

tion of certain surface-active biological or synthetic polymers [6]. It is likely that our findings are also based upon an alteration of the isolated PRG and Fc portions due to their exposure to hydrophobic surfaces. Our results furthermore suggest that this alteration is markedly reduced when the Fab fragment is present. These findings may provide an explanation for the stability of the complete preparation. Thus, the PRG in the digest – which appears to be the most important constituent for clinical purposes [2] – and the Fc fragment are stabilized by the Fab fragment during all stages in production and in the final preparation as well.

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Short Communication

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Immunological Partial Identity and *in vitro* Inhibitory Effect of Two Major Timothy Pollen Allergens to Whole Pollen Extract of Four Grasses

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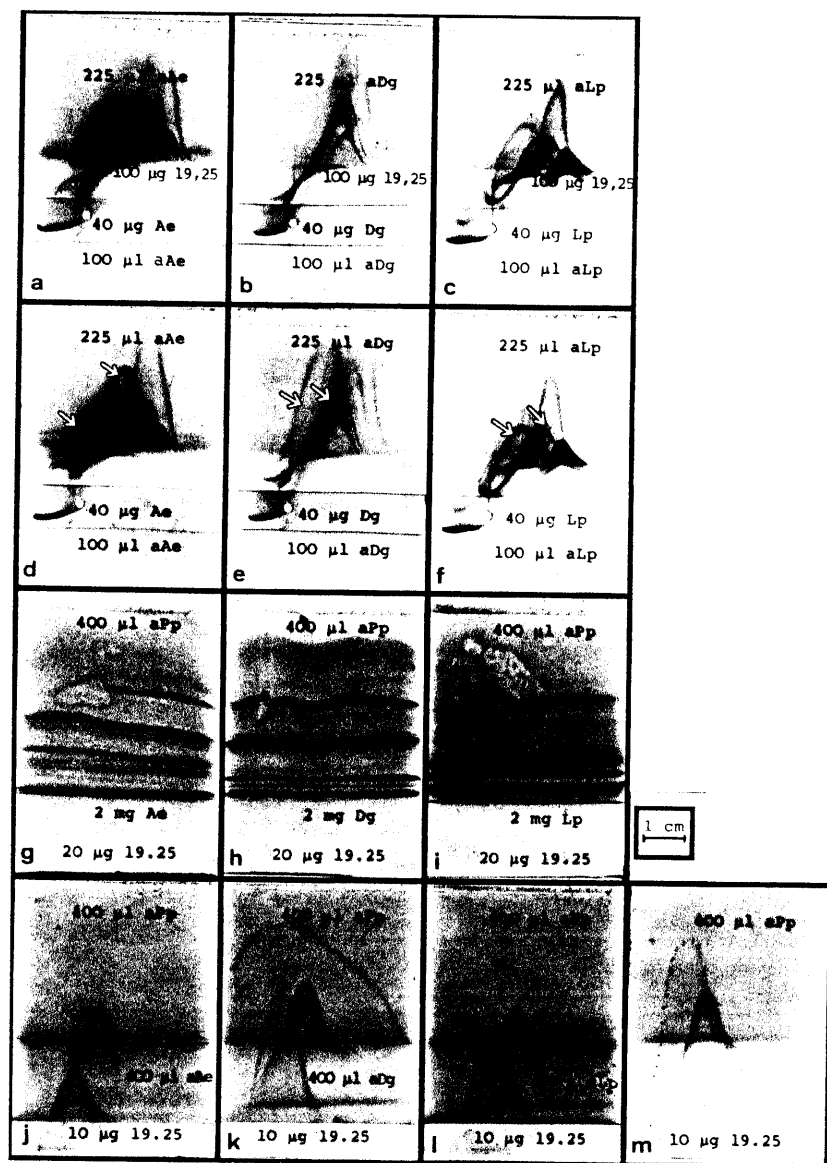
Abstract. Crossed immunoelectrophoresis in various modifications has directly revealed the immunological partial identity between isolated major allergens of timothy pollen (antigens 19 and 25) and the corresponding pollen allergens of false oat, orchard and rye grass, using rabbit antibodies raised against all four grasses. The inhibitory effects of antigens 19 and 25 relative to those of whole pollen extracts have been determined by means of RAST, inhibiting serum pools from selected grass-pollen-allergic patients and using insolubilized whole pollen extracts from the four grasses as allergosorbents. The inhibitory effects of antigens 19 and 25 were determined to be 97, 91, 92 and 73% of those of whole extracts of timothy, false oat, orchard and rye grass, respectively.

An extensive cross-reactivity between various whole extracts of grass pollen has been demonstrated by means of the RAST technique [1, 2]. These studies showed the existence of unique allergenic determinants indirectly. A more direct comparison between electrophoretically separated proteins from seven different grass pollen extracts was performed by means of immunodiffusion and skin tests whereby partial immunological identity between related allergens of the different grasses was demonstrated [3]. Recently, four major allergens of timothy pollen have been isolated [4] two of which were shown to possess about 90% of the allergenic activity as compared with whole timothy pollen extracts. For optimal diagnosis and treatment of sensitive patients,

highly purified or even isolated allergens may be preferable; furthermore, it is a clinical experience that most individuals who are allergic to grass pollen are sensitive to many (if not all) antigenically related grasses. Thus it is relevant to determine the degree of allergenic activity exerted by these isolated timothy allergens on whole pollen extracts of various grasses.

In this study we have analyzed for partial identity timothy antigens 19 and 25 and the corresponding allergens in four common Scandinavian grasses by means of quantitative immunoelectrophoresis. The inhibitory effects of these allergens on the whole grass pollen extracts were tested by means of RAST inhibition technique.

Dissolved (10 g·l⁻¹) freeze-dried allergen



extract of grass pollen from *Avena elatior* (Ae; false oat), *Dactylis glomerata* (Dg; orchard), *Lolium perenne* (Lp; rye grass) and *Phleum pratense* (Pp; timothy) as well as rabbit antibodies against these extracts (aAe, aDg and aLp) were prepared as described for timothy (Pp 0375) and aPp 0375 in Løwenstein [4]. Dissolved ($1.25 \text{ g} \cdot \text{l}^{-1}$) timothy antigens 19 and 25 were obtained as described in Løwenstein [4]. Equipments, reagents and methodology for crossed-line immunoelectrophoresis (CLIE) and crossed immunoelectrophoresis (CIE) with intermediate gel were performed as described in Axelsen *et al.* [5], details given in Løwenstein [4]. RAST inhibitions were performed as described in Løwenstein [4]; allergosorbents of immobilized Ae, Dg, Lp and Pp-paper discs were produced. Pools of sera from 11, 9, 13 untreated, allergic patients, class 4 in RAST with the same allergosorbent as above and reactions in prick tests corresponding to at least 1 HEP [6] with the extract diluted 1:100 were inhibited in the Ae, Dg and Pp experiments, respectively. A pool from 6 untreated, Rye-grass-

allergic patients having a 2+ intradermal skin reaction to less than $10^{-3} \mu\text{g}$ rye group I (kindly supplied by Dr. D. G. Marsh) was inhibited in the Lp experiments. The inhibitions were carried out with (1) buffer, (2) antigens 19, 25 ($1.25 \text{ g} \cdot \text{l}^{-1}$), and (3) whole extracts (Ae, Dg, Lp or Pp) on the respective serum pools and allergosorbents. Negative controls using a 1,000-donor pool of human serum were subtracted from the experimental results. The percent inhibition was expressed as

$$\frac{\text{CPM buffer} - \text{CPM antigen 19, 25}}{\text{CPM buffer} - \text{CPM whole extract}} \times 100,$$

where CPM are counts per minute after inhibition with buffer, antigens 19, 25 and whole extract. ^{125}I -anti IgE (Pharmacia, Uppsala, Sweden) was used. All experiments were performed twice in duplicate.

The antigenic relationship between timothy antigens 19 and 25 and the corresponding antigens from three other grasses is demonstrated in figure 1.

In figure 1a-f, the corresponding antigens (marked by arrows in the controls) of Ae, Dg and Lp are absorbed *in situ* by antigens 19 and 25; furthermore, *in situ* absorption with whole extract of Pp elevated all the precipitates in similar experiments (not shown); in contrast in figure 1g-i, antigens 19 and 25 are *in situ* absorbed by the corresponding antigens of Ae, Dg and Lp (only horizontal lines left; figure 1m is the control); finally, in figure 1j-l, the intermediate gel technique illustrates the cross-reactivity between aPp and aAe, aDg and aLp which significantly precipitate antigen 25 and less significantly antigen 19; figure 1m is the control. Thus a significant antigenic relationship of distinct grass pollen antigens to antigen 25 and a relatively weaker relation-

Fig. 1. Identity/partial identity between two major allergens of timothy pollen (antigens No. 19 and 25) and pollen allergens of four common grasses demonstrated by CLIE (a-i) and CIE with intermediate gel (j-m) in 1% (w/v) agarose gel containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate and 0.003 M sodium azide (pH 8.6, 25°C) at 15°C . Dimensions of the first, intermediate, anodic, and cathodic gels are indicated. Electrophoretic conditions: first-dimension electrophoresis for 25 min at $10 \text{ V} \cdot \text{cm}^{-1}$; anode to the right; second-dimension electrophoresis for 15 h at $2 \text{ V} \cdot \text{cm}^{-1}$; anode uppermost. The arrows in plates d-f indicate precipitates which are absorbed *in situ* by antigens No. 19 and 25 in the corresponding plates a-c. Essential but weak precipitates are stipulated. m control.

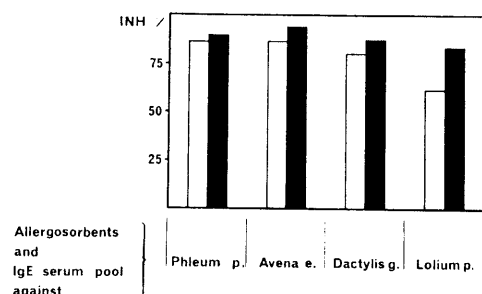


Fig. 2. RAST inhibition of four different whole pollen extracts and allergosorbents. □ = Antigen No. 19 and 25; ■ = whole pollen extracts of Pp, Ae, Dg and Lp.

ship to antigen 19 were demonstrated. The inhibitory effects of the isolated allergens are demonstrated in figure 2 which shows that they are able to exert 97 ± 8 , 91 ± 11 , 92 ± 9 and $73 \pm 12\%$ inhibition relative to that of the whole extracts of Pp, Ae, Dg and Lp, respectively. This method was preferred to the determination of concentrations corresponding to 50% inhibition because these are only relevant when comparing allergenic extracts of almost identical qualitative composition. In 1 case (Pp), the whole extract and antigens 19 and 25 contained the same amount of antigen 25, but in the 3 other cases, this quantitative relationship is unknown. Furthermore, for Pp ten times higher concentrations of antigens No. 19 and 25 or of the whole extracts as inhibitors gave comparable results [4, fig. 3]. In principle, the results are dependent on the serum pools which were selected on the basis of patients' sensitivity to the extract in question and there is no guarantee that IgE against all minor components is present in the pool. Nevertheless, it is shown that antigens 19 and 25 isolated from timothy pollen and their partially identical antigens from the other grasses make a significant contribu-

tion to the total grass pollen allergenic activity. The possible practical value of this knowledge in specific immunotherapy of grass pollen allergies will have to be studied in the future.

Contrary to the previous study of Marsh *et al.* [3] on the immunologically partial identity between antigens of grass pollens, we are handling isolated physicochemically characterized allergens in a very sensitive system (fig. 1); furthermore, these two allergens have been shown to be the main components in Malleys AgB [4] although in quite different proportions than in our mixture. This explains previous difficulties in detecting partial identity between AgB and antigens from other grasses. In our experiments, significant identities were demonstrated, although weaker for antigen 19 than for antigen 25.

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