

# T-cell epitopes of Phl p 1, major pollen allergen of timothy grass (*Phleum pratense*): Evidence for crossreacting and non-crossreacting T-cell epitopes within grass group I allergens

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**Background:** The use of peptides representing T-cell epitopes of allergens is a modern concept for improvement of specific immunotherapy. A prerequisite for this approach is the identification of T-cell epitopes of atopic allergens.

**Methods:** T-cell lines and 40 T-cell clones (TCC) specific for Phl p 1, the group I allergen of timothy grass (*Phleum pratense*), were established from the peripheral blood of nine patients allergic to grass pollen and mapped for epitope specificity by using overlapping dodecapeptides. Phenotype and cytokine production profile of TCC were investigated. Representative TCC were analyzed for HLA-restriction, T-cell receptor V $\beta$  gene usage, and crossreactivity with grass pollen extracts from *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Secale cereale*, and selected amino acid sequence-derived peptides.

**Results:** Patients displayed IgE binding to all grass species investigated. Forty TCC were established. Fifteen T-cell epitopes could be identified on Phl p 1. Of 40 TCC, 39 displayed the helper cell (Th) phenotype; one clone was CD8<sup>+</sup>. Specific stimulation induced a Th2-like type of cytokine production in 20 of 39 TCC. Crossreactivity studies revealed crossreacting and non-crossreacting T-cell epitopes.

**Conclusion:** Phl p 1, a major grass pollen allergen, harbors multiple T-cell epitopes. Species-specific and crossreacting T-cell epitopes exist among group I allergens of grasses. Epitope recognition patterns could not be correlated with particular HLA haplotypes. A restricted T-cell receptor V $\beta$  gene usage was not observed. (*J ALLERGY CLIN IMMUNOL* 1995;96:986-96.)

**Key words:** Type I allergy, recombinant Phl p 1, T-cell clone, T-cell epitope, crossreactivity

Up to 20% of the population of industrialized countries suffers from type I allergy.<sup>1,2</sup> Several

atopic allergens have been investigated by means of molecular biologic techniques.<sup>3,4</sup> cDNA cloning and sequencing have provided complete deduced amino acid (AA) sequences of these allergens, some of which were further expressed as recombinant non-fusion proteins.<sup>5</sup> Therefore molecular biology of atopic allergens provided the possibility of analyzing T-cell epitopes on the respective proteins.<sup>6,7</sup>

Type I allergy to pollens is a common disease.<sup>8-11</sup> Pollens of grasses are the most important sources of allergens.<sup>9-11</sup> Group I allergens of grass pollens are glycoproteins of a molecular mass of about 26 to 32 kd and consist of several highly homologous and immunologically related isoforms<sup>9,10</sup> (isoallergens). Recently, the group I pollen allergens Phl p

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#### Abbreviations used:

|       |                                    |
|-------|------------------------------------|
| AA:   | Amino acid(s)                      |
| APC:  | Antigen-presenting cells           |
| cpm:  | Counts per minute                  |
| IL:   | Interleukin                        |
| IT:   | Immunotherapy                      |
| PBMC: | Peripheral blood mononuclear cells |
| r:    | Recombinant                        |
| SI:   | Stimulation index                  |
| TCC:  | T-cell clone                       |
| TCL:  | T-cell line                        |
| TCR:  | T-cell receptor                    |
| Th:   | T helper (cell)                    |

1 from timothy grass (*Phleum pratense*), several isoforms of Lol p 1 from ryegrass (*Lolium perenne*), and Sec c 1 from rye (*Secale cereale*; Laffler S, unpublished results) were characterized by molecular cloning techniques and were found to display a high degree of similarity in AA sequence.<sup>11-13</sup> IgE antibodies specific for group I allergens of grass pollens are known to crossreact<sup>10</sup>; therefore patients have allergic symptoms during the whole flowering season of grasses. T lymphocytes were found to play an important role in the induction of IgE synthesis, the crucial event in the development of type I allergy.<sup>14-16</sup> It was shown that allergen-specific T-cell clones (TCC) derived from atopic patients typically belong to the Th2 helper cell subset, which is characterized by a high production of interleukin (IL)-4, the most potent inducer of IgE synthesis in B lymphocytes.<sup>17</sup> With use of peripheral blood mononuclear cells (PBMC), T-cell lines (TCL) and TCC, T-cell epitopes of Lol p 1 from ryegrass have been described.<sup>12, 18, 19</sup>

In this study we investigated humoral and cellular responses to recombinant Phl p 1. To determine T-cell epitopes of Phl p 1, we tested TCL and TCC established from individuals allergic to grass pollens for reactivity with peptides synthesized according to the AA sequence of this allergen. We showed IgE reactivity patterns of patients allergic to grass pollen and evaluated the extent of cross-reactivity on the helper T cell level. Knowledge of major or crossreacting T-cell epitopes of common allergens might have an impact on the development of vaccines for immunotherapy in the future.

## METHODS

### Patients

Peripheral blood was collected from nine persons allergic to grass pollen (E.C., G.Z., H.E.R., H.N., K.H., L.V., N.O., P.S., R.H.). Type I allergy to grass pollen was

documented by typical case history, positive skin test results, and positive RAST finding (RAST class 3 to 6). HLA typing was performed as previously described,<sup>20</sup> and it revealed the following MHC class II phenotypes: E.C.: DRB1\*07, DRB1\*16; DRB4, DRB5; DQB1\*01; DQB1\*02; G.Z.: DRB1\*07, DRB1\*08; DRB4; DQB1\*03, DQB1\*04; H.E.R.: DRB1\*01, DRB1\*04; DRB4; DQB1\*01, DQB1\*03; H.N.: DRB1\*01, DRB1\*13; DRB3; DQB1\*01, DQB1\*03; K.H.: DRB1\*11, DRB1\*17; DRB3; DQB1\*02; DQB1\*03; L.V.: DRB1\*04, DRB1\*15; DRB4, DRB5; DQB1\*01, DQB1\*03; N.O.: DRB1\*04, DRB1\*13; DRB3, DRB4; DQB1\*01, DQB1\*03; P.S.: DRB1\*07, DRB1\*15; DRB4, DRB5; DQB1\*01, DQB1\*02; R.H.: DRB1\*08, DRB1\*13; DRB3; DQB1\*01, DQB1\*04.

### Grass pollen extracts

Pollens from *Phleum pratense* (timothy grass), *Dactylis glomerata* (cocksfoot grass), *Poa pratensis* (Kentucky bluegrass), *Lolium perenne* (ryegrass), and *Secale cereale* (rye) were purchased from Allergon AB (Engelholm, Sweden). Aqueous extracts of these pollens were prepared as previously described.<sup>8</sup> Lyophilized extracts were dissolved in tissue culture medium, sterile filtrated, and used for cell culture in a final concentration of 25 µg/ml.

### Expression and purification of recombinant non-fusion Phl p 1

Recombinant (r) Phl p 1 protein expressed in *Escherichia coli* was purified by ion exchange chromatography (Susani M, unpublished results). For quantification, the molecular extinction coefficient was calculated from the DNA-derived AA sequence,<sup>11</sup> as previously described.<sup>21</sup> For use in cell culture, lyophilized material was dissolved in culture medium and sterile filtrated.

### Peptides

A panel of 76 dodecapeptides overlapping for three residues (neighbors share nine AA) was synthesized according to the Phl p 1 sequence (Cambridge Research Biochemicals Limited, Cambridge, U.K.).<sup>11, 22</sup> Epitope mapping of TCL and TCC was performed by using these peptides at a concentration of 5 µg/ml. The following peptides, representing AA sequences of Lol p 1 and of Sec c 1 homologous to Phl p 1 epitopes, were synthesized (Cambridge Research Biochemicals Limited) and used for crossreactivity studies (bold letters indicate AA exchanges between the corresponding peptides):

|              |                                |
|--------------|--------------------------------|
| FEIKCTKPEACS | (Phl p 1; AA 73-84)            |
| FEIKCTKPESCS | (Lol p 1; AA 73-84)            |
| FELKCTKPEACS | (Sec c 1; AA 73-84)            |
| IAPYHFDLSGHA | (Phl p 1, Lol p 1; AA 100-111) |
| IDPYHFDLSGHA | (Sec c 1; AA 100-111)          |

### IgE immunoblots

Plasma samples (derived from the cell separation process) were tested for IgE reactivity with r Phl p 1 and IgE binding to aqueous extracts of *Phleum pratense*, *D.*

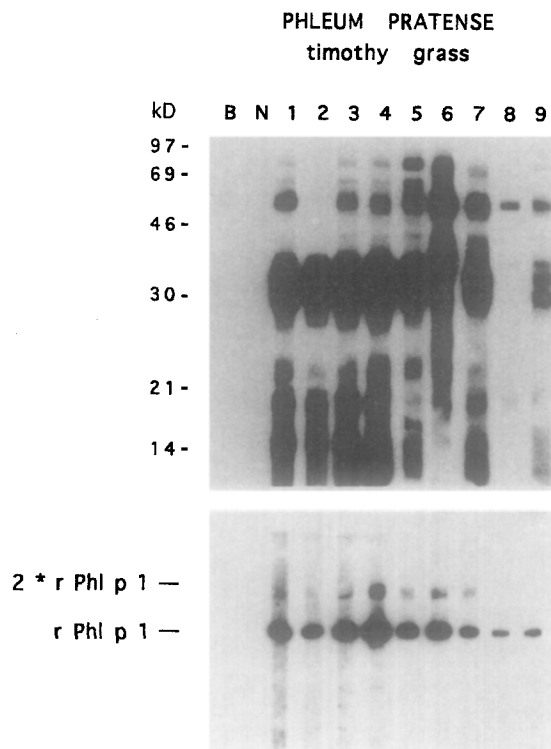


**FIG. 1.** IgE immunoblot experiments. Reactivity of patients' plasma with nitrocellulose-blotted aqueous extract from timothy grass (*Phleum pratense*) pollen and with purified recombinant Phl p 1 (2\*r Phl p 1: Phl p 1-dimer). Numbers 1-9, patients' plasma: R.H., K.H., G.Z., N.O., L.V., E.C., H.E.R., P.S., H.N.; B, buffer control; N, normal human serum pool.

*glomerata*, *Poa pratensis*, *L. perenne*, and *S. cereale*. IgE immunoblots were performed according to procedures previously described.<sup>8</sup> A serum pool of 13 donors without allergy and buffer alone were used as controls. IgE binding to proteins was detected with an iodine 125-labeled antihuman IgE antibody (Pharmacia Diagnostics, Uppsala, Sweden).

### Allergen-specific TCL and TCC

Allergen-specific TCL and TCC were established according to methods previously described.<sup>7</sup> Briefly,  $10^6$  PBMC were stimulated with individual optimal r Phl p 1 concentrations (10 to 20  $\mu$ g/ml) in Ultra Culture medium (BioWhittaker, Walkersville, Md.) supplemented with 2 mmol/L glutamine and  $2 \times 10^{-5}$  mol/L 2-mercaptoethanol in 24-well, flat-bottomed culture plates (3524; Costar, Cambridge, Mass.). After 5 days of culturing, human r IL-2 (10 to 20 U/ml, Boehringer, Mannheim, Germany) was added, and the culture was continued for an additional 7 days. Viable T-cell blasts were separated by means of density centrifugation (Ficoll/Paque; Pharmacia), and

Phl p 1 specificity of TCL was assessed by an [<sup>3</sup>H]-thymidine (Amersham, Amersham, U.K.) incorporation assay.

For TCC, T-cell blasts were seeded in limiting dilution (0.3 cells/well) in 96-well round-bottomed plates (Nunc, Nunc, Denmark) in the presence of  $2 \times 10^5$  irradiated (50 Gy) allogenic PBMC as "feeder cells," 1% phytohemagglutinin v/v (Gibco, Grand Island, N.Y.) and r IL-2 (4 U/well) in the medium described above. Growing microcultures were expanded at weekly intervals with fresh feeder cells and r IL-2. The specificity of TCC was assessed as previously described.<sup>7</sup> Briefly, 2 to  $5 \times 10^4$  T-cell blasts were incubated in triplicate cultures in 96-well plates in the presence of  $10^5$  autologous PBMC as antigen-presenting cells (APC), r Phl p 1 and whole *Phleum pratense* pollen extract for 48 hours at 37°C, 5% CO<sub>2</sub> in humidified atmosphere. After pulsing for 16 hours with 0.5  $\mu$ Ci per well of [<sup>3</sup>H]-thymidine, cells were harvested and the incorporated radioactivity was measured by scintillation counting. Results are displayed as counts per minute (cpm). When the stimulation index (SI; ratio between cpm obtained in cultures with TCC plus autologous irradiated APC plus antigen, and cpm obtained in cultures containing TCC and APC alone) was greater than 10, responses were considered as positive.

### Analysis of the phenotype of TCC

The phenotype of TCC was analyzed by means of flow cytometry, using a fluorescence-activated cell sorter (FACScan; Becton-Dickinson, Mountain View, Calif.). For analyses the FITC-labeled monoclonal antibodies anti-Leu 4/CD3, anti-Leu 3a/CD4, anti-Leu 2a/CD8, anti-TCR  $\alpha/\beta$  WT 31 and anti-TCR  $\gamma/\delta$  I (Becton-Dickinson) were used.<sup>7</sup>

### Specific stimulation of TCC for cytokine production

For cytokine production,  $10^6$  clone cells were incubated with  $5 \times 10^5$  irradiated (50 Gy) autologous APC and r Phl p 1 (10  $\mu$ g/ml) in 1 ml of culture medium. Supernatants were collected after 24 hours of incubation. Cytokine levels in the supernatants were measured using ELISA systems purchased from Quantakine R&D systems, Minneapolis, Minn. (IL-4, sensitivity threshold: 3 pg/ml), and Medgenix Diagnostix, Fleurus, The Netherlands (interferon-gamma [IFN- $\gamma$ ] sensitivity threshold: 1.5 pg/ml; IL-2, sensitivity threshold: 0.1 U/ml). Cultures containing APC alone and TCC plus APC without allergen were used as negative controls.

### T-cell receptor analysis

Analysis of the T-cell receptor (TCR) V $\beta$  region was performed to prove the monoclonality of the TCC (single V $\beta$ -gene usage). Single-stranded cDNA derived

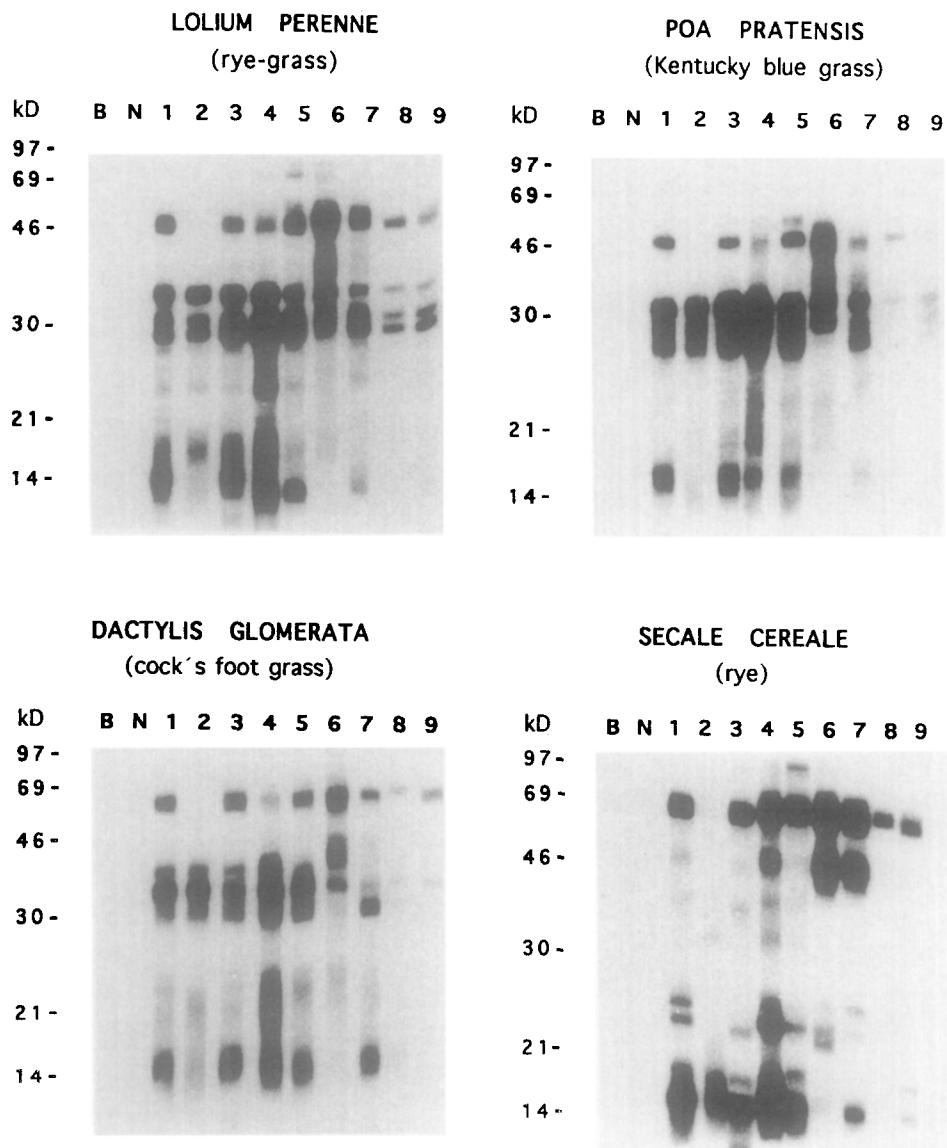


**FIG. 2.** IgE immunoblot experiments. Reactivity of patients' plasma with aqueous extracts from ryegrass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), cocksfoot grass (*Dactylis glomerata*), and rye (*Secale cereale*). Numbers 1-9, patients' plasma: R.H., K.H., G.Z., N.O., L.V., E.C., H.E.R., P.S., H.N.; B, buffer control; N, normal human serum pool.

from the clones' RNA and V $\beta$  family-specific primers plus a C $\beta$ -specific primer were used in the polymerase chain reaction as described.<sup>23</sup>

### HLA restriction

Murine monoclonal antibodies against human MHC class II determinants L 243 [anti-HLA-DR (IgG2a)], Leu 10 [anti-HLA-DQ (IgG1)], and B7/21 [anti-HLA-DP (IgG1)] were purchased from Becton-Dickinson. Proliferation assays were performed in 96-well plates as described.<sup>24</sup> TCC ( $2 \times 10^4$  cells/ml) were cultured with autologous irradiated PBMC ( $5 \times 10^4$  cells/ml) in the

presence of r Phl p 1 (5  $\mu$ g/ml). The antibodies directed against framework antigens of HLA-D-region molecules were used at a final concentration of 25  $\mu$ g/ml. Proliferation assays that revealed blocking of more than 50% of antigen-dependent proliferation were considered as positive.

## RESULTS

### Purification of Phl p 1

Analysis of purified Phl p 1 revealed purity of >98%. SDS-PAGE and protein staining revealed a single band in the relative molecular mass range



**TABLE I.** Epitope specificities, stimulation indices (stimulation with epitope peptides), and cytokine production of Phl p 1-specific TCC.

| T-cell clone | Epitope AA on Phl p 1 | Stimulation index | Cytokine production |             |                       | Th subset |
|--------------|-----------------------|-------------------|---------------------|-------------|-----------------------|-----------|
|              |                       |                   | IL-4 (pg/ml)        | IL-2 (U/ml) | IFN- $\gamma$ (pg/ml) |           |
| E.C. 2       | 73-84                 | 66                | 114                 | <0.1        | <1.5                  | 2         |
| E.C. 26      | 97-111                | 475               | <3                  | 5           | 100                   | 1         |
| E.C. 27      | 157-168               | 190               | 100                 | 2.8         | 150                   | 0         |
| E.C. 29      | 97-111                | 257               | 725                 | 6           | 110                   | 0         |
| E.C. 55      | 91-102                | 207               | 290                 | <0.1        | 100                   | 0         |
| G.Z. 3       | 70-84                 | 101               | 937                 | <0.1        | 50                    | 2         |
| G.Z. 52      | 70-84                 | 21                | >4000               | 27          | 70                    | 2         |
| G.Z. 53      | 70-84                 | 16                | 2157                | 0.2         | <1.5                  | 2         |
| G.Z. 69      | 157-168               | 25                | 3843                | 18          | <1.5                  | 2         |
| H.E.R. 30    | 226-240               | 418               | 2130                | 1.2         | 200                   | 2         |
| H.E.R. 32    | 100-114               | 76                | <3                  | <0.1        | 3300                  | 1         |
| H.E.R. 38    | 130-141               | 122               | 742                 | <0.1        | 400                   | —         |
| H.E.R. 47    | 100-114               | 65                | <3                  | 0.6         | 3000                  | 1         |
| H.E.R. 81    | 34-45                 | 100               | 1502                | 21          | 210                   | 0         |
| H.N. 6       | 22-36                 | 72                | 28                  | <0.1        | 120                   | 0         |
| K.H. 8       | 25-39                 | 82                | <3                  | 8           | <1.5                  | 1         |
| K.H. 27      | 70-84                 | 220               | 860                 | 12          | 2400                  | 0         |
| L.V. 24      | 100-114               | 364               | 2876                | <0.1        | 180                   | 2         |
| L.V. 32      | —                     | —                 | 456                 | <0.1        | <1.5                  | 2         |
| N.O. 5       | 100-114               | 50                | >4000               | >60         | 170                   | 2         |
| N.O. 6       | 100-114               | 47                | 2600                | 0.5         | <1.5                  | 2         |
| N.O. 9       | 100-114               | 60                | >4000               | <0.1        | <1.5                  | 2         |
| N.O. 14      | 100-114               | 128               | 1320                | 1.5         | 800                   | 0         |
| N.O. 20      | 100-114               | 122               | >4000               | 48          | 160                   | 2         |
| N.O. 21      | 211-225               | 61                | 130                 | <0.1        | 100                   | 0         |
| N.O. 24      | 211-225               | 22                | 150                 | <0.1        | 120                   | 0         |
| N.O. 29      | 100-114               | 25                | >4000               | 48          | 140                   | 2         |
| N.O. 31      | 127-138               | 234               | 314                 | <0.1        | 170                   | 0         |
| N.O. 32      | 73-84                 | 35                | 584                 | 4           | 200                   | 0         |
| N.O. 34      | 127-138               | 212               | 58                  | <0.1        | <1.5                  | 2         |
| N.O. 37      | 127-138               | 144               | 818                 | 0.5         | 1300                  | 0         |
| N.O. 54      | 127-138               | 89                | 1334                | 3.8         | 300                   | 0         |
| N.O. 55      | 169-183               | 16                | 880                 | 13          | <1.5                  | 2         |
| P.S. 8       | 73-84                 | 70                | 2600                | 2.6         | 80                    | 2         |
| P.S. 10      | 121-134               | 110               | 394                 | <0.1        | <1.5                  | 2         |
| P.S. 11      | 142-155               | 34                | 574                 | 16          | 160                   | 0         |
| P.S. 15      | 142-155               | 125               | 62                  | <0.1        | <1.5                  | 2         |
| P.S. 16      | 142-155               | 32                | 26                  | <0.1        | <1.5                  | 2         |
| R.H. 5       | 109-123               | 166               | 1700                | <0.1        | 150                   | 2         |
| R.H. 18      | 109-123               | 31                | 425                 | 6           | 300                   | 0         |

ation on stimulation with pollen extract of *Phleum pratense*. Looking at crossreactivity, 16 of 19 TCC (84%) could be stimulated with *D. glomerata* extract; 18 of 19 TCC (95%) reacted with *Poa pratensis* extract; 16 of 19 TCC (84%) proliferated in response to *L. perenne* extract; 9 of 19 TCC

(47%) displayed crossreactivity with *S. cereale* extract; 8 of 19 TCC (42%) displayed reactivity with all five grass pollen extracts tested. In some TCC proliferation induced by the grass pollen extracts differed considerably. Crossreactivity data with grass pollen extracts are summarized in Table II.

**TABLE II.** Proliferation of Phl p 1-specific TCC in response to stimulation with aqueous extracts from timothy grass (*Phleum pratense*), ryegrass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), orchard grass (*Dactylis glomerata*), and rye (*Secale cereale*). (+, Stimulation index >10; (+), stimulation index 3-10; -, no proliferation.)

| TCC       | T-cell epitope AA on Phl p 1 | Grass pollen extracts |                 |            |               |               |
|-----------|------------------------------|-----------------------|-----------------|------------|---------------|---------------|
|           |                              | <i>Phleum</i>         | <i>Dactylis</i> | <i>Poa</i> | <i>Lolium</i> | <i>Secale</i> |
| K.H. 8    | 25-39                        | +                     | +               | +          | +             | +             |
| H.E.R. 81 | 34-45                        | +                     | +               | +          | +             | (+)           |
| K.H. 27   |                              | +                     | +               | +          | +             | (+)           |
| G.Z. 53   |                              | +                     | +               | +          | +             | -             |
| E.C. 2    | 73-84                        | +                     | +               | +          | +             | +             |
| P.S. 8    |                              | +                     | +               | +          | +             | (+)           |
| N.O. 32   |                              | +                     | -               | (+)        | (+)           | -             |
| E.C. 55   | 91-102                       | +                     | +               | +          | +             | -             |
| E.C. 29   |                              | +                     | +               | +          | +             | (+)           |
| L.V. 24   |                              | +                     | -               | +          | -             | -             |
| N.O. 14   | 100-111                      | +                     | +               | +          | +             | +             |
| N.O. 29   |                              | +                     | +               | -          | +             | +             |
| R.H. 5    | 109-133                      | +                     | -               | +          | -             | -             |
| P.S. 10   | 121-135                      | +                     | +               | +          | +             | (+)           |
| N.O. 37   |                              | +                     | (+)             | +          | +             | -             |
| N.O. 54   | 127-138                      | +                     | (+)             | +          | (+)           | -             |
| E.C. 27   | 157-168                      | +                     | (+)             | +          | +             | -             |
| N.O. 55   | 169-183                      | +                     | (+)             | +          | +             | -             |
| H.E.R. 30 | 226-240                      | +                     | (+)             | (+)        | -             | -             |

#### Proliferation assays using peptides synthesized according to the sequences of Phl p 1, Lol p 1, and Sec c 1

Nine TCC reacting with two major epitopes on Phl p 1 were tested for crossreactivity with peptides synthesized according to corresponding regions on Lol p 1 and Sec c 1. Five TCC established from TCL of different individuals were specific for the Phl p 1 epitope AA 73-84. These TCC displayed reactivity with peptides derived from Phl p 1 and Lol p 1; 2 of 5 TCC also reacted with the Sec c 1-derived peptide, though lower SI were observed (Fig. 4). Four other TCC reacting with AA 100-111 of Phl p 1 were investigated. All these TCC could be stimulated with peptide IAPYHFDLSGHA (position 100-111 in Phl p 1 and Lol p 1); decreased reactivity was found with the Sec c 1 peptide displaying an AA exchange at position 101 (Ala > Asp; Fig. 5).

#### Phenotype and cytokine production of Phl p 1-specific TCC

Fluorescence-activated cell sorter analysis revealed the Th phenotype (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>-</sup>)

for 39 of 40 TCC. One clone (H.E.R. 38) was found to express CD8. The TCR could be identified as TCR  $\alpha/\beta$  in all cases. All TCC were analyzed for IL-4, IL-2, and IFN- $\gamma$  secretion on specific stimulation. Of Th clones, 20 of 39 revealed a high IL-4/IFN- $\gamma$  ratio (Th2 cells), whereas 15 of 39 TCC were classified as Th0 clones (secretion of both IL-4 and IFN- $\gamma$ ). In supernatants of 4 of 39 TCC IFN- $\gamma$ , but no IL-4, could be measured (Th1 type of cytokine production). TCC H.E.R. 38 (CD8<sup>+</sup>) secreted considerable amounts of both IL-4 and IFN- $\gamma$ . Tests were performed twice in duplicates and were reproducible. The values of cytokines detected are summarized in Table I.

#### T-cell receptor analysis and HLA restriction

The TCR of all TCC could be identified as TCR  $\alpha/\beta$ . Analysis of TCC-derived cDNAs for TCR-V $\beta$  gene usage was performed for representative TCC (TCC used for detailed crossreactivity studies with peptides derived from AA sequences of selected group I grass allergens) reacting with Phl p 1 epitope AA 73-84 (E.C.2: V $\beta$ 18; G.Z.53: V $\beta$ 13.1; K.H.27:

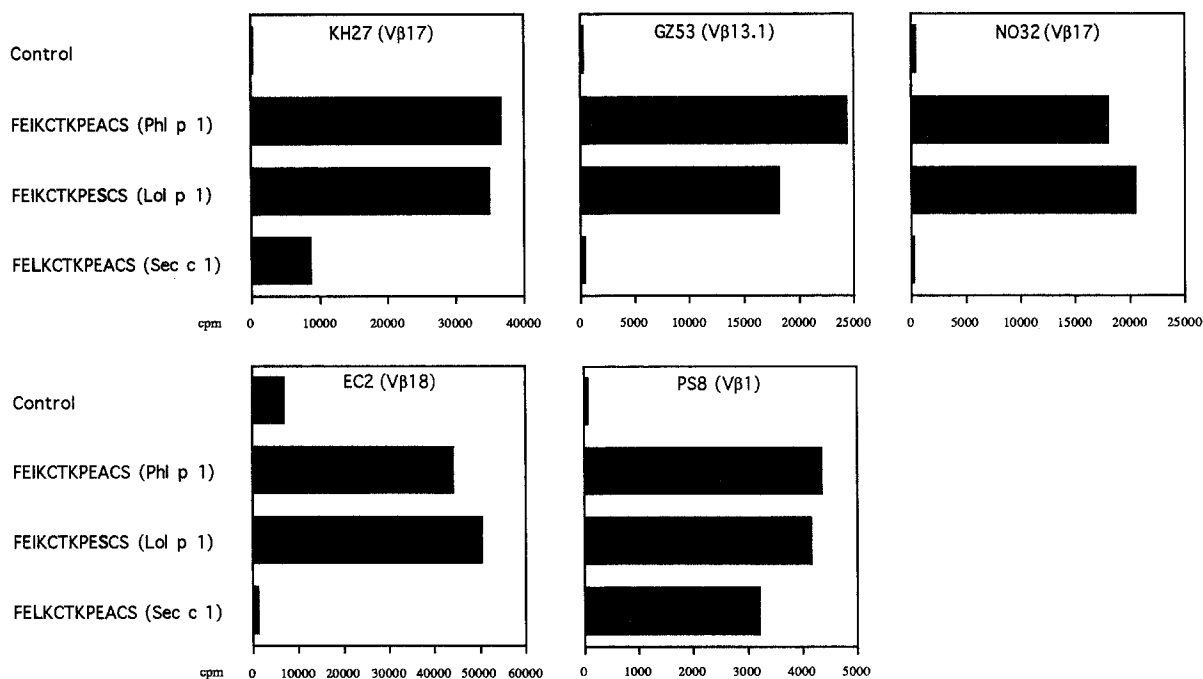


**FIG. 4.** TCR Vβ gene usage and proliferative responses of five selected TCC reactive with Phl p 1 epitope AA 73-84 to peptides synthesized according to corresponding regions on Lol p 1 and Sec c 1. (cpm, Counts per minute after [<sup>3</sup>H]-thymidine uptake.)

Vβ17; N.O.32: Vβ17; P.S.8: Vβ1), and for TCC reacting with Phl p 1 peptide AA 100-111 (E.C.29: Vβ5.1; L.V.24: Vβ20; N.O.14: Vβ13.1 and N.O.29: Vβ4). In all cases blocking studies using monoclonal antibodies directed against framework antigens of HLA-D region molecules revealed inhibition in the presence of monoclonal antibody L243, indicating peptide presentation by HLA-DR molecules.

## DISCUSSION

Proteins from pollens are frequently the cause of type I allergy. Among other sources, grass pollens contain a number of important allergens.<sup>9-11</sup> In this study we investigated the T cell-activating epitopes of Phl p 1, the major allergen of *Phleum pratense* (timothy grass). This allergen was chosen because of the worldwide occurrence of timothy grass and its particular importance as a source of grass pollen allergens. Phl p 1 has recently been cloned and sequenced.<sup>11</sup> Purified r Phl p 1 expressed as non-fusion protein was used for this investigation. Dodecapeptides, synthesized according to the sequence of Phl p 1 and overlapping for three residues (neighbors shared nine AA), were used to map T-cell epitopes of TCL and TCC established from the peripheral blood of persons allergic to

grass pollen. In accordance with investigations dealing with other allergens,<sup>6, 19, 25-30</sup> we could detect multiple T-cell epitopes, which were scattered over the whole AA sequence. Some T-cell specificities were detected in several patients and therefore seemed to represent "dominant epitopes"<sup>28-31</sup> (Fig. 3, Table I).

In some individuals the T-cell response seemed to be directed against a very limited number of epitopes, whereas up to seven distinct T-cell specificities were found within other patients. Lol p 1, the group I allergen from ryegrass, was investigated,<sup>12, 18, 19</sup> and data for comparison were available: Spiegelberg et al.<sup>19</sup> described 16 T-cell epitopes on Lol p 1. Comparison of these T-cell epitopes with our results revealed epitopes at corresponding positions; however, we could identify additional T cell-activating peptides.

All patients investigated in this study showed IgE reactivity to pollen extracts of several grasses, including *Phleum*, *Lolium*, *Dactylis*, *Poa*, *Secale* (Figs. 1 and 2). To investigate whether crossreactivity also occurs on the T-cell level,<sup>32</sup> Phl p 1-specific TCC were tested for proliferation on stimulation with extracts of these grasses. Different patterns of crossreactivity could be found (Table II). These results matched

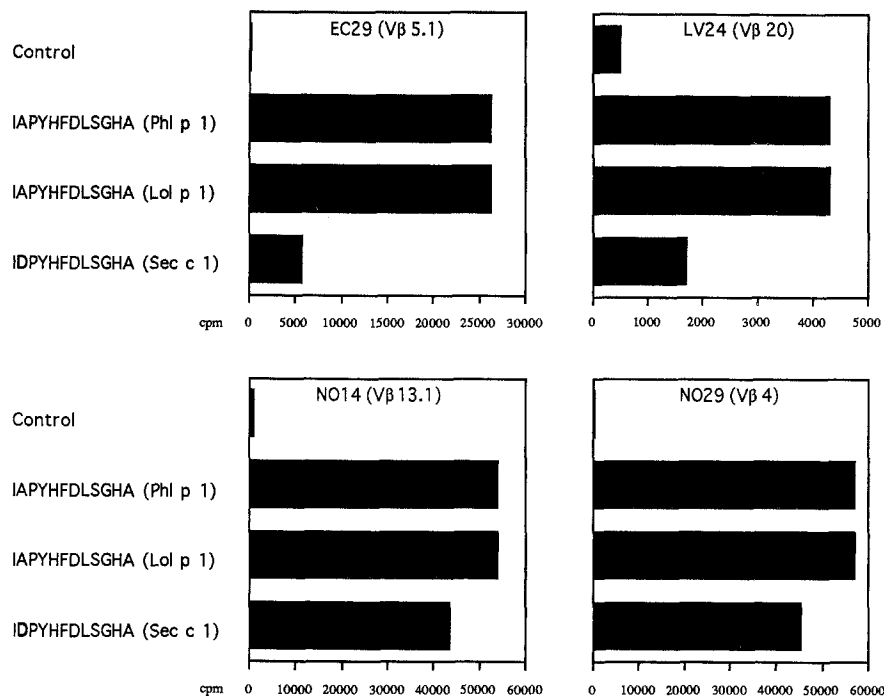


**FIG. 5.** TCR V $\beta$  gene usage and proliferative responses of four selected TCC reactive with Phl p 1 epitope AA 100-111 to peptides synthesized according to corresponding regions on Lol p 1 and Sec c 1. (cpm, Counts per minute after [ $^3$ H]-thymidine uptake.)

well with sequence similarities or differences on the corresponding positions of the respective group I allergens. Two major epitopes, recognized by several patients, were analyzed in detail. Peptides were synthesized according to the homologous regions of group I allergens from *Phleum*, *Lolium*, and *Secale*, where sequence data were available.<sup>11, 12</sup> Nine TCC reacted with these epitopes on the Phl p 1 molecule. TCR and MHC analysis of TCC was performed to ensure specificity of the crossreactivity experiments.<sup>23, 24</sup> Again, crossreactive and non-crossreactive TCC could be observed (Figs. 4 and 5). In two TCC, crossreactivities seen with total pollen extracts could not be confirmed using peptides from the respective group I allergens (E.C.2: *Secale*, N.O.32: *Lolium*). In one TCC proliferation with *Lolium*- and *Secale*-derived peptides could be observed, but no reactivity with total extracts of these sources. This is obviously due to the fact that cDNA-derived AA sequences represent only one of multiple protein isoforms occurring in natural grass pollens. Similar observations have been described for other allergens.<sup>33</sup>

IL-4, a T helper cell product, is the most important inducer of IgE synthesis in rodents and human beings.<sup>14-17</sup> Indeed, a large propor-

tion of the T helper cell clones investigated in this study (20 of 39) displayed a high IL-4 versus IFN- $\gamma$  ratio (Table I), indicating their importance in the pathogenesis of the disease. Specific immunotherapy (i.e., treatment of allergy by the injection of increasing doses of specific allergen) is a well-established method in treatment of type I allergy resulting from airborne allergens.<sup>34, 35</sup> It has recently been shown, that successful immunotherapy modulates cytokine production patterns produced by allergen-specific T lymphocytes.<sup>36</sup> Until now, vaccines containing a mixture of allergens have been used for immunotherapy. However, treatment of patients with peptides representing T-cell epitopes could improve the efficacy of immunotherapy.<sup>31, 37-39</sup> These peptides might modulate the T-cell response to allergens, which provides necessary help for specific B cells to induce the production of the antibody isotype IgE.<sup>31, 37-39</sup> The utilization of such peptides for immunotherapy would eliminate the risk of anaphylactic side effects present during conventional immunotherapy, as they are not capable of binding IgE antibodies,<sup>7</sup> allowing higher doses to be applied. It has been reported that treatment with single peptides rep-

resenting immunodominant T-cell epitopes is able to modulate the immune response to the complete allergen.<sup>31, 37, 38</sup> Therefore crossreacting major T-cell epitopes might represent tools for immunotherapy of grass pollen allergy in the future.

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