

## Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies

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### Summary

**Background** Allergen-specific immunotherapy represents a causal form of treatment for IgE-mediated allergies. The allergen extract-based analyses of immunotherapy-induced effects yielded highly controversial results regarding a beneficial role of therapy-induced IgG antibodies.

**Objective** We analysed allergen-specific IgE, IgG subclass, and IgM responses in patients treated with a grass pollen allergy vaccine adjuvanted with monophosphoryl lipid A (MPL), a Th1-inducing agent, and in a placebo group using recombinant timothy grass pollen allergen molecules (rPhl p 1, rPhl p 2, rPhl p 5).

**Results** The strong induction of allergen-specific IgG<sub>1</sub> and IgG<sub>4</sub> antibodies observed only in the actively treated group was associated with significant clinical improvement. Therapy-induced allergen-specific IgM and IgG<sub>2</sub> responses were also noted in several actively treated patients. An inhibition of allergen-dependent basophil histamine release was only obtained with sera containing therapy-induced allergen-specific IgG, but not with sera obtained before therapy or from placebo-treated patients. Moreover, patients with therapy-induced allergen-specific IgG antibodies showed a reduced induction of allergen-specific IgE responses during seasonal grass pollen exposure.

**Conclusion** Successful immunotherapy with the MPL-adjuvanted grass pollen allergy vaccine is associated with the production of allergen-specific IgG antibodies. These blocking antibodies may have protective effects by inhibiting immediate-type reactions and systemic increases of IgE responses caused by seasonal allergen exposure.

**Keywords** blocking antibodies, immunotherapy, recombinant grass pollen allergens

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### Introduction

The only causal and specific therapy for IgE-mediated allergies is allergen-specific immunotherapy [1]. It was developed long before the discovery and characterization of IgE antibodies and is based on the administration of the disease-eliciting allergens or modifications thereof in order to reduce allergic symptoms in an allergen-specific manner [2]. The potential mechanisms underlying specific immunotherapy include the induction of blocking antibodies, a shift from Th2 to Th1 responses, and the induction of tolerance [3, 4]. Since its introduction into clinical practice in 1911, injection immunotherapy is developing more and more towards the principle of vaccination, mainly by using depot adjuvant-bound allergen, extracts, allergen-derived peptides, recombi-

nant allergens and their derivatives [1, 3, 4]. The fact that the imbalance within the Th1/Th2 system observed in allergic patients gives rise to the preferential production of IgE antibodies to otherwise harmless antigens (i.e. allergens) has been a major argument to seek for therapeutic strategies or adjuvants that may promote Th1 immune responses [5–7].

Besides strategies that are based on the use of allergen-encoding DNA [8] or allergen-coupled oligonucleotides (CpG motifs) [9] to induce Th1-like cytotoxic immune responses, liposome-entrapped allergen extracts have been suggested for immunotherapy [10].

Recently it has been demonstrated that monophosphoryl lipid A (MPL), a 3-deacylated monophosphoryl lipid A, derived from the lipopolysaccharide (LPS) of *Salmonella minnesota*, in conjugation with antigens adsorbed to L-tyrosine, induced a Th1-skewed immune response and also led to enhanced IgG responses [11, 12].

In this study humoral immune responses (IgE, IgG<sub>1–4</sub>, and IgM) were investigated in patients who had received grass pollen-specific immunotherapy with a tyrosine-adsorbed,

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glutaraldehyde-modified grass pollen extract containing MPL as an adjuvant [13]. Recombinant major timothy grass pollen allergens, rPhl p 1, rPhl p 2, and rPhl p 5 [14], were used in order to measure immune responses towards defined allergen molecules. It was investigated whether therapy-induced allergen-specific IgG antibodies can inhibit allergen-dependent histamine release from human basophils. Furthermore, the increases of allergen-specific IgE antibodies induced by seasonal allergen exposure were compared between patients who had received active or placebo treatment.

## Methods

### *Patients and clinical data*

Sera analysed in this study were obtained from 20 grass pollen allergic patients (active treatment:  $n = 11$ ; placebo:  $n = 9$ ) who had participated in a randomized, double-blind, placebo-controlled multicentre immunotherapy study that was conducted strictly according to the declaration of Helsinki [13]. The study employed a preseasonal immunotherapy regimen comprising a course of four subcutaneous injections of L-tyrosine-adsorbed, glutaraldehyde-modified grass pollen extract containing the Th1-driving adjuvant MPL or, for control purposes (placebo group), injections with L-tyrosine (2% w/v) in an excipient solution alone [13]. The aqueous grass pollen extract was prepared from a mixture of equal amounts of 13 temperate grasses (*Secale cereale*: rye; *Alopecurus pratensis*: Meadow foxtail; *Cynosurus cristatus*: Dogtail grass; *Dactylis glomerata*: Orchard grass; *Lolium perenne*: Rye grass; *Poa pratensis*: Kentucky bluegrass; *Anthoxanthum odoratum*: Vernal grass; *Festuca pratensis*: Fescue meadow; *Agrostis tenuis*: Bentgrass; *Phleum pratense*: Timothy grass; *Bromus spp.*: Brome grass; *Arrhenatherum elatius*: Oat grass; *Holcus lanatus*: Velvet grass) and has been standardized for group 1 allergens. The IgE reactivity of the extract was reduced by glutaraldehyde treatments as described [15]. The last injection was given approximately 2–4 weeks before the start of the main grass pollen season. Blood samples were taken before treatment (point 1), after treatment, (point 2), in the middle of the assessment period (i.e. grass pollen season: May/June) (point 3), and in autumn (point 4).

### *Assessment of clinical efficacy*

Over 4 weeks during the main grass pollen season, clinical efficacy was determined according to diary cards containing the patients' daily assessment of eye, nose, and lung symptoms (scores: no (0), mild (1), moderate (2), severe (3) symptoms) [13]. In parallel medication, intake was scored [13]. Symptom scores as well as symptom medication scores of the verum- and the placebo-treated groups were analysed before (time-point 1) and after treatment (time-point 3: grass pollen season).

### *ELISA measurements of IgE, IgG subclass, and IgM responses*

ELISA plates (GREINER Labortechnik GmbH, Kremsmünster, Austria) were coated either with natural timothy grass

pollen extract (100 µg/mL in PBS) (phosphate-buffered saline) or rPhl p 1, rPhl p 2, or rPhl p 5 (5 µg/mL in PBS) at 4 °C overnight. Plates were washed twice with PBS/0.05% v/v Tween 20 (buffer A) and blocked with PBS/1% w/v BSA/0.05% v/v Tween 20 (bovine serum albumin) at 37 °C for 2.5 h. Sera were diluted in buffer B as follows: IgE 1 : 5, IgG<sub>1-4</sub> 1 : 50, IgM 1 : 100. Plate-bound allergens were incubated with 100 µL/well of the diluted sera at 4 °C overnight. After five washes with 250 µL/well of buffer A, bound antibodies were detected with mouse monoclonal anti-human IgE, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgM antibodies (PharMingen, San Diego, CA, USA), and a horseradish peroxidase-labelled sheep anti-mouse antiserum (Amersham, Pharmacia) as described [14].

The differences in antibody levels between time-points 1 and 3 were calculated ( $OD \pm SD$  time-point 3 –  $OD \pm SD$  time-point 1) (optical density) and analysed with the Wilcoxon two-sample test. The alterations in OD levels corresponding to antibody levels between time-points 1 and 3 were expressed as mean percentage increases for the placebo and verum group.

### *Basophil histamine release experiments*

Heparinized blood samples from grass pollen allergic patients were obtained by venipuncture after informed consent was given. Granulocytes were isolated by dextran sedimentation [16]. Purified rPhl p 5 was dissolved in histamine release buffer (Immunotech, Marseille, France) at different concentrations ( $10^{-4}$  µg/mL,  $10^{-3}$  µg/mL,  $10^{-2}$  µg/mL,  $10^{-1}$  µg/mL, 1 µg/mL). In one set of experiments, allergen dilutions were incubated with equal volumes of sera obtained from patients before (time-point 1) or after (time-point 3) treatment, for 30 min at 37 °C as described [17]. Granulocytes were then exposed to the allergen-containing samples and histamine release into the cell-free supernatants was determined by radioimmunoassay (Immunotech). Histamine releases were measured in triplicate and were expressed as percentages of total histamine determined after cell lysis [16].

### *Statistical analysis*

The comparison between treatment groups was analysed with a test for independent groups, i.e. the Wilcoxon two-sample test.

## Results

### *Effects of the grass pollen allergy vaccine on antibody responses as monitored with natural and recombinant timothy grass pollen allergens*

Most of the patients (10/11) who had received active treatment (e.g., patients #6, #3) (Figs 1 and 2), but not the placebo-treated patients (e.g. patients #15, #13) (Figs 3 and 4), exhibited a strong induction of IgG<sub>1</sub> and/or IgG<sub>4</sub> responses to natural timothy grass pollen extract and to rPhl p 5 in the course of treatment. IgG subclass responses induced by the vaccine were mainly directed against rPhl p 5, whereas rPhl p 1- and rPhl p 2-specific IgG subclass responses were low and found in only a few patients (Figs 1 and 2).

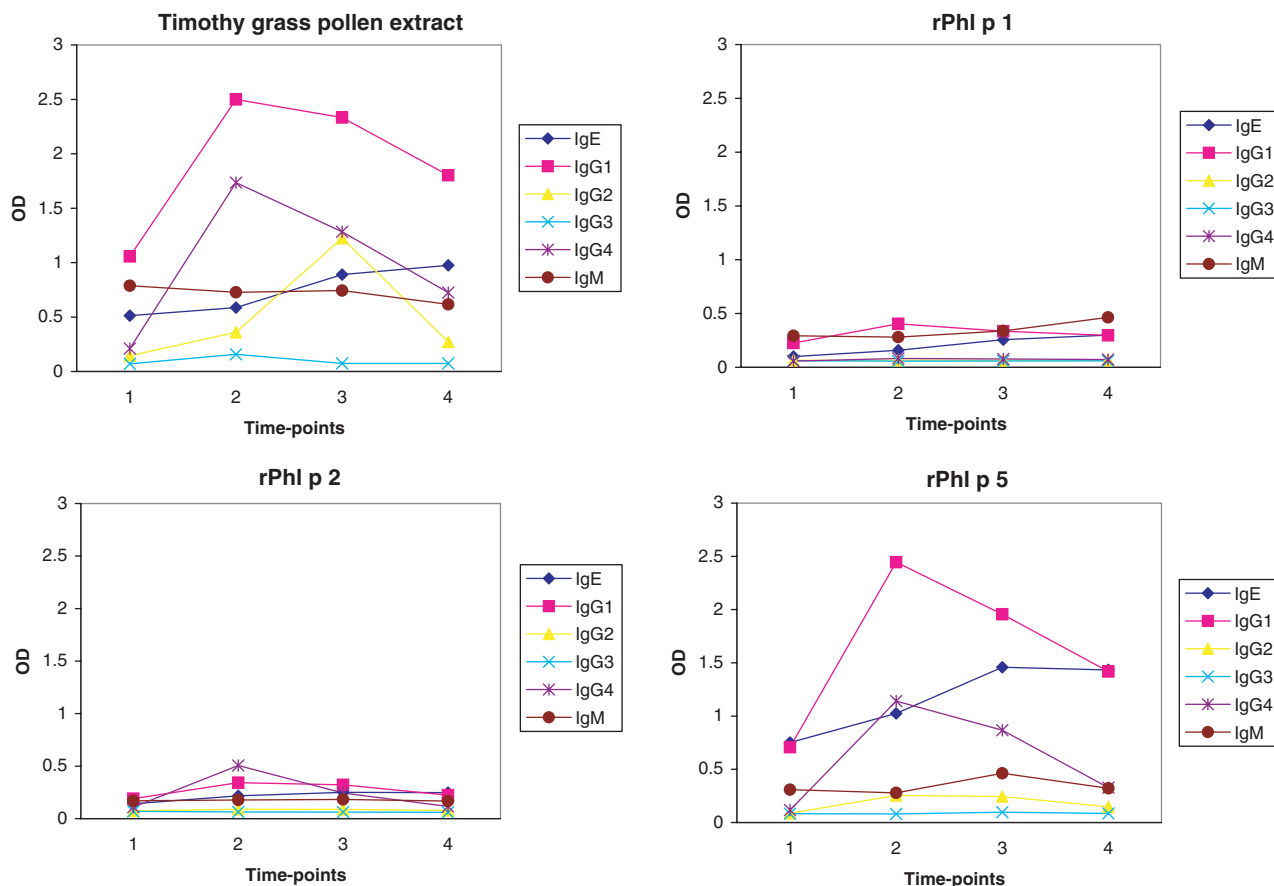


**Fig. 1.** Courses of IgE, IgG<sub>1-4</sub>, and IgM responses to timothy grass pollen extract and recombinant timothy pollen allergens. Patient #6 who had received active treatment is shown. The optical densities corresponding to the amounts of bound IgE, IgG<sub>1-4</sub>, and IgM antibodies are displayed on the y-axis. The time-points of serum collection are displayed on the x-axis.

An induction of IgG<sub>2</sub> antibodies occurred in certain cases (4/11 patients) to components of timothy grass pollen extract other than major allergens (Phl p 1, Phl p 5, Phl p 2) (e.g. patient #6, Fig 1) and to rPhl p 5 (patient #7, data not shown).

In most of the patients, timothy grass pollen-specific IgE antibodies were directed mainly against rPhl p 5. The patterns of alteration in the IgE responses were different from those observed for IgG subclasses. Patients who had received active treatment (e.g. patients #3, #6) showed a reduced increase of allergen-specific IgE antibodies after the beginning of the grass pollen season compared to patients who had received placebo treatment (e.g. patients #13, #15) (Figs 1–4).

#### *Treatment with MPL-adjuvanted grass pollen allergoid induces allergen-specific IgM and pollen extract-specific IgG<sub>2</sub> responses*

MPL has been mainly used as an adjuvant for vaccines against viral infections [18–20]. During immunotherapy with MPL-adjuvanted grass pollen allergoid, an induction of IgM responses to timothy grass pollen extract as well as to recombinant timothy grass pollen allergens (e.g. patient #3, rPhl p 1, and rPhl p 5, Fig 2) was observed in five out of the

11 actively treated patients. In agreement with previous data, showing that MPL induces a Th1 immune response in animal models [12], we observed an induction of IgG<sub>2</sub> antibodies to natural timothy grass pollen extract in four out of the 11 actively treated patients (e.g. patient #6, Fig 1).

#### *Actively treated patients exhibit a strong induction of allergen-specific IgG<sub>1</sub> and IgG<sub>4</sub>, which is associated with significant clinical improvement*

Optical density values corresponding to allergen-specific IgG<sub>1</sub> and IgG<sub>4</sub> levels in serum samples obtained before and after therapy were compared in patients who had received active or placebo treatment (Fig. 5). After treatment (time point 3), timothy grass pollen extract-specific IgG<sub>1</sub> levels had increased on an average for 208.1% in the active group (Fig 5a) whereas in the placebo group only a 46% average increase was observed (Fig. 5b). A statistically significant difference between the alterations in the placebo ( $0.32 \pm 0.17$ ) and verum ( $1.00 \pm 0.51$ ) group was found ( $P < 0.001$ ). An even more pronounced increase of rPhl p 5-specific IgG<sub>1</sub> was found for the treated group (218.6% vs. 38.6% in the placebo group) (Figs 5c and d). Again, the difference between the placebo

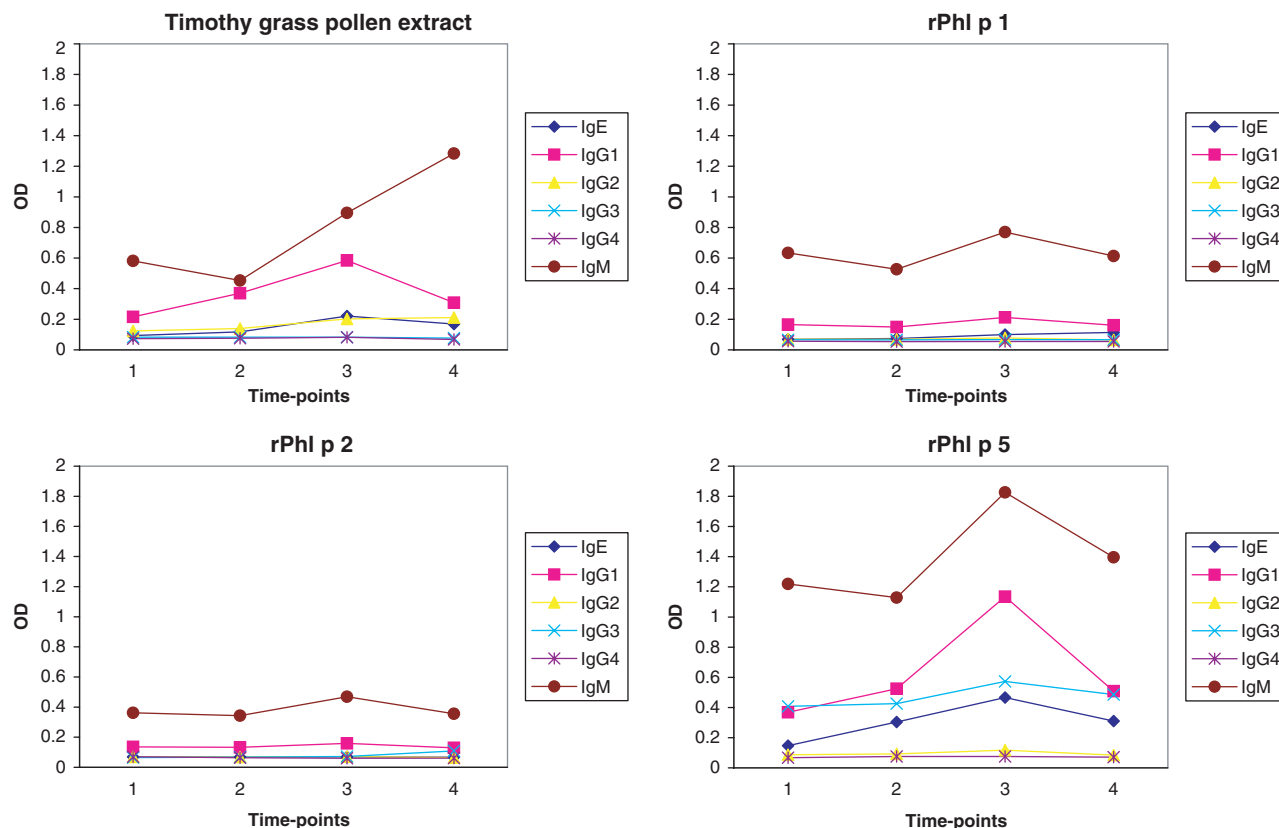


**Fig. 2.** Courses of IgE, IgG<sub>1–4</sub>, and IgM responses to timothy grass pollen extract and recombinant timothy pollen allergens. Patient #3 who had received active is shown. The optical densities corresponding to the amounts of bound IgE, IgG<sub>1–4</sub>, and IgM antibodies are displayed on the y-axis. The time-points of serum collection are displayed on the x-axis.

( $0.20 \pm 0.22$ ) and verum group ( $0.86 \pm 0.41$ ) was statistically significant ( $P < 0.001$ ).

Phl p 1-specific IgG<sub>1</sub> responses in both groups (active, placebo) were lower than rPhl p 5-specific IgG<sub>1</sub> responses before therapy. Only low increases of Phl p 1-specific IgG<sub>1</sub> of approximately 30% were observed, and there was no statistically significant difference between placebo and verum groups (Figs 5e and f).

Alterations of allergen-specific IgG<sub>4</sub> responses were similar to those observed for IgG<sub>1</sub>: a 427.7% average increase of IgG<sub>4</sub> against natural timothy grass pollen extract occurred in actively treated patients (Fig. 6a) compared to a 50.7% increase in placebo-treated patients (Fig. 6b). The difference between the groups (placebo:  $0.15 \pm 0.19$ ; verum:  $0.94 \pm 0.67$ ) was significant ( $P < 0.01$ ). rPhl p 5-specific IgG<sub>4</sub> antibodies increased to 532.3% in the active group, but only a 32.9% increase was observed in the placebo group (Figs 6c and d). Again, the difference between the groups (placebo:  $0.04 \pm 0.06$ ; verum:  $0.86 \pm 0.71$ ) was significant ( $P < 0.01$ ). rPhl p 1-specific IgG<sub>4</sub> levels showed a 133% increase in the active group and a 45% increase in the placebo group and there was no significant difference between the groups (Figs 6e and f). In conclusion, none of the placebo-treated patients showed increases of IgG as observed in the active group.

After treatment (time-point 3), a statistically significant difference ( $P < 0.01$ ) between the placebo ( $2.2 \pm 0.5$ ) and

verum group ( $1.6 \pm 0.6$ ) was demonstrated for symptom scores. Verum-treated patients also showed a significant improvement in their symptom medication score ( $1.7 \pm 0.5$ ) when compared with the placebo-treated patients ( $2.4 \pm 0.7$ ) ( $P = 0.04$ ). No significant difference regarding symptoms and medication intakes between the placebo- and verum-treated patients was observed during the previous pollen season.

#### *Actively treated patients show a reduced boost of IgE production during the grass pollen season*

It is well established that allergic patients exhibit strong increases of allergen-specific IgE levels after seasonal allergen exposure [21]. In order to study whether specific immunotherapy may reduce boosts of IgE production caused by seasonal allergen exposure, we compared specific IgE levels in serum samples of actively and placebo-treated patients before and at the end of the grass pollen season. Figures 7a and b showed a 72.4% increase in IgE antibodies against timothy grass pollen extract in the actively treated group and an 85% increase in the placebo group, respectively. rPhl p 5-specific IgE antibodies showed an increase of 144.7% in the active group and a 195% increase in placebo-treated patients (Figs 7c and d). rPhl p 1-specific IgE levels rose 51.3%, whereas placebo-treated patients displayed a 77.7% increase of Phl p 1-specific IgE antibodies (Figs 7e and f). Although statistically not

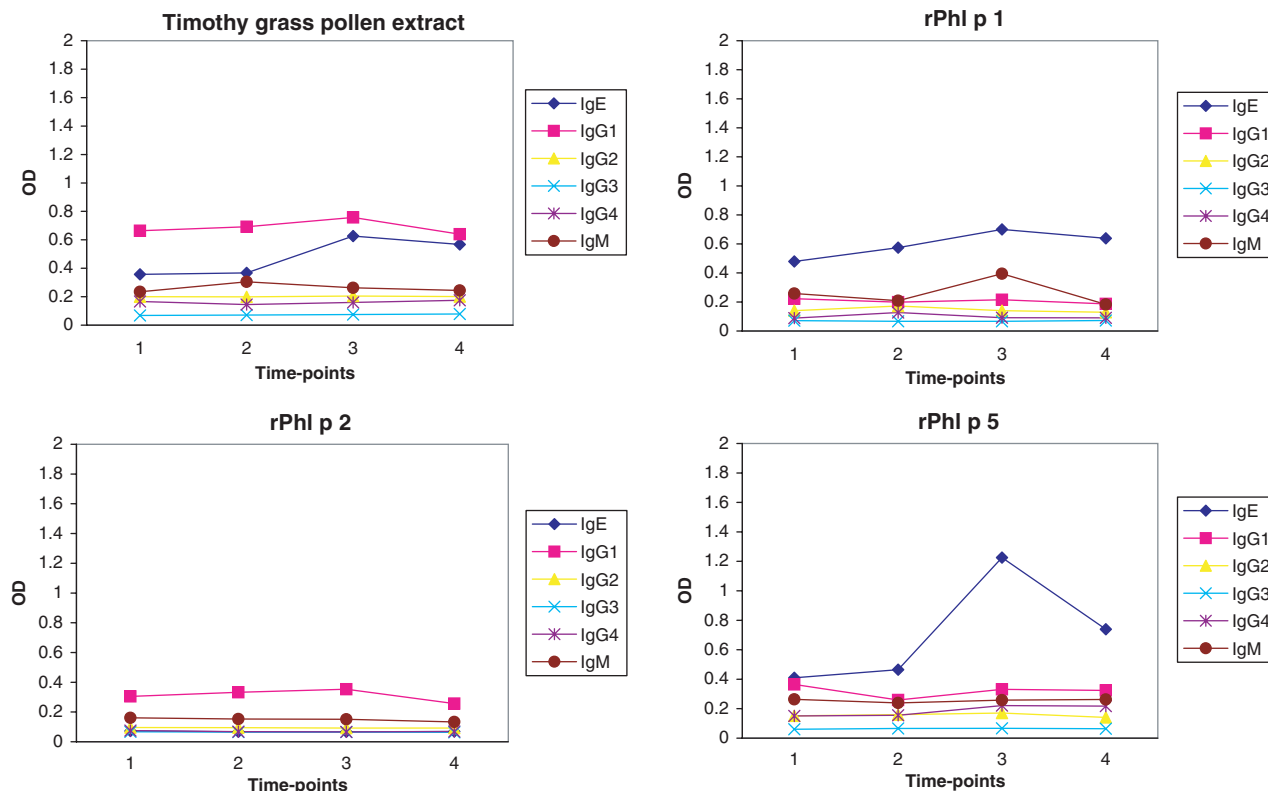


**Fig. 3.** Courses of IgE, IgG<sub>1-4</sub>, and IgM responses to timothy grass pollen extract and recombinant timothy pollen allergens. Patient #15 who had received placebo treatment is shown. The optical densities corresponding to the amounts of bound IgE, IgG<sub>1-4</sub>, and IgM antibodies are displayed on the y-axis. The time-points of serum collection are displayed on the x-axis (in the middle of the assessment period, June 99).

significant, increases of allergen-specific IgE were always lower in the actively treated patients compared to the group who had received placebo (Fig. 7).

#### *Sera containing therapy-induced IgG antibodies inhibit allergen-dependent basophil histamine release*

In order to study whether therapy-induced IgG antibodies can inhibit allergen-induced immediate allergic reactions, basophil histamine release experiments were performed as described earlier for venom allergy [22]. rPhl p 5 was preincubated with two sera obtained before or after active treatment and then exposed to basophils from a Phl p 5-allergic person (Figs 8a and b). Sera containing therapy-induced rPhl p 5-specific IgG<sub>1</sub> and IgG<sub>4</sub> antibodies inhibited the Phl p 5-dependent histamine release strongly: when rPhl p 5 was preincubated with patients' sera obtained before treatment, a 30–50% induction of histamine release was observed at a concentration of  $10^{-4}$  µg/mL allergen. Pre-incubation of rPhl p 5 with sera from the same patients containing therapy-induced rPhl p 5-specific IgG antibodies reduced the histamine release at the  $10^{-4}$  µg/mL allergen dose down to 0–5% (Figs 8a and b: patients #5, #7). Furthermore, sera containing therapy-induced IgG antibodies shifted the point of maximal histamine release to 10-(Fig. 8b: patient #7) or to 100-fold (Fig. 8a: patient #5) higher

allergen concentrations. Sera from Phl p 5-sensitized, placebo-treated patients had no significant effects on allergen-induced histamine release (e.g. Fig. 8c: patient #13). The spontaneous histamine release observed in these experiments never exceeded 10% (Fig 8a–c).

#### **Discussion**

In this study, we used recombinant major timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) [15] to study allergen-specific IgE, IgG<sub>1-4</sub>, and IgM responses in grass pollen allergic patients who had received a preseasonal course of four injections with MPL-adsorbed, tyrosine-adsorbed grass pollen allergoid or placebo. The strong induction of IgG<sub>4</sub> and IgG<sub>1</sub> antibodies and, to a lower degree, of IgG<sub>2</sub> and IgM against timothy grass pollen extract in the actively but not in the placebo-treated group indicates the vaccination character of this treatment. However, a detailed analysis with recombinant timothy grass pollen allergens revealed a great variation of IgG responses against the individual grass pollen allergen molecules.

In our opinion, the heterogeneous induction of IgG antibodies to different components in a therapeutic allergen extract deserves attention for several reasons. It demonstrates that it is impossible to correlate IgG antibody levels and

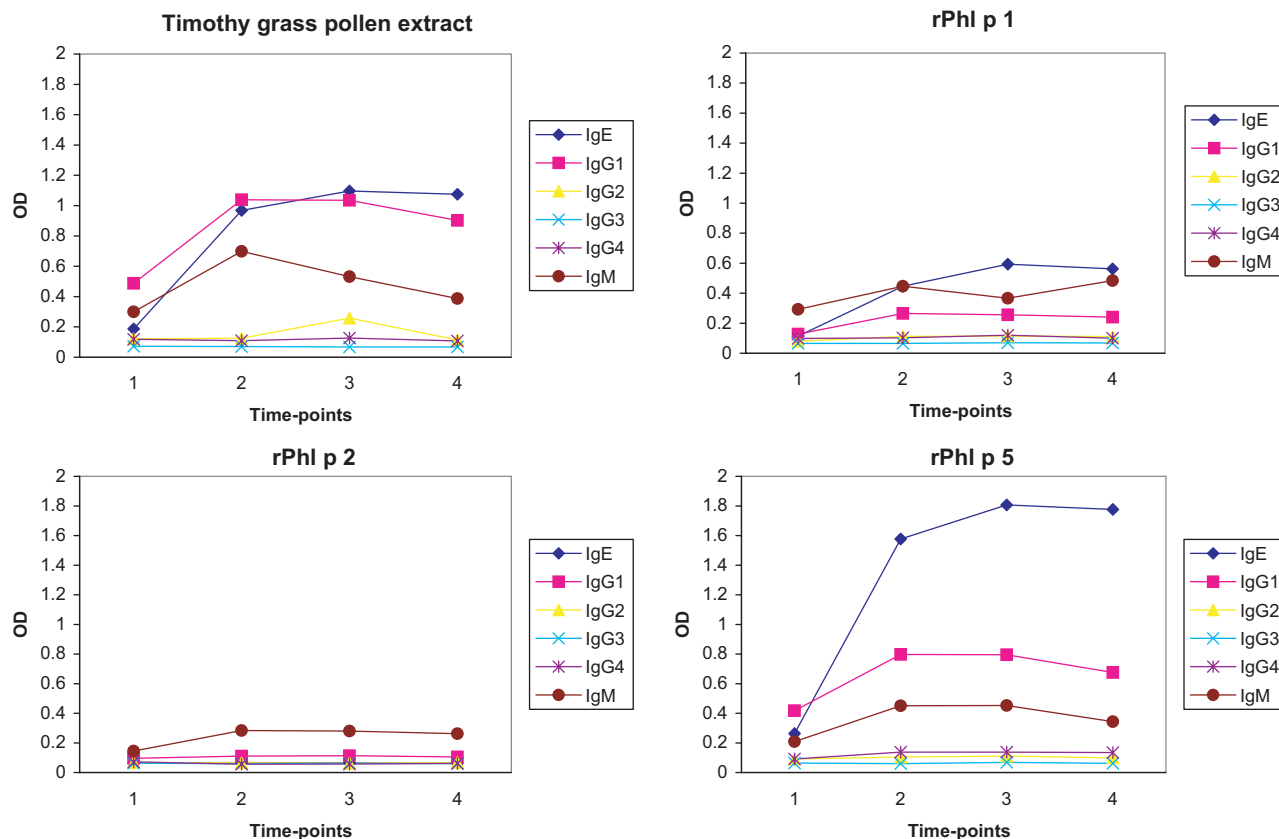


**Fig. 4.** Courses of IgE, IgG<sub>1-4</sub>, and IgM responses to timothy grass pollen extract and recombinant timothy pollen allergens. Patient #13 who had received placebo treatment is shown. The optical densities corresponding to the amounts of bound IgE, IgG<sub>1-4</sub>, and IgM antibodies are displayed on the y-axis. The time-points of serum collection are displayed on the x-axis (after the pollen season, September/October 99).

clinical improvement using crude allergen extracts because the proportion of allergen extract-specific IgG directed to the individual disease-eliciting allergens cannot be determined and evaluated using allergen extracts. In this context, we found it noteworthy that in some patients IgG antibodies were induced primarily against extract components other than the major grass pollen allergen groups (e.g. Fig. 1: patient #6, IgG<sub>2</sub>) and very low or undetectable IgG responses were induced against certain major allergens (e.g. Phl p 1, Phl p 2). The reason for the low induction of antibody levels to certain allergens may be their low representation in the extract, their intrinsic low immunogenicity, or a glutaraldehyde-induced reduction of their immunogenicity. Our data thus suggest that the outcome of specific immunotherapy might be improved if allergy vaccines would contain equally immunogenic allergens or allergen derivatives in equivalent and defined molarities [23].

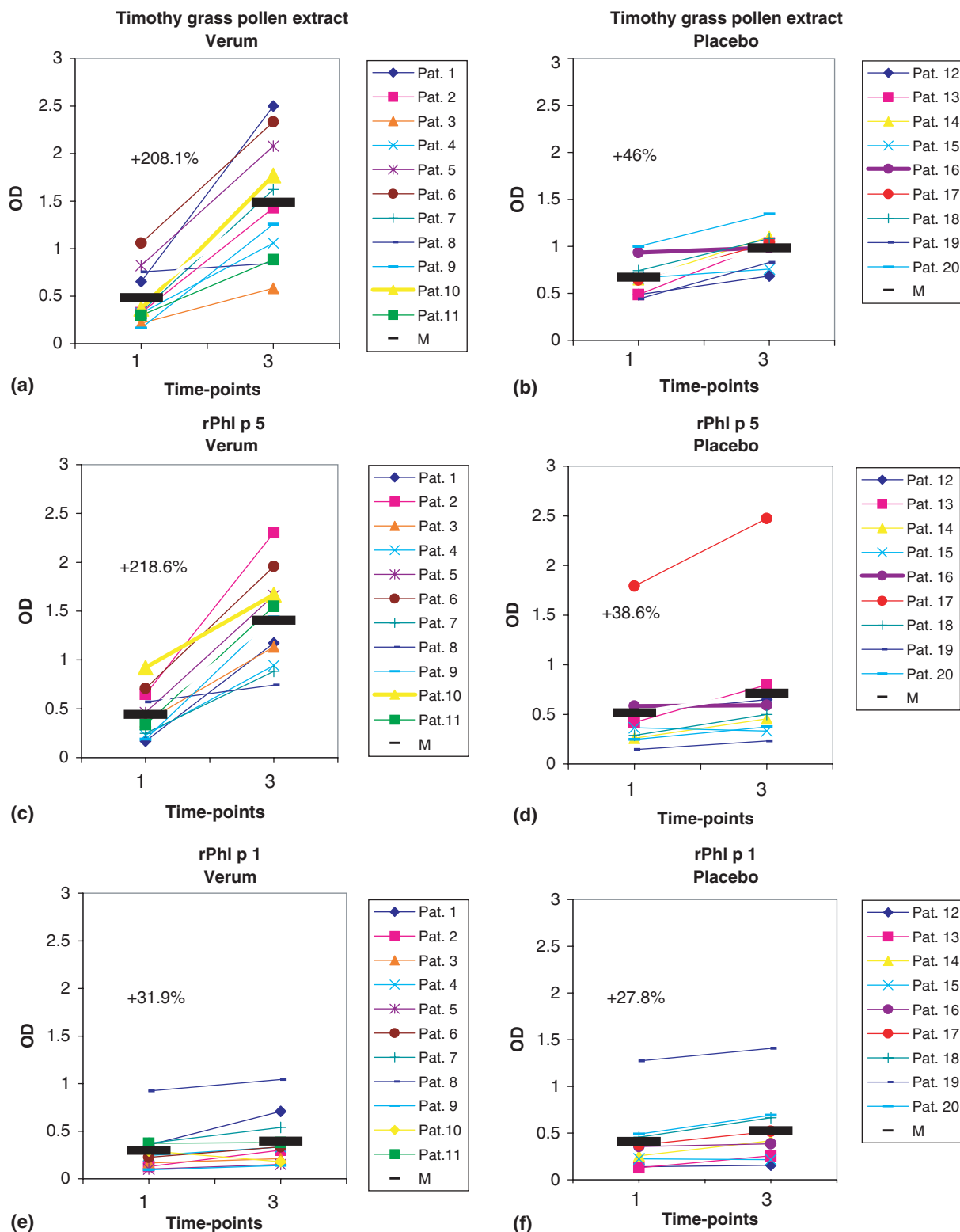
Even if the analysis of antibody reactivities and clinical data performed with single-allergen molecules, the protective activity of IgG antibodies may vary, depending on other factors (e.g. epitope specificity, affinity/avidity, level/titre, class/subclass). It is thus possible that many of the controversial results obtained regarding the role of 'blocking antibodies' were caused by the use of natural allergen extracts for treatment and analysis [4, 24–26].

In this study, the strongest induction of IgG antibodies was observed against rPhl p 5, a finding that may be explained by

the fact that group 5 allergens are stronger inducers of antibodies than group 1 allergens [23, 27]. Group 5 allergens are major allergens for more than 80% of the grass pollen allergic patients. They induce high IgE antibody levels in patients and belong to the most potent allergen molecules described to date [28–31].

For Phl p 5, our data strongly suggest that therapy-induced blocking antibodies have protective activity if they compete with IgE antibodies with respect to their epitopes. Support for the latter assumption comes from the following observations made in this study. First, we found a significant improvement of symptom as well as symptom medication scores in actively treated patients who developed Phl p 5-specific IgG responses. Second, we could demonstrate that only sera containing therapy-induced allergen-specific IgG, but not sera without such IgG (i.e. sera obtained before treatment or from patients who had received placebo), inhibited allergen-induced basophil histamine release. This finding is in agreement with previous results showing that sera containing therapy-induced allergen-specific IgG inhibited basophil degranulation [17], but it cannot be excluded at this point that in addition to allergen-specific IgG antibodies other therapy-induced, allergen-specific humoral factors may also contribute to the inhibition of immediate type-reactions.

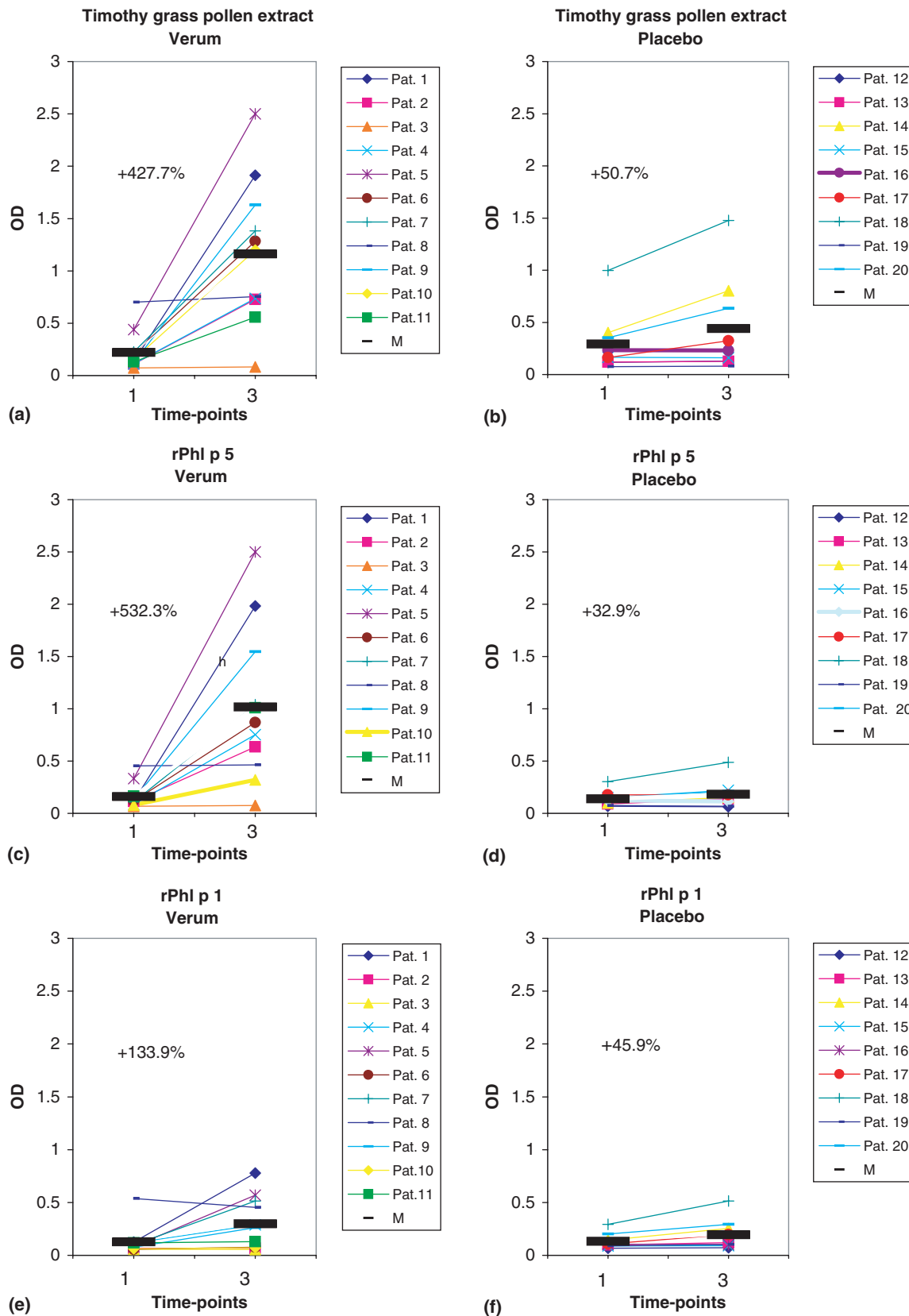
Therapy-induced blocking antibodies may have beneficial effects by reducing immediate allergic symptoms (e.g. suppression of basophil histamine release). As previously



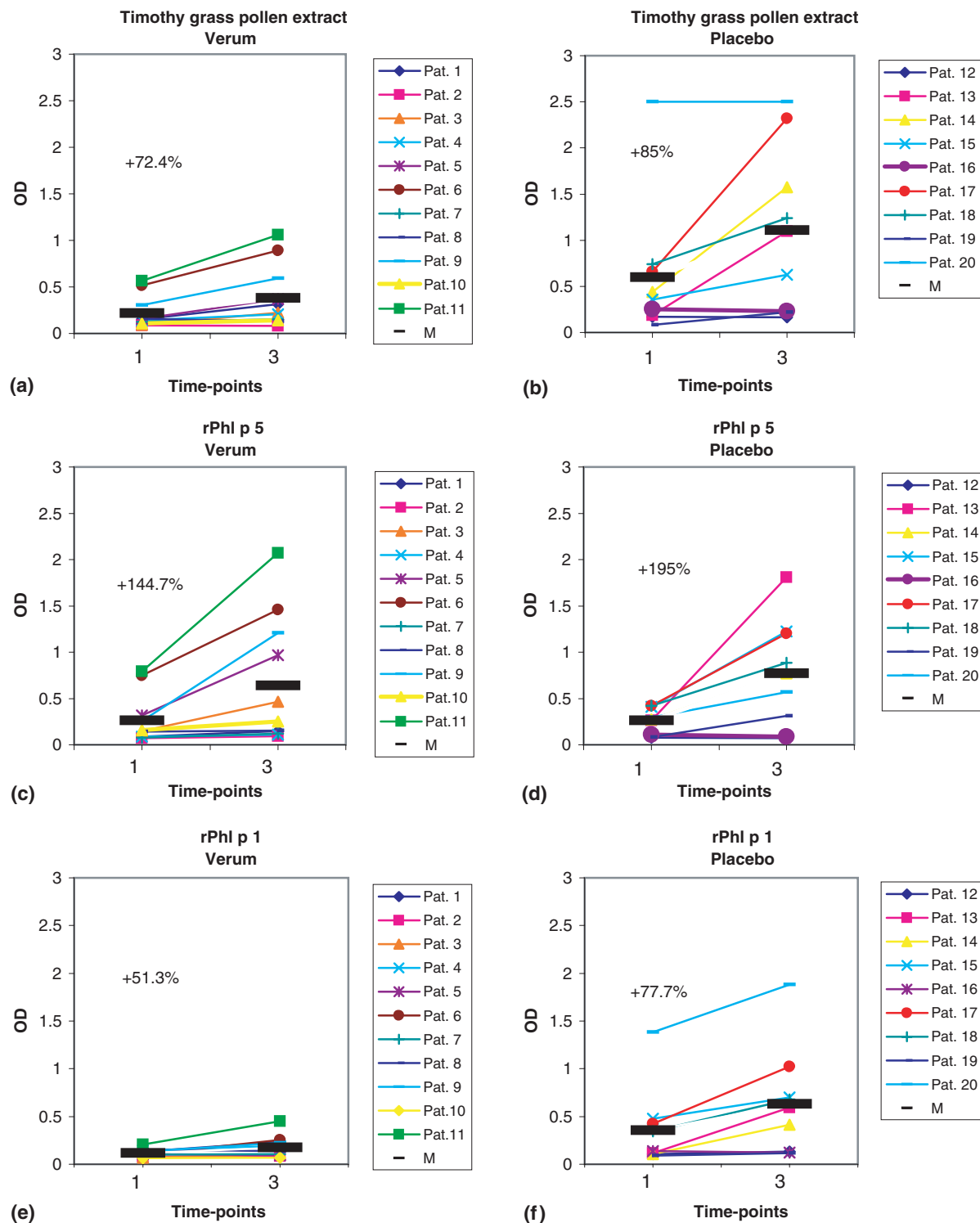
**Figs. 5.** Levels of IgG<sub>1</sub> to timothy grass pollen extract and recombinant allergens. Patients who had received active (active: patients 1–11) or placebo treatment (placebo: patients 12–20) before (left: point 1) and during the pollen season (right: point 3, May/June) are shown. The horizontal black bars represent the means of the IgG<sub>1</sub> responses. The OD values are displayed on the y-axis. Percentage increases of antibody levels are shown in the diagrams.

reported for birch pollen immunotherapy, it is possible that they may also down-regulate IgE-mediated allergen presentation to T cells and eosinophil activation and thus decrease the late-phase symptoms of allergy [32]. The following finding

may perhaps be important for the long-term effects of immunotherapy: that patients having therapy-induced allergen-specific IgG already exhibited in the first grass pollen season, immediately after the first treatment course, a lower



**Figs. 6.** Levels of IgG<sub>4</sub> to timothy grass pollen extract and recombinant allergens. Patients who had received active (active: patients 1–11) or placebo treatment (placebo: patients 12–20) before (left: point 1) and during the pollen season (right: point 3, May/June) are shown. The horizontal black bars represent the means of the IgG<sub>4</sub> responses. The OD values are displayed on the y-axis. Percentage increases of antibody levels are shown in the diagrams.



**Figs. 7.** Levels of IgE to timothy grass pollen extract and recombinant allergens. Patients who had received active (active: patients 1–11) or placebo treatment (placebo: patients 12–20) before (left: point 1) and during the pollen season (right: point 3, May/June) are shown. The horizontal black bars represent the means of the IgE responses. The OD values are displayed on the y-axis. Percentage increases of antibody levels are shown in the diagrams.

induction of IgE responses by seasonal allergen contact than the placebo group. The lower increase of IgE in the actively treated group during the grass pollen season may be caused by blocking antibodies preventing the stimulation of IgE memory cells or may result from a modulation of the Th2/

Th1 balance caused by the MPL-adjuvanted grass pollen allergoid. The latter explanation (i.e. a modification of the memory Th2 response) is, however, less likely because an allergen-specific IgG<sub>2</sub> response indicating a shift to a Th1-like immunity was noted in only few patients [33–35].

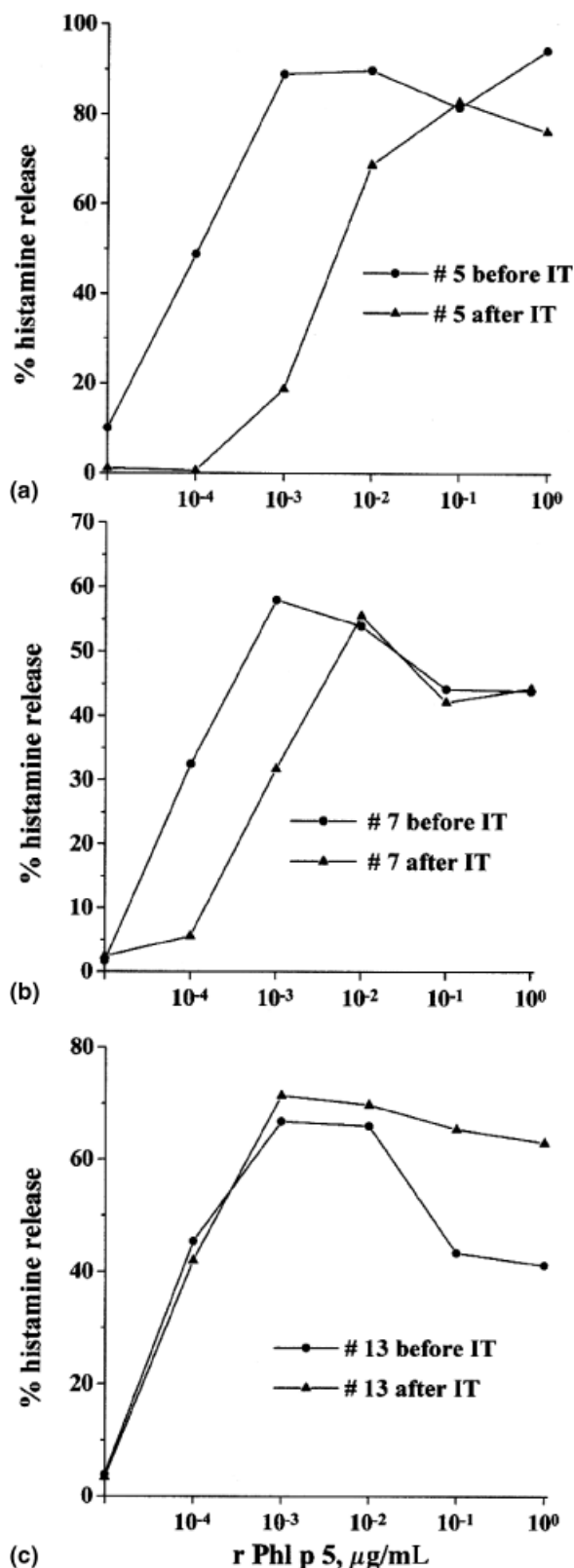


**Fig. 8.** Preincubation of rPhl p 5 with sera containing therapy-induced IgG antibodies inhibits histamine release. Different concentrations of rPhl p 5 were preincubated with sera obtained before (dots) and after (triangles) immunotherapy from two patients who had received active treatment (a: #5; b: #7) or from a placebo-treated patient (c: #13). Results in a–c represent the percentage values of total histamine and are expressed as the mean  $\pm$  SD of triplicate determinations (y-axis).

In summary, our study demonstrates that a preseasonal course of four injections of MPL-adsorbed, tyrosine-adsorbed grass pollen allergoid has vaccination character. Clinical improvement was associated with the production of allergen-specific IgG antibodies, which may inhibit allergen-induced immediate reactions. Furthermore, treated patients having allergen-specific IgG exhibited reduced increases in allergen-specific IgE after seasonal allergen exposure.

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### References

- 1 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102:558–62.
- 2 Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911; 1:1572–3.
- 3 Durham SR, Till SJ. Immunological changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; 102: 157–64.
- 4 Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002; 2:446–53.
- 5 Romagnani S. The Th1/Th2 paradigm. *Immunol Today* 1997; 18:263–6.
- 6 Ebner C. Immunological mechanisms operative in allergen-specific immunotherapy. *Int Arch Allergy Immunol* 1999; 119:1–5.
- 7 Durham SR, Kay AB, Hamid Q. Changes in allergic inflammation associated with successful immunotherapy. *Int Arch Allergy Immunol* 1995; 107:282–4.
- 8 Hsu CH, Chua KY, Tao MH et al. Immuno-prophylaxis of allergen-induced immunoglobulin E synthesis and airway hyper-responsiveness *in vivo* by genetic immunization. *Nat Med* 1996; 2:540–4.
- 9 van Uden J, Raz E. Immunostimulatory DNA and applications to allergic disease. *J Allergy Clin Immunol* 1999; 104:902–10.
- 10 Sehra S, Chugh L, Gangal SV. Polarized Th1 responses by liposome-entrapped allergen and its potential in immunotherapy of allergic disorders. *Clin Exp Allergy* 1998; 28:1530–7.
- 11 Ulrich JT, Myers KR. Monophosphoryl lipid A as an adjuvant. Past experiences and new directions. In: Powell MF, Newman MJ, eds. *Vaccine design: the subunit and adjuvant approach*. New York: Plenum Press, 1995:495–524.
- 12 Wheeler AW, Marshall JS, Ulrich JT. A Th1-inducing adjuvant, MPL<sup>®</sup> enhances antibody profiles in experimental animals suggesting it has the potential to improve the efficacy of allergy vaccines. *Int Arch Allergy Immunol* 2001; 126:135–9.
- 13 Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 2001; 56:498–505.
- 14 Vrtala S, Susani M, Sperr WR et al. Immunologic characterization of purified recombinant timothy grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5). *J Allergy Clin Immunol* 1996; 97:781–7.
- 15 Moran DM, Wheeler AW, Overell BG, Woroniecki SR. Chemical modification of crude timothy grass pollen extract. III. The effect of glutaraldehyde-induced aggregation on antigenic and immuno-

- genic properties. *Int Arch Allergy Appl Immunol* 1977; 55: 315–321.
- 16 Valent P, Besemer J, Muhm M et al. Interleukin-3 activates human blood basophils via high affinity binding sites. *Proc Natl Acad Sci USA* 1989; 86:5542–6.
  - 17 Ball T, Fuchs T, Sperr WR et al. B cell epitopes of the major timothy grass pollen allergen, rPhl p 1, revealed by gene fragmentation as candidates for immunotherapy. *FASEB J* 1999; 13:1277–90.
  - 18 Thoelen S. An overview on a novel adjuvanted prophylactic hepatitis B vaccine. *Acta Gastrenterol Belg* 2000; 63:185–6.
  - 19 Thoelen S, Van Damme P, Mathei C et al. Safety and immunogenicity of a hepatitis B vaccine formulated with a novel adjuvant system. *Vaccine* 1998; 16:708–14.
  - 20 Heppner DG, Gordon DM, Gross M et al. Safety immunogenicity and efficacy of *Plasmodium falciparum* repeatless circumsporozoite protein vaccine encapsulated in liposomes. *J Infect Dis* 1996; 174:361–6.
  - 21 Henderson LL, Larson JB, Gleich GJ. Maximal rise in IgE antibody following ragweed pollination season. *J Allergy Clin Immunol* 1975; 55:10–5.
  - 22 Clinton PM, Kemeny DM, Youlten LJ, Lessof MH. Histamine release from peripheral blood leukocytes with purified bee venom allergens: effect of hyperimmune beekeeper plasma. *Int Arch Allergy Immunol* 1989; 89:43–8.
  - 23 Linhart B, Jahn-Schmid B, Verdino P et al. Combination vaccines for the treatment of grass pollen allergy consisting of genetically engineered hybrid molecules with increased immunogenicity: *FASEB J*. (21 June, 2002) 10.1096/fj.01-1012fje.
  - 24 Djurup R, Malling HJ. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin Allergy* 1987; 17:459–68.
  - 25 Boluda L, Fernandez-Caldas E, Berrens L. The role of IgG in Type I allergy: an unsolved problem. *J Invest Allergol Clin Immunol* 1997; 7:205–10.
  - 26 Denepoux S, Eibensteiner PB, Steinberger P et al. Molecular characterization of human IgG monoclonal antibodies specific for the major birch pollen allergen Bet v 1. Anti-allergen IgG can enhance the anaphylactic reaction. *FEBS Lett* 2000; 465:39–46.
  - 27 Vrtala S, Ball T, Spitzauer S et al. Immunization with purified natural and recombinant allergens induces mouse IgG<sub>1</sub> antibodies that recognize similar epitopes as human IgE and inhibit the human IgE–allergen interaction and allergen-induced basophil degranulation. *J Immunol* 1998; 160:6137–44.
  - 28 Vrtala S, Sperr WR, Reimitzer I et al. cDNA cloning of a major allergen from timothy grass (*Phleum pratense*) pollen; characterization of the recombinant Phl p V allergen. *J Immunol* 1993; 151:4773–81.
  - 29 Laffer S, Spitzauer S, Susani M et al. Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J Allergy Clin Immunol* 1996; 98:652–8.
  - 30 Bufe A, Gehlhar K, Schramm G, Schlaak M, Becker WM. Allergenic activity of a major grass pollen allergen is elevated in the presence of nasal secretion. *Am J Respir Crit Care Med* 1998; 157:1269–76.
  - 31 Flicker S, Vrtala S, Steinberger P et al. A human monoclonal IgE antibody defines a highly allergenic fragment of the major timothy grass pollen allergen, Phl p 5: molecular, immunological and structural characterization of the epitope-containing domain. *J Immunol* 2000; 165:3849–59.
  - 32 Van Neerven RJ, Wikborg T, Lund G et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4<sup>+</sup> T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; 163:2944–52.
  - 33 Kawano Y, Noma T, Yata J. Regulation of human IgG subclass production by cytokines IFN- $\gamma$  and IL-6 act antagonistically in the induction of human IgG<sub>1</sub> but additively in the induction of IgG<sub>2</sub>. *J Immunol* 1994; 153:4948–58.
  - 34 Inoue R, Kondo N, Kobayashi Y, Fukutomi O, Orii T. IgG<sub>2</sub> deficiency associated with defects in production of interferon- $\gamma$ . *Scand J Immunol* 1995; 41:130–4.
  - 35 Servet-Delprat C, Bridon JM, Djossou O, Yahia SA, Banchereau J, Briere F Delayed IgG<sub>2</sub> humoral response in infants is not due to intrinsic T or B cell defects. *Int Immunol* 1996; 8:1495–502.