out in the same manner as in group 5. Two days after the last combined sensitization hot-labelled birch pollen was given intracardially two times with an interval of 3 days. Blood sampling and determination of PCA antibodies against birch pollen and egg albumin followed 14 days after the last sensitization.

RESULTS

Combined sensitization of rabbits by means of an intravenous and pernasal administration of birch pollen allergen led to the formation of skin sensitizing PCA antibodies and haemagglutinating antibodies. Radioactive ¹²⁵I alone in no way impaired the subsequent sensitization.

Radioactively labelled birch pollen allergen (750–1000 μCi) administered before the combined sensitization was capable of suppressing very significantly the following sensitization.

Radioactively labelled birch pollen allergen (750–1000 μCi) was capable of suppressing the immunization which had already been started.

The combined immunization of rabbits with birch pollen and egg albumin resulted in the production of anti-birch pollen and anti-egg albumin PCA antibodies.

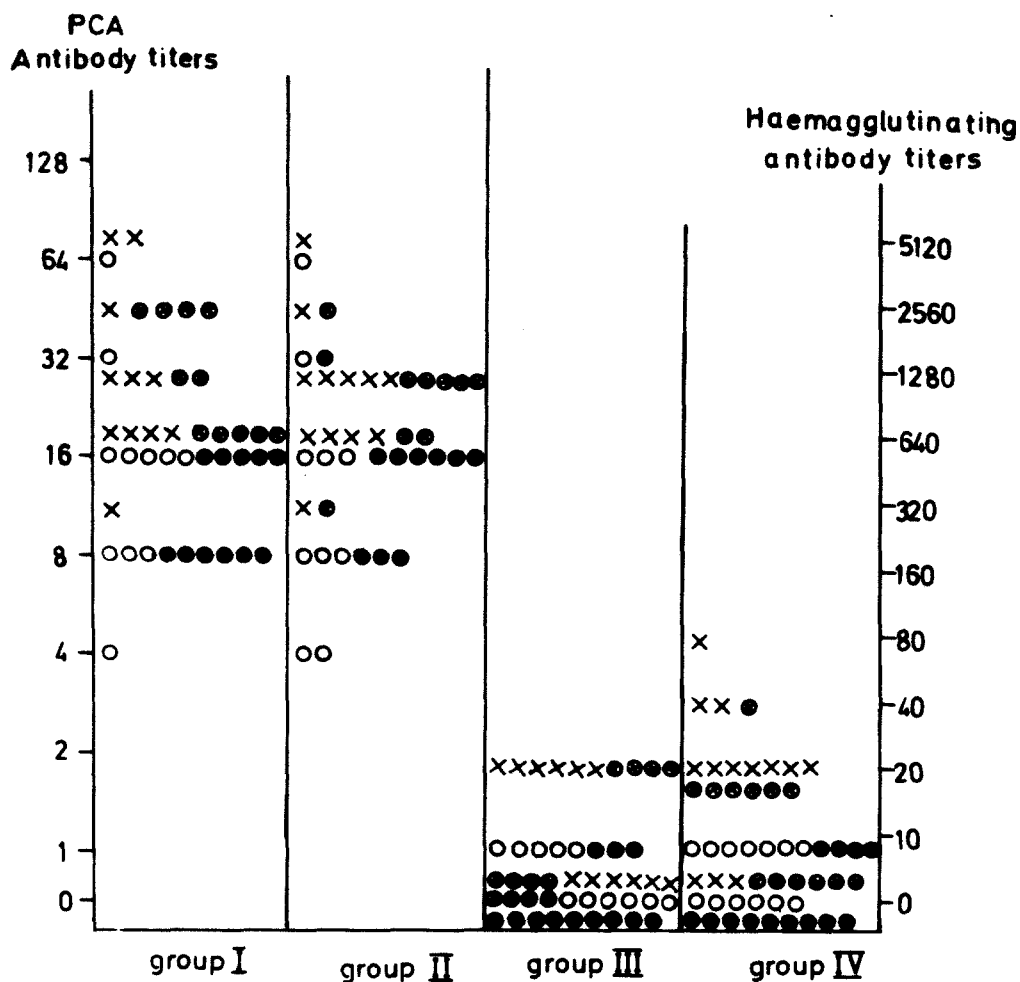


Fig. 1.
○ = blood PCA antibodies against birch pollen
● = secretory PCA antibodies against birch pollen
× = blood haemagglutinating antibodies against birch pollen
⊗ = secretory haemagglutinating antibodies against birch pollen.

Hot-labelled birch pollen (750–1000 μ Ci) applied before or after the combined immunization suppressed selectively the production of anti-birch pollen antibodies. The formation of PCA antibodies directed against the egg albumin remained intact. (See Fig. 1, 2).

Statistical evaluation of the results was made with the Wilcoxon-test. There were no significant differences between groups 1 and 2; the same is true of groups 3 and 4. There were, however, statistically very significant differences ($P < 0.01$) when groups 1 and 2 were compared with groups 3 and 4.

The results of the statistical evaluation of groups 5, 6, and 7 were the following: Regarding the formation of antibodies against egg albumin there was no significant difference between groups 5, 6, and 7. There was, however, a

very strong significant difference ($P < 0.01$) when group 5 was compared with groups 6 and 7 with regard to the formation of antibodies against birch pollen allergen.

DISCUSSION

Combined sensitization of rabbits by means of an intravenous and pernasal administration of birch pollen allergen led to the formation of skin-sensitizing PCA antibodies and haemagglutinating antibodies.

We succeeded in suppressing selectively with the aid of hot-labelled birch pollen allergen the production of anti-birch pollen antibodies intravenously administered before the pollen sensitization. The basis of this effect is the fixation of the radiolabelled birch pollen allergens to the

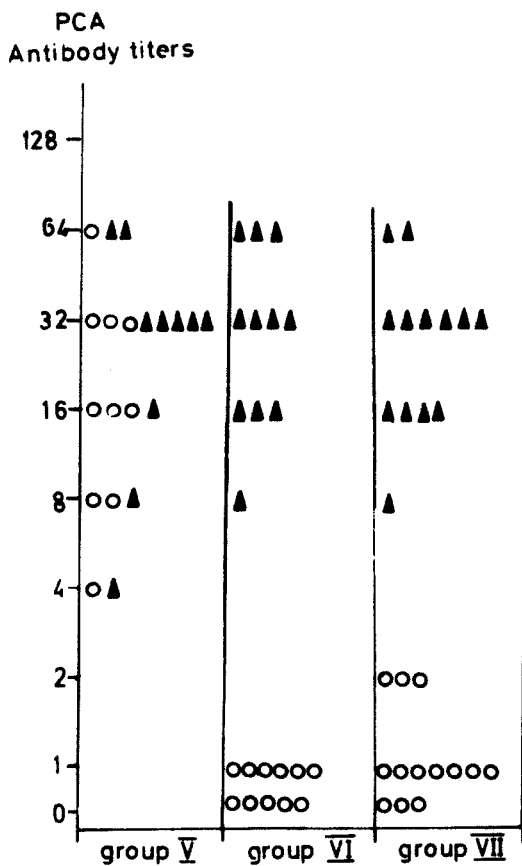


Fig. 2
○ = blood PCA antibodies against birch pollen
▲ = blood PCA antibodies against egg albumin.

receptors of the genetically determined B cell clone and the radioactive damage of the latter.

The suppressive effect of the application of labelled allergen was also detectable when the sensitization had already been initiated. Combined immunization with birch pollen allergen and egg albumin resulted in the production of PCA antibodies to both antigens. Hot-labelled birch pollen applied before or after the combined sensitization selectively suppressed the synthesis of anti-birch pollen antibodies whereas the synthesis of anti-egg albumin antibodies remained unaffected.

Guided by the obtained results, we intend to design a specific therapy for exogenous allergic diseases of the IgE type. Experiments to counteract the development of experimental allergic encephalomyelitis in guinea pigs, by pretreating the animals with radioactively labelled basic myelin protein, are already successfully completed.

Similar experiments concerning the suppression of experimental immune thyroiditis and experimental immunological aspermia are also in progress.

Our results in the present study imply a theoretical possibility of selectively suppressing practically all immunological and immunopathological phenomena where there is a possibility of applying radioactive hot-labelled antigens.

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