Analysis of Common Antigenicities among *Rosaceae* Pollen Allergens.

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Common antigenicities among ten kinds of pollen extract of *Rosaceae* four *Prunoideae* three *Rosoideae* and three *Maloideae* were investigated by means of RAST inhibition test using a pooled serum from three patients with cherry pollinosis, and immunodiffusion method using anti-cherry and anti-apple pollen rabbit sera. Strong common antigenicities were found among the allergens from plants belonged to the same subfamily but weak common antigenicities among the allergens from plants belonged to the different subfamily.

Key words: Common antigenicity, Rosaceae, RAST inhibition, Ouchterlony immunodiffusion

Introduction

In the previous report [1], we examined the mutual relationship among the antigenicities of fruit pollen allergens, four species of cherry subfamily (*Prunoideae*) and two species of pear subfamily (*Maloideae*), and suggested that strong common antigenicities existed in the allergens from plants belonged to the same subfamily but weak common antigenicities among the allergens from plants belonged to the different subfamily.

In addition to the pollen of *Prunoideae* and *Maloideae*, to pollens of *Rosoideae* also cause occupational pollinosis, e.g. rose or strawberry pollinosis [2,3].

In this paper, we attempted to elucidate the mutual relationship among the pollen allergens of *Rosoideae*, *Prunoideae* and *Maloideae* by means of RAST inhibition test using a pooled serm from the cherry pollinosis patients and an immunodiffusion technique using anti-cherry and anti-apple pollen rabbit sera.

Materials and methods

The following pollens were used in this study; *Prunus persica* (peach), *Prunus Yamasakura* (Yamasakura cherry), *Prunus lannesiana cv. Imose* (double flowered cherry) and *Prunus grayana* (bird cherry) belonged to *Prunoideae*; *Kerria japonica* (yellow rose), *Fragaria chiloensis* (strawberry) and *Rosa hybrida* (rose) belonged to *Rosoideae*; *Sorbus commixta* (mountain ash), *Pyracantha angustifolia* (firethorn), and *Chaenomeles lagenaria* (Japanese quince) belonged to *Maloideae*. *Quercus serrata* (Japanese oak) belonged to *Fagales* and *Viburnum dilatatum* (viburnum) belonged to *Sympetalae* were used as control. Pollen extracts were prepared by mixing the pollens in 0.125M ammonium bicarbonate solution (pollens(wt)/ solution

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(vol):1/10) at 4°C for 48hr. Serum was obtained from three patients allergic to cherry pollen. All of them had pollinosis symptoms. They showed positive intradermal test reaction to a 1:100,000w/v dilution of pollen extract, and positive provocation test to a 1:20 w/v dilution of pollen extract, and elevated levels of IgE antibodies to the allergens in an extract of cherry pollen by RAST test[4].

RAST disci were prepared according to the method of Shafiee et al[5]. RAST inhibition tests were carried out as follows; 100μl of 100-fold diluted pollen extract with 0.05M phosphate buffered saline, pH7.2 was added to 50μl of the pooled serum from three patients, and the mixture was kept overnight at room temperature, and then RAST test was performed by the RAST kit (Phadezym RAST, Shionogi LTD). Ouchterlony immunodiffusion analysis was performed in an agarose gel plate. Anti-cherry or anti-apple pollen serum was made by rabbits as follows; 0.4 ml of the mixture of pollen extract and Freund's complete adjuvant (1 : 1) was injected subcutaneously into the back of albino rabbits every week. After twelve injections, hyperimmune serum to cherry or apple pollen allergen was obtained.

Results and Discussion

On the RAST inhibition test (Table 1), strong inhibitory activities (about 80% inhibition) were observed by the pollen extracts of *Prunus persica*, *Prunus lannesiana cv. Imose*, *Prunus Yamasakura* and *Prunus grayana* belonged to *Prunoideae*. On the other hand, inhibitory activities of the pollen extracts from both *Rosoideae* and *Maloideae* were weaker than those of the extracts from *Prunoideae*, 20.9 ~ 64.8% in *Rosoideae* and 13.9 ~ 53.6% in *Maloideae*. No inhibitory activity was found in the pollen extracts of *Quercus serrata* of *Fagales* and *Viburnum dilatatum* of *Sympetalae* used as control.

Reactivities of pollen extracts to anti-cherry and anti-apple pollen rabbit sera in the agarose gel were shown in Fig.1. Clear precipitation lines were found between anti-cherry pollen rabbit serum and *Prunoideae* pollen extracts, *Prunus persica*, *Prunus Yamasakura*, *Prunus lannesiana cv. Imose* and *Prunus grayana*, and these precipitation line fused to each other (Fig. 1a). No precipitation line was visible in every other pollen extract (Fig.1b). On the contrary,

Table 1 RAST inhibition test of the different kinds of pollen extracts using pooled serum from three patients with cherry pollinosis.

Pollen species				Absorbance (420nm)	Inhibition (%)
Family	Subfamily	Genus	Species		
Rosaceae	Prunoideae	Prunus	persica	0.128	80.6
		Prunus	lannesiana	0.130	80.3
		Prunus	Yamasakura	0.142	78.5
		Prunus	grayana	0.152	77.0
	Rosoideae	Kerria	japonica	0.232	64.8
		Fragaria	chiloensis	0.352	46.7
		Rosa	hybrida	0.522	20.9
	Maloideae	Sorbus	commixta	0.306	53.6
		Pyracantha	angustifolia	0.568	13.9
		Chaenomeles	lagenaria	0.480	27.3
Fagaceae		Quercus	serrata	0.624	5.5
Caprifoliaceae		Viburnum	dilatatum	0.628	4.8
		control		0.660	—

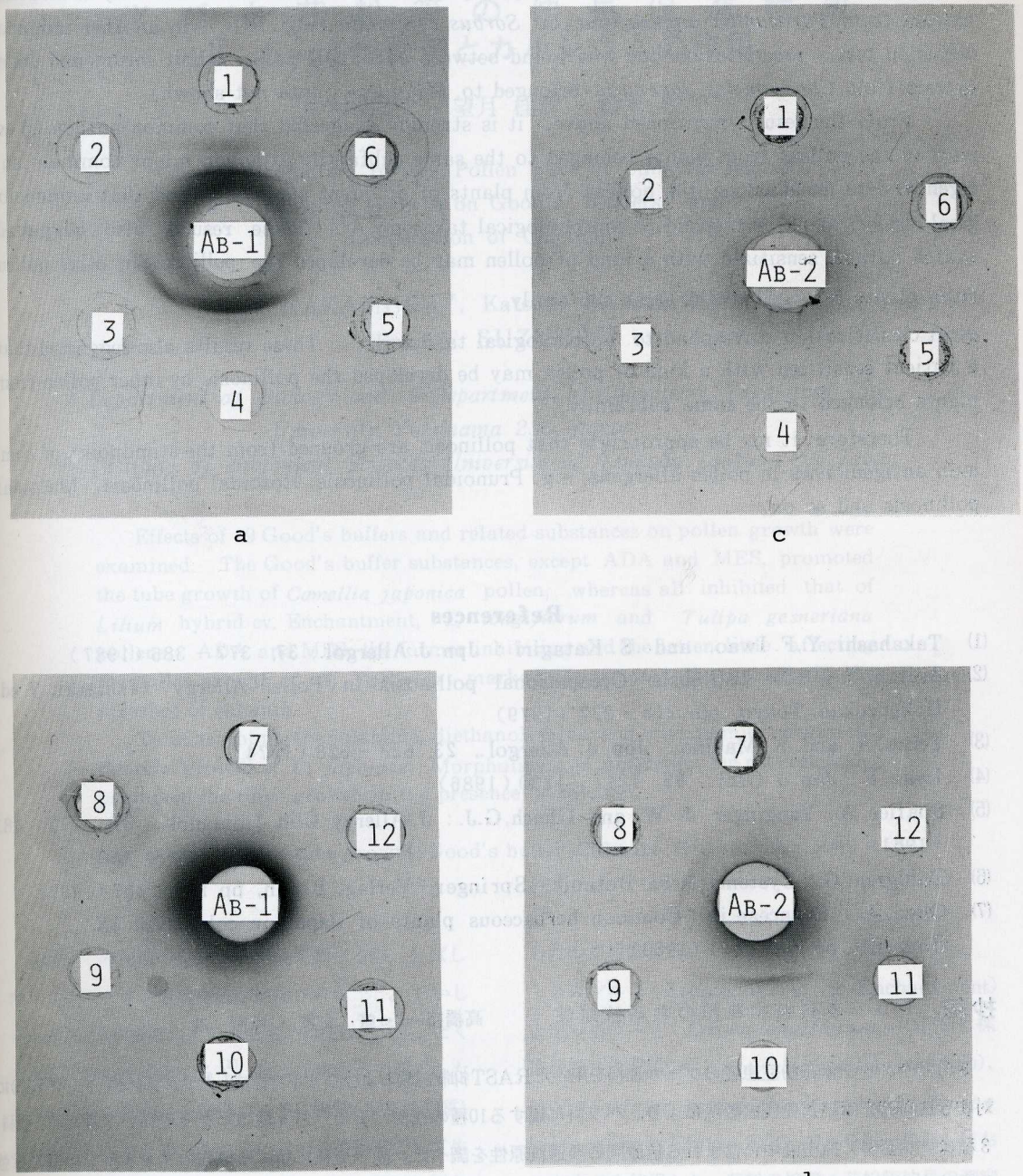

Fig. 1 Ouchterlony immunodiffusion test on an agarose gel plate. AB−1; anti−cherry pollen serum, AB−2; anti−apple pollen serum. Pollen extracts; 1 *Prunus persica*, 2 *Prunus Yamasakura*, 3 *Prunus lannesiana cv. Imose*, 4 *Prunus grayana*, 5 control(saline), 6 *Quercus serrata*, 7 *Kerria japonica*, 8 *Sorbus commixta*, 9 *Fragaria chiloensis*, 10 *Pyracantha angustifolia*, 11 *Rosa hybrida*, and 12 *Viburnum dilatatum*.

reactivities of the pollen extracts to anti-apple pollen rabbit serm were found only in the pollen extract from *Pyracantha angustifolia* or *Sorbus commixta* (Fig. 1d). In another immunodiffusion test, a precipitation line was found between anti-apple pollen rabbit serum and pollen extract from *Chaenomeles lagenaria* belonged to *Maloideae* (data not shown).

From the results mentioned above, it is strongly suggested that common antigen(s) existed in the pollens from plants belonged to the same subfamily although minor common antigen(s) were found among the pollens from plants of different subfamily, and that immunological classification correspond to morphological taxonomy[6,7]. These results also suggested that a patient sensitized with a kind of pollen may be developed the pollinosis by other pollen from plants belonged to the same subfamily.

gical classification correspond to morphological taxonomy. These results also suggested that a patient sensitized with a kind of pollen may be developed the pollinosis by other pollen from plants belonged to the same subfamily.

Therefore, it my be appropriate that pollinosis are grouped from the standpoint of common antigenicities in pollen allergens, e.g. Prunoidal pollinosis, Rosoidal pollinosis, Maloidal pollinosis and so on.

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抄録 バラ科植物花粉の共通抗原性 高橋裕一・厳 文雄・片桐 進

3名のサクランボ花粉症患者のプール血清を用いたRAST抑制試験およびウサギで作成したサクランボとリンゴ花粉に対する抗血清を用いた免疫拡散法により、バラ科に属する10種の花粉（そのうち4種はサクラ亜科、3種はバラ亜科、3種はナシ亜科）抽出液中に含まれる抗原間の共通抗原性を調べた。同一亜科に属する植物のアレルゲンの間には強い共通抗原性がみいだされたのに対して、亜科が異なる植物のアレルゲンの間には弱い共通抗原性がみいだされた。

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