

## Original article

# Cross-reactivity between the major *Parietaria* allergen and rotavirus VP4 protein

**Background:** The present study investigates immunological cross-reactivity between Par o 1, the major pollen allergen of *Parietaria*, and the VP4 protein of rotavirus, a microorganism that is world-wide the main etiological agent of gastroenteritis in children.

**Methods:** IgG and IgE cross-reactivity was assessed by direct binding and competitive inhibition assays (ELISA and DARIA), using recombinant VP4 from rhesus infectious rotavirus (RR), synthetic peptides and Par o 1-specific antibodies affinity purified from pooled and individual human sera.

**Results:** Antibodies specifically binding Par o 1, affinity purified from the sera of 35 individuals with skin test positivity to *Parietaria* and from 14 pools, were extensively cross-reactive with RRVP4. Cross-reactive binding was specifically inhibited by synthetic peptides derived from the C-terminal sequences of the VP4 proteins from human and rhesus infectious rotavirus.

**Conclusions:** This study reports the first evidence of cross-reactivity between an allergen and a viral antigen.

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Key words: cross-reactivity; *Parietaria*; rotavirus; VP4.

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The present study investigates immunological cross-reactivity between the allergen Par o 1 and the VP4 protein of rotavirus (R), a microorganism that is world-wide the main etiological agent of gastroenteritis in children and in many animal species (1). Par o 1 is the major pollen allergen of *Parietaria officinalis*, a weed of the family of *Urticaceae*, which causes a pollenosis highly prevalent in Europe (2). Synthetic peptides representing dominant epitopes of Par o 1 for antibodies and T cells have been identified (3–5). This study was prompted by the results of a computer search through protein sequence databases indicating that the C-terminal sequence of the RVP4 protein, conserved between several virus strains, shares a motif with a nonapeptide containing a dominant epitope.

Rotaviruses belong to the reoviridae family and their presence in the environment is ubiquitous (1). VP4 is the viral hemagglutinin and is present on the virion in form of spikes. VP4 is cleaved by trypsin in two subfragments: VP8, which contains the hemagglutination domain and

VP5 (6–9). The putative cross-reactive epitope is located in the C-terminal portion of VP4 (which is also the C-terminal portion of VP5), in a region which appears to be conserved between VP4 proteins from human and animal-infective virus strains. In the present study, we used the VP4 protein from rhesus-infective rotavirus (RR) strains. RRVP4 is extensively cross-reactive with antibodies elicited by the homologous protein from human-infective (HR) strains and induces in humans an antibody response cross-reactive with HRVP4 (10).

Cross-reactivity between unrelated antigens has been studied mainly in the field of autoimmunity, where infection by microorganisms possessing antigens cross-reacting with self-proteins can contribute to the pathogenesis (11, 12). Recently, it has also been postulated that cross-reactivity of allergens with endogenous proteins is a pathogenic factor in atopic diseases (13). Recent epidemiological and *in vitro* studies suggest that interaction of the immune system with microbial antigens influences the nature and development of the immune response and could play a role in the development of allergy (14–17). The mechanisms involved are largely unknown. Cross-reactivity could represent one mechanism by which certain common infections influence the outcome of the immune response to specific environmental allergens.

**Abbreviations:** DARIA, double antibody radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; HRV, human rotavirus; HRVP4, human rotavirus VP4 protein; RRV, rhesus rotavirus; RRVP4/RRVP5\*, rhesus rotavirus VP4/VP5\* proteins; ST, skin prick test.

## Materials and methods

### Allergens

The major allergens from the species *P. judaica* and *P. officinalis*, Par j 1 and Par o 1, respectively, are glycoproteins of 12–14 kDa and are extensively cross-reactive (18, 19).

*P. officinalis* pollen was purchased from Allergon AB (Sweden). Purified Par o 1 used to perform enzyme-linked immunosorbent assay (ELISA) and affinity chromatography was obtained by SDS-PAGE fractionation of the aqueous pollen extract (18) followed by electroelution (Prep Cell, Biorad, USA) of the 14-kDa band. The allergen obtained by electroelution was compared by ELISA (IgG antibodies) and double antibody radioimmunoassay (DARIA) (IgE antibodies) inhibition to the standard preparation of highly purified Par o 1 (18): reciprocal inhibition was more than 95%, indicating that the two preparations contained essentially the same allergenic and antigenic components.

### RRV proteins

RRVP4 and RRVP5\*, a VP4 fragment, containing amino acid residues 274–474 and lacking the putative cross-reacting region, were expressed in baculovirus and purified by ammonium sulfate precipitation and affinity chromatography using specific mouse monoclonal antibodies (Mabs), as already described (8, 20).

### Peptides and proteins

The peptides p96 (EFGTSSCRL), pQM (NRIEQLIMQCRL), pQM9 (EQLIMQCRL), pKV (NRIEQLILQCKL), pQL (NRIEQLILQCRL) and pNS (NRLENLISQCRL) were synthesized using an ABI automated synthesizer according to the manufacturers' instructions and purified by HPLC. The peptide pQM (with the derived pQM9) represents the C-terminal sequence (aa 765–776) of RRVP4, and the peptides pKV, pQL and pNS the C-terminal sequences (aa 764–775) of VP4 proteins from the most common human infective virus strains.

The peptide p96 contains epitope(s) of Par o 1 and Par j 1 for IgG and IgE antibodies and for T cells (3–5). Its sequence, deduced from that of a cDNA fragment, could not be localized in the published sequences of Par j 1 isoforms (21). The peptides pKV, pQM (with pQM9), pQL and pNS share 3, 4, 4 and 5 aminoacid residues, respectively, with p96.

Neurotensin (ELIENKPRRPYIL), cytochrome C and insulin A chain were purchased from Sigma and recombinant Bet v 1 from Allergon.

### Serum donors

Unless otherwise stated, sera from allergic patients were obtained from allergy clinics and sera from healthy subjects from the general population. Sera from donors typed for HLA-DRB1\* alleles were obtained as previously described (22, 23). Sera from birch atopic donors were a gift from Prof. C. Lahoz (Madrid). Sera from North American (USA) donors were donated by Prof. D. G. Marsh (Baltimore, MD). Clinical history was recorded using a standard questionnaire. All serum donors were informed volunteers.

Atopy was defined as skin prick test (ST) positivity to at least one common allergenic extract (24). Skin test positivity was defined by a wheal diameter more than 3 mm greater than that of the negative control. Donors were analyzed for ST reactivity towards a panel of 18 glycerinated extracts of common inhalant allergens (Hollister-

Stier Laboratories, USA). ST reactivity to *Parietaria* was tested using *P. judaica* and *P. officinalis* pollen extracts: results will be expressed as presence/absence of ST reactivity to *Parietaria* as reactivity to these extracts was always concordant. Maryland donors had not been tested for ST reactivity to *Parietaria*, as this plant is not reported to grow in Maryland; however, sera did not contain IgE antibodies to Par o 1 (DARIA).

### Serum pools

Pools consisted of equal amounts of four sera. Thirteen pools were obtained by addition of sera from selected donors. Pool nos. 1–12 were from donors with sensitization to multiple allergenic extracts including (nos. 1–6) or excluding (nos. 9–12) *Parietaria* extract or with monosensitization to *Parietaria* extract (nos. 7 and 8); pool nos. 13 and 14 were from nonatopics. Seven pools were from donors with known HLA-DRB1\* genotype, that was: HLA-DRB1\*1100 (nos. 5, 6, 8, 12 and 13) or -DRB1\*03 (pool no. 9) or different from -DRB1\*1100 and DRB1\*\*03 (no. 7). Donors were Italians, with the exception of donors of pools nos. 10 and 11, who were Americans from Baltimore.

### Antibody purification

Antibodies were affinity purified from pools using nitrocellulose-filter-coupled antigens. For the coupling, 50 µg of antigen dissolved in 50–100 µl of phosphate-buffered saline (PBS), pH 7.2 were incubated at room temperature with a nitrocellulose filter (15 mm diameter) for 20–30 min. When dry, the filter was incubated for 30 min to 1 h in 10 mM Tris-HCl buffer (pH 8), 150 mM NaCl, 0.5% Tween 20 (TBST) + 1% BSA to block further binding and subsequently exhaustively washed (TBST).

For the purification of antibodies, 5 ml of 1/5 dilution (TBST) of serum were incubated overnight at 4°C with filter-coupled antigen. After extensive washing (TBST); antibodies were eluted from the filter by addition of six aliquots of 500 µl each of 0.1 M glycine-HCl buffer, pH 2.9, 0.5 M NaCl. The eluate (3 ml, containing approximately 0.4 µg of protein/ml, as evaluated by determining the OD at 280 nm, was neutralized with 1 M Tris, dialysed (PBS). Aliquots (300 µl) were stored at –20°C. Affinity purified antibodies are indicated as antigen specific.

### Antisera and immunoglobulins

Goat antiserum to human IgE was purchased from DAKO (Denmark), alkaline phosphatase-labeled goat antiserum to rotavirus antigens from Chemicon, alkaline phosphatase-labeled goat antisera to human or mouse IgG from Promega (USA).

### Mouse monoclonal antibodies

Mouse monoclonal antibodies (Mabs) 7A12 and 2G4, specific for RRVP4 and RRVP5\*, respectively, were kindly supplied by Prof. Harry Greenberg, Stanford University, Stanford, CA (20).

### Radiolabelling

Highly purified Par o 1 (18) (14–40 µg) was labeled with 2 mCi of <sup>125</sup>I (3.7 GBq/ml, Amersham, UK) using the chloramine T method (25). Five hundred microliter aliquots (1 µg/ml; 10–20 × 10<sup>6</sup> cpm) in borate buffered saline (BBS), pH 8.4 containing 5% bovine serum albumin (BSA) were stored at –20°C.

## SDS-PAGE and Western blotting

SDS-PAGE and Western blotting were performed using 12% polyacrylamide gel and established protocols (26).

## ELISA

The ELISA was performed according to established protocols (27). Briefly, 1 µg of protein or 5 g of peptide, dissolved in 50 µl of 0.1 M bicarbonate buffer (pH 9.6) or in PBS, respectively, were added to each well of a polystyrene microtiter plate and the plate was incubated overnight at 37°C. After washing (PBS containing 0.1% BSA, 0.1% azide, 0.05% Tween 20) and addition to each well of 50 µl of PBS containing 1% BSA, 0.1% Na azide, the plate was incubated at room temperature for 1 h, to block the remaining binding sites. Fifty microliters per well of antiserum or of antibody solution (first antibody) at appropriate dilution (PBS) were added to each well and the plate was incubated at 4°C for 1 h. After washing (PBS containing 1.5 mM MgCl<sub>2</sub>, 2 mM beta-mercaptoethanol, 0.1% Na azide, 0.1% BSA), bound IgG antibodies were revealed with alkaline-phosphatase-conjugate goat antiserum to human or goat IgG (second antibody), according to manufacturer's instructions. Absorbance at 405 nm was revealed by an automated ELISA reader.

Assays were performed in duplicate and the accepted difference between duplicates was ≤11%. Controls, consisting of wells where the addition of the first antibody was omitted (background) or where the well-bound antigen was an irrelevant protein (nonspecific binding) were run with each experiment. Binding was considered significant when, after subtraction of the background value, the value of absorbance at 405 nm obtained for the tested antigen was more than 1.5-fold greater than the value obtained for nonspecific binding.

## DARIA

DARIA was carried out as previously described (28). Serum (50 µl) was mixed with 50 µl of the appropriate dilution of an IgE myeloma protein (IgE carrier). The mixture was incubated at room temperature for 4 h with 100 µl of BBS, pH 8.4 containing 1 ng of highly purified radiolabeled Par o I (10–20 000 cpm) (19). Antigen–antibody complexes were precipitated by addition of the appropriate amount of anti-human IgE, followed by incubation at 4°C for 16 h. Precipitates were collected on Whatman GF/A glass fiber paper filters and, after washing, the radioactivity retained was measured by a gamma counter. Each experiment included a negative control (background) and a reference dose-response curve, obtained with a standard serum. Assays were performed in duplicate and the accepted difference between duplicates was ≤11%.

## Competitive inhibition immunoassays

DARIA and ELISA inhibition assays were performed at antibody dilutions, which, in direct assays, achieved approximately 50% of the maximal binding to the antigen. Fifty microliters of serum or of antibody eluate were absorbed with serial amounts of the proteins used as inhibitors at room temperature for 1 h (DARIA) or overnight at 4°C (ELISA). Inhibition was expressed as:  $(1 - (a - b)/(c - b)) \times 100$ , where  $a$  and  $c$  are the cps bound (DARIA) or the values of absorbance at 405 nm (ELISA) obtained in essays performed in the presence and in the absence of the inhibitor tested, respectively, and  $b$  the background value.

## Computer searches and statistical analyses

Databases containing sequences of viral and bacterial proteins were generated from All-Prot (using the STRINGSEARCH software) and screened using the FINDPATTERN software.

## Results

### Cross-reactive binding of Par o 1- and RRVP4-specific antibodies

Human IgG antibodies Par o 1-, RRVP4- and RRVP5\*-specific were purified from a pool of sera obtained from donors ST positive to *Parietaria*. The mouse monoclonal antibodies Mab 7A12 and Mab 2G4, specific for RRVP4 and RRVP5\*, respectively, and a polyclonal goat antiserum specific for human rotavirus antigens were used as controls. The ELISA binding profiles reported in Fig. 1A show that Par o 1 was significantly bound by RRVP4- but not by RRVP5\*-specific antibodies; similarly the RRVP4 protein but not the RRVP5\* fragment reacted with Par o 1-specific antibodies.

The specificity of cross-reactive binding was confirmed by the results of ELISA inhibition assays reported in Fig. 2. Absorption of Par o 1- (Fig. 2A) or of RRVP4- (Fig. 2B) specific antibodies with RRVP4 or with Par o 1, respectively, extensively inhibited cross-reactive binding, whereas absorption with a control protein (the birch allergen Bet v 1) had no effect.

### Inhibition of the cross-reactive binding by peptides

Figure 2A and B shows also the dose–response inhibition curves obtained by absorption of Par o 1- and RRVP4-specific antibodies with serial amounts of the nonapeptides p96 or pQM9, derived from Par o 1 and RRVP4, respectively. Absorption with p96 pQM9 inhibited 75 and 60%, respectively, the binding of Par o 1-specific antibodies to RRVP4, and 67 and 64%, respectively, the binding of RRVP4-specific antibodies to Par o 1. No inhibition was observed following absorption with the control peptide neurotensin.

To further investigate peptide specificity of the cross-reactive antibodies, four dodecapeptides representing the C-terminal region of RRVP4 (pQM) and of different serotypes of HRVP4 (pQL, pNS and pKV) were synthesized and tested for the ability to inhibit the binding of Par o 1- and of RRVP4-specific antibodies to the cross-reactive antigen.

Par o 1- or RRVP4-specific antibodies were absorbed with serial amounts of the peptides pQL, or pQM, or pNS or pKV or neurotensin (Fig. 1B). Absorption with pQL, pQM, pNS and pKV ( $9400 \times 10^{-7}$  mol/l) inhibited 20, 51, 60 and 25%, respectively, the binding of Par o 1-specific antibodies to RRVP4 and 49, 72, 62 and 53%, respectively, the binding of RRVP4-specific antibodies to Par o 1. Absorption with neurotensin or with cytochrome C had no effect.

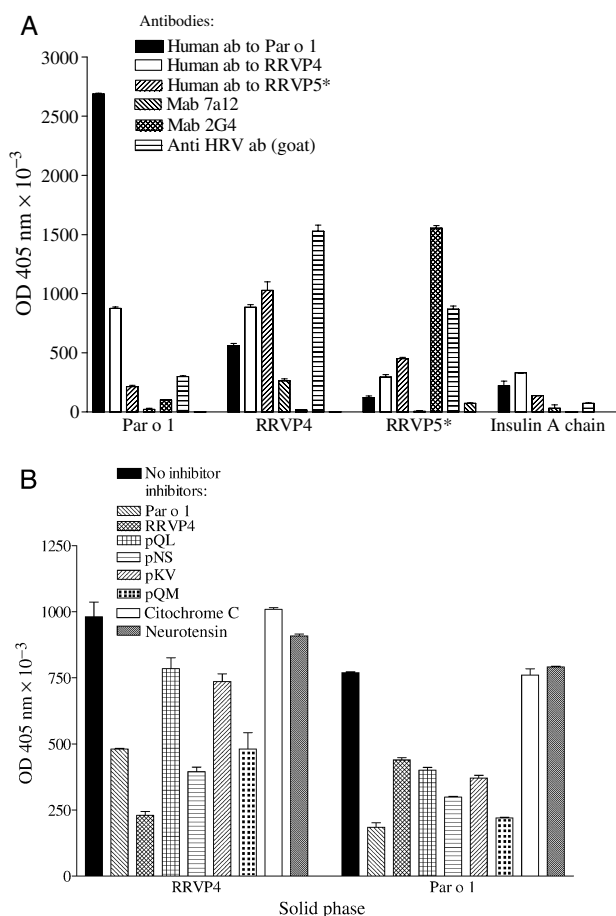


**Figure 1.** (A) Direct ELISA. Binding of human IgG antibodies (ab) Par o 1-, RRVP4- and RRVP5\*-specific, of mouse monoclonal antibodies RRVP4- (Mab 7a12) and RRVP5\*- (Mab 2G4) specific and of HRV-specific alkaline phosphatase-labeled goat antiserum to the solid phase antigens indicated in the abscissae. (B) ELISA inhibition. Binding of Par o 1 (left)- and RVP4- (right) specific human IgG antibodies to the cross-reactive antigens RRVP4 (left) and Par o 1 (right). Fifty microliters of antibody eluate were absorbed with peptides ( $9400 \times 10^{-7}$  mol/l) or Par o 1 ( $1.6 \times 10^{-7}$  mol/l), or RRVP4 ( $0.3 \times 10^{-7}$  mol/l) or cytochrome C ( $2 \times 10^{-7}$  mol/l) prior to the performance of the assay. Human antibodies were affinity purified from a pool of sera from individuals ST positive to *Parietaria*, as described under 'Materials and methods'. Antibody binding was revealed using alkaline phosphatase-labeled goat antiserum specific for human or mouse IgG.

Taken together, the results of these experiments indicate that the epitope shared between Par o 1 and RRVP4 is located in VP4 C-terminal region.

#### Inhibition of Par o 1-specific DARIA (IgE antibodies) by RRVP4

To assess whether Par o 1-specific IgE antibodies cross-reacted with RRVP4, 100  $\mu$ l of 1/5 dilution of serum pool were absorbed with serial amounts of RRVP4 or

of RRVP5\* or of insulin A chain before performing the DARIA. The inhibition curves reported in Fig. 2C show that the binding to radiolabeled Par o 1 was inhibited (up to 66%) by absorption with RRVP4, according to a dose-response curve, whereas absorption with RRVP5\* or with insulin A chain had no effect.

#### Inhibition of Par o 1-specific ELISA (IgG antibodies) by RRVP4

Previous studies had shown that the HLA class II alleles DRB1\*1100 and DRB1\*03 are predisposing and protecting, respectively, towards developing ST positivity to *Parietaria* and responsiveness to Par o 1 (23). To investigate whether cross-reactivity of Par o 1-specific IgG antibodies with RRVP4 was influenced by the atopic condition of serum donors, and/or by their DRB1\* genotype, IgG antibodies were affinity purified from 14 pools formed by sera from selected donors, as described under 'Materials and methods'.

The eluate from each of the 14 pools contained antibodies with reactivity to Par o 1, irrespective of donor's atopic status or HLA-DRB1\* genotype (Fig. 3). Interestingly, Par o 1-specific antibodies were recovered also from pool nos. 10 and 11, obtained from donors living in Maryland (USA) where the presence of *Parietaria* has not been reported. The eluates from 12 pools bound RRVP4 (binding was not significant for eluates from pool nos. 4 and 5). It is interesting to note that binding to Par o 1 was comparatively higher than binding to RRVP4 in the eluates from seven of the eight pools from donors ST positive to *Parietaria* (pool nos. 1–7) and in one of the six pools from donors ST negative to this pollen extract (pool no. 11).

Antibodies eluted from pool nos. 1, 4–6, 9, 11 and 12 were further analyzed by ELISA inhibition (Table 1). Fifty microliters of eluate were absorbed with serial amounts of Par o 1, or RRVP4, or Bet v 1, or insulin A chain and tested for binding to Par o 1. Absorption with RRVP4 inhibited Par o 1 binding, whereas absorption with Bet v 1 had no effect. Table 1 summarizes these experiments, reporting, for each eluate, the amounts of Par o 1 and RRVP4 required to inhibit 50% of Par o 1 binding (columns a and b, respectively) and the percent inhibition achieved with the highest amount of inhibitor (columns a1 and b1, respectively). Interestingly, the ratio between the amounts of Par o 1 and of RRVP4 corresponding to 50% binding inhibition (column a/b) tended to be decreased in tests performed with antibodies from donors ST positive to *Parietaria* (pool nos. 1, 4–6) as compared to tests performed with antibodies from donors ST negative to *Parietaria* (pool nos. 9–12). As a control, we tested the ability of RRVP4 to inhibit the binding between Bet v 1 and Bet v 1-specific antibodies, affinity purified from a pool of subjects allergic to birch. The results of this experiment confirm the RRVP4 specifically inhibits Par o 1 binding (Fig. 2D).

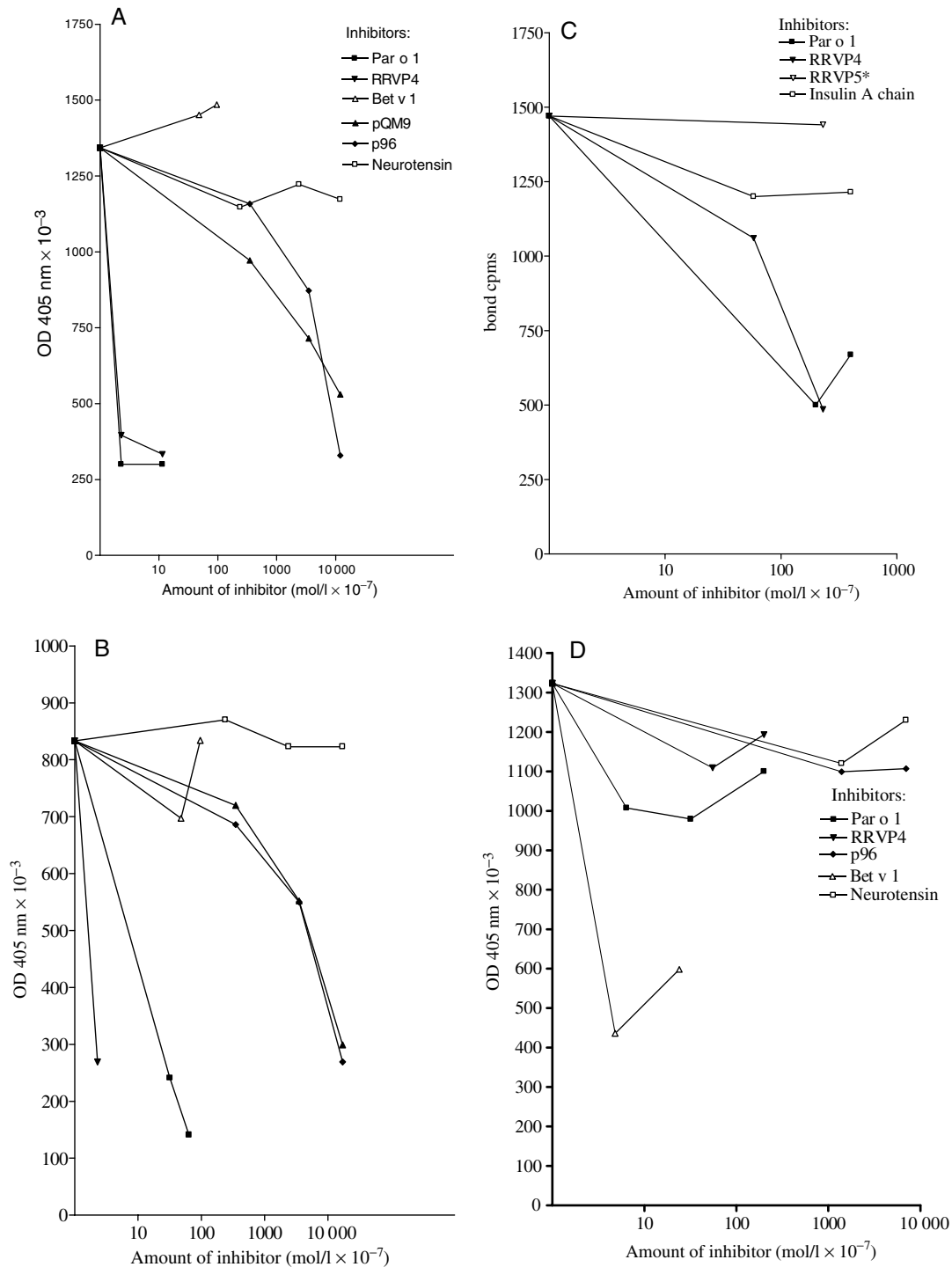


Figure 2. (A, B, D) ELISA inhibition. Binding of human Par o 1- (A), or RRVP4- (B), or Bet v 1-specific (D) IgG antibodies to RRVP4 (A), or Par o 1 (B), or Bet v 1 (D). Fifty microliters of antibody eluate were absorbed with the amounts of inhibitors indicated in the abscissae. Antibodies were affinity purified from a pool of sera from individuals ST positive to *Parietaria* (A, B), or to Birch (D) as described under 'Materials and methods'. Antibody binding was revealed using alkaline phosphatase-labeled goat antiserum specific for human or mouse IgG. (C) DARIA inhibition. Binding of IgE antibodies from a human pool to radiolabeled Par o 1, as described under 'Materials and methods'. One hundred microliters of 1/5 dilution of serum were absorbed with the amount of inhibitors indicated in the abscissae prior to the performance of the assay. Allergen-antibody complexes were precipitated using goat antiserum specific for human IgE.

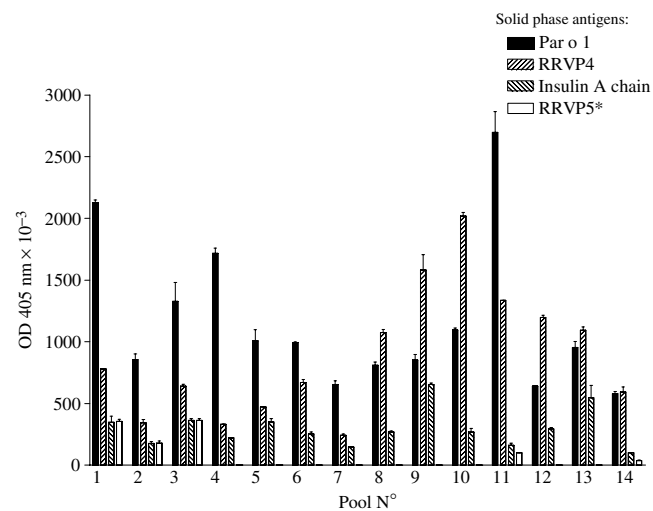


Figure 3. Direct ELISA. Binding of Par o 1-specific IgG antibodies affinity purified from pools. Binding was revealed using phosphatase-labeled goat anti serum specific for human IgG. This figure is a summary of two experiments; binding to RRVP5\* was determined only for the eluates from pool nos. 1–3, 11 and 14.

Table 1. ELISA inhibition. Binding of Par o 1-specific human IgG antibodies to solid phase Par o 1

| Pool no. | a    | a <sup>1</sup> | b    | b <sup>1</sup> | a/b  |
|----------|------|----------------|------|----------------|------|
| 1        | 20.3 | 71%            | 81.3 | 78%            | 0.25 |
| 4        | 8.3  | 94%            | 27.5 | 89%            | 0.30 |
| 5        | 6.0  | 62%            | 45.5 | 60%            | 0.13 |
| 6        | 2.6  | 69%            | 41.7 | 76%            | 0.06 |
| 9        | 19.5 | 71%            | 40.4 | 66%            | 0.49 |
| 11       | 17.2 | 96%            | 42.8 | 65%            | 0.40 |
| 12       | 6.4  | 63%            | 9.3  | 75%            | 0.67 |

Par o 1-specific antibodies affinity were purified from the pools described under ‘Materials and methods’. Antibody eluates were grouped according to donor’s characteristics. Fifty microliters of 1/5 dilution of eluate were absorbed with serial amounts of Par o 1 or of RRVP4 ( $1\text{--}100 \times 10^{-7}$  mol/l) and tested in ELISA for binding to Par o 1.

a, b:  $1 \times 10^{-7}$  mol/l of Par o 1 (a) or RRVP4 (b) required for 50% binding inhibition (extrapolated from dose–response curves).

a<sup>1</sup>, b<sup>1</sup>: percent inhibition achieved by absorption with the maximum amount ( $100 \times 10^{-7}$  mol/l) of Par o 1 (a<sup>1</sup>) or RRVP4 (b<sup>1</sup>).

a/b: ratio between values in column a and b.

Cross-reactivity of IgG antibodies from individual sera

To further investigate whether the presence of cross-reacting antibodies was correlated to phenotypic or genotypic characteristic of the serum donors, par o 1-specific IgG antibodies were purified from 50 individual sera. Sera were subdivided into four categories, according to donor’s characteristics. Group A–C donors were Italians with (group A) or without (groups B and C) ST

Table 2. ELISA. Reactivity of Par o 1-specific antibodies from individual donors

|                     | Solid phase antigen |                    |                    |                    |                      |
|---------------------|---------------------|--------------------|--------------------|--------------------|----------------------|
|                     | Par o 1             | RRVP4              | Insulin A chain    | RRVP5*             | R                    |
| Group A<br>(n = 16) | 786 ± 626<br>(575)  | 507 ± 555<br>(363) | 235 ± 235<br>(150) | 229 ± 272<br>(150) | 4.04 ± 4.1<br>(2.62) |
| Group B<br>(n = 19) | 556 ± 305<br>(492)  | 641 ± 447<br>(467) | 263 ± 162<br>(198) | 232 ± 238<br>(224) | 1.04 ± 0.6<br>(1.22) |
| Group C<br>(n = 3)  | 506 ± 40<br>(467)   | 421 ± 10<br>(422)  | 236 ± 21<br>(226)  | nd                 | 1.1 ± 01<br>(1.1)    |
| Group D<br>(n = 12) | 604 ± 195<br>(553)  | 550 ± 370<br>(380) | 217 ± 73<br>(210)  | nd                 | 1.16 ± 0.9<br>(1.25) |

Par o 1-specific antibodies affinity purified from individual sera and grouped according to donor’s characteristics. Eluates were tested for binding to Par o 1, RRVP4, RRVP5\* and insulin A chain. Donors in groups A–C were Italians, ST positive (group A) or negative (groups B and C) to *Parietaria*. Donors in groups A and B were HLA-DRB1\*1100 and donors in group C HLA-DRB1\*03. Donors in group D were from USA.

The table reports, for each group, the arithmetic means (medians) of the value (absorbance at 405 nm) obtained for binding to the solid phase antigens. R: arithmetic means (medians) of the ratios between values obtained for binding to Par o 1 and to RRVP4 (calculated after subtracting unspecific binding to insulin A chain).

positivity to *Parietaria*. Group A and B donors had the genotype HLA-DRB1\*1100 and group C donors the genotype -DRB1\*03, positively and negatively correlated, respectively, to development of atopy to *Parietaria* (23). Sera in group D were from the atopic donors from Baltimore.

The eluates were analyzed for binding to Par o 1, RRVP4, RRVP5\* and insulin A chain. The results of this analysis, summarized in Table 2, confirmed that the presence of cross-reacting antibodies was not limited to subjects with atopy to *Parietaria*. Each of groups A–C eluates showed significant binding to Par o 1 and to RRVP4. Ten of the 12 group D eluates significantly bound Par o 1 and RRVP4 and two were negative for both antigens. Table 2 reports, for each group, the arithmetic means and medians of the absorbance values at 405 nm and ratio between the absorbance values obtained for binding to Par o 1 and to RRVP4. Interestingly, this ratio was significantly increased in essays performed with eluates from donors ST positive as compared to donors ST negative to *Parietaria* (Mann–Whitney rank test,  $P = 0.002$ ).

Discussion

The cross-reactivity between Par o 1 and RRVP4 is amply documented by ELISA and DARIA performed with human IgG and IgE antibodies, respectively. The presence of cross-reacting antibodies is not sporadic and does not correlate with donor’s atopic phenotype or with HLA-DRB1\* genotypes associated to *Parietaria* specific atopy (Fig. 3, Tables 1 and 2).

The postulated localization of the cross-reactive epitope at the C-terminal end of the VP4 protein is supported by the observation that the bindings of cross-reactive antibodies was specifically inhibited by synthetic peptides representing the C-terminal segments of RRV4 (Figs 1B and 2A, B). Moreover, the binding of Par o 1-specific IgG and IgE antibodies to Par o 1 was inhibited by recombinant RRV4 but not by its fragment RRV5\* (6), which contains amino acid residues 274–474 and lacks the putative cross-reacting region (Fig. 2C).

The consistently extensive inhibition of Par o 1 binding achieved by absorption with RRV4 in competitive immunoassays suggests that the cross-reacting epitope(s) dominate the antibody response to Par o 1 (Fig. 2A–C and Table 2). Immunodominance of protein epitopes represented by synthetic peptides has been reported in a number of systems (29, 30). However, it should be emphasized that it is often difficult to prove that the repertoire of antibodies monitored is complete, as the antigen used in the assay could be partially denatured. An additional factor which may restrict the repertoire of Par o 1 epitopes monitored, is that the allergen preparation used in the assay, obtained from commercial pollen, could represent only a fraction of the array of molecules (Par o 1 isoforms, local variants) to which serum donors were exposed and against which they developed antibodies.

The observation that synthetic peptides representing the C-terminal regions of VP4 proteins from different serotypes of human infective rotavirus inhibit the cross-reactive binding, suggests that Par o 1 crossreacts also with the VP4 protein from human-infective rotavirus (Fig. 1B). Antibodies directed at the HRVP4 region

identified in this study have not been previously described. RRV4 epitopes for humans are located at the amino-terminal region (10). HRVP4 C-terminal region contains epitopes identified by mouse monoclonal antibodies, the role of which in virus neutralization has not been established (20).

The presence of antibodies with reactivity to Par o 1 in the sera from the Baltimore donors could be explained by priming of the immune system by rotavirus infection. This hypothesis is supported by the observation that the affinity of cross-reacting antibodies for Par o 1 was increased in *Parietaria* ST positive, as compared to *Parietaria* ST negative donors (Tables 1 and 2), perhaps reflecting the effect, in the first, of exposure to the pollen allergens and Par o 1-driven antibody repertoire selection.

The results of the present study do not provide information on the biological significance of the cross-reactivity between Par o 1 and RVP4. Interaction of the human immune system with *Parietaria* and rotavirus antigens occurs at very early age repertoires. In areas of diffusion of *Parietaria*, the large majority of adults develop IgG antibody response to its pollen allergens and, according to a recent survey, almost all children are seropositive to rotavirus by 2 years of age (31–33). It could be hypothesized that cross-reactivity between these antigens could influence the cytokine profile and/or the epitope specificity of individual immune response (15–17, 34).

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