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Immunology

Amino acid sequence of Fel dI, the major allergen of the domestic cat: Protein sequence analysis and cDNA cloning

(polymerase chain reaction/N-glycosylation)

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ABSTRACT **The complete primary structure of Fel dI (International Union of Immunological Societies nomenclature), the major allergen produced by the domestic cat, *Felis domesticus*, was determined by protein sequence analysis and cDNA cloning. Protein sequencing of Fel dI from an immunoaffinity-purified extract of house dust revealed that the allergen is composed of two polypeptide chains. Degenerate oligonucleotides derived from the protein sequence were used in polymerase chain reaction amplification of cat salivary gland cDNA to demonstrate that the two chains are encoded by different genes. Chain 1 of Fel dI shares amino acid homology with rabbit uteroglobin, while chain 2 is a glycoprotein with N-linked oligosaccharides.**

The house cat (*Felis domesticus*) is a significant source of proteins known to elicit the symptoms of allergic disease. These symptoms range in severity from the relatively minor discomforts of rhinitis and conjunctivitis to potentially life-threatening asthmatic episodes. The frequency of cat allergy in the United States is on the order of 10% (1). A substantially greater proportion (20–30%) of asthmatics respond with immediate hypersensitivity upon skin test challenge with cat allergens (2, 3), and this hypersensitivity is a significant risk factor associated with the disease (4, 5).

Although allergic patient serum reacts with a number of proteins found in cat pelt extracts (6, 7), the dominant allergen is Fel dI (ref. 8; International Union of Immunological Societies nomenclature; ref. 9). Specifically, radioallergosorbent test (RAST) and crossed immunoelectrophoresis (CIE)/crossed radioimmunoelectrophoresis (CRIE) analysis of IgE antibodies in the serum of allergic patients have shown that the majority of this antibody response is directed against Fel dI (7, 10–13). The clinical relevance of these findings has been reinforced by studies detecting Fel dI-specific IgE in the serum of at least 80% of cat-allergic patients (6, 7, 10). Airborne levels of Fel dI found in houses with cats are often in excess of those required to provoke an asthmatic response in experimental aerosol bronchial challenge (14, 15). In addition, cat-allergic patients administered partially purified Fel dI in immunotherapy regimens experienced significantly diminished bronchial reactivity upon antigen challenge relative to control patients (16, 17).

Fel dI, found in the saliva, sebaceous glands, and pelts of cats (18–20), has been purified via biochemical (12) and immunoaffinity techniques (13, 21) and characterized as an acidic glycoprotein with a molecular mass of approximately 38 kDa. Under reducing conditions the apparent molecular mass of Fel dI shifts to ≈18 kDa, implying a multimeric structure for the antigen (12, 22).

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T-cell recognition is believed to play a pivotal role in the immunological response to allergens. Upon activation by an allergen, T cells secrete cytokines whose effects include both the activation of inflammatory cells and the supply of help to B cells for IgE production (23). T-cell epitopes within a protein are, for the most part, defined by the primary sequence of short peptides derived from intact protein. It is this primary sequence that determines the nature of the interaction of a peptide with the T-cell receptor and HLA molecules (24–26). Hence, the goal of the work presented in this manuscript was to define the primary structure of Fel dI.§ Such knowledge should aid the study and treatment of the human allergic response to cats.

MATERIALS AND METHODS

Immunoblot Analysis. Gel electrophoresis and electroblot transfer/immunodetection were performed as described (27).

Biotinylated goat anti-rabbit immunoglobulin (Southern Biotechnology Associates, Birmingham, AL) or biotinylated goat anti-human IgE (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used as the second antibody, and ^{125}I-labeled streptavidin (Amersham) was used as a reporter ligand for autoradiography at −80°C with intensifying screen.

Histamine Release Analysis. Release of histamine from basophils was measured with a RIA kit (AMAC, Westbrook, ME) with a monoclonal antibody (mAb) specific for an acylated derivative of histamine (28).

Protein Sequence Analysis. A 10% (wt/vol) aqueous extract of dust collected from a house with four pet cats was affinity-purified via a mAb anti-Fel dI column (21). Sequence analyses were performed with an Applied Biosystems 477A protein sequenator connected to an on-line phenylthiohydantoin analyzer. N-terminal sequence analysis of intact Fel dI was performed after concomitant pretreatment of the protein sample *in situ* with tributylphosphine and 4-vinylpyridine, giving repetitive yields > 93% (29). *o*-Phthalaldehyde (OPA) (30) was applied to block N termini except those with prolines (positions 4 and 32 of chain 1 or positions 7 and 37 of chain 2).

Internal sequence was determined by proteolytic digestion of intact protein. Reduced and pyridylethylated Fel dI was digested with endopeptidase Lys-C, endopeptidase Asp-N, or endoproteinase Glu-C (Boehringer Mannheim) or was cleaved with 2% (wt/vol) CNBr in 70% (vol/vol) formic acid (31) overnight at room temperature. Time dependent *in situ* CNBr digestion of Fel dI on the sequencer glass filter disk (32) was performed after five sequencer cycles, preventing

Abbreviations: Fel dI, *Felis domesticus* allergen I; OPA, *o*-phthalaldehyde; PCR, polymerase chain reaction; mAb, monoclonal antibody.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession nos. M74952, M74953, and M77341).

the formation of a CNBr fragment between Met-3 and Met-42 of chain 2, after which the sample was blocked with acetic anhydride to reduce spurious sequence signals. Alternatively, tryptic peptides were obtained by enzymatic digestion (Worthington) of unmodified Fel dI. The peptides were separated on an Applied Biosystems 151A HPLC system with an Aquapore RP300 column and subjected to sequence analysis.

Anti-Peptide Antisera. Peptides Fel 1 (chain 1, residues 1–17), Fel 2 (chain 1, residues 9–25), Fel 4 (chain 2, residues 37–55), and Fel 18 (chain 2, residues 23–48) were made with Applied Biosystems 430 or Milligen 9050 peptide synthesizers. Peptides were conjugated to keyhole limpet hemocyaninin via ethyldicyclohexylcarbodiimide and injected into rabbits with complete Freund's adjuvant, followed by one booster. Anti-peptide antibodies were affinity-purified by absorption/elution with cognate peptide-Sepharose columns.

RNA Preparation and cDNA Synthesis. Mandibular and parotid glands from five cats (BioMedical Associates, Friedensburg, PA) were pooled and frozen in liquid N_2. Total RNA was prepared by grinding the frozen tissue in guanidine thiocyanate and forming a pellet through a CsCl cushion (33). cDNA was synthesized using Superscript reverse transcriptase (Bethesda Research Laboratories). Second-strand cDNA synthesis (34) was carried out with a kit reaction mixture from Bethesda Research Laboratories.

Polymerase Chain Reaction (PCR). An MJ Research Programmable Thermal Controller was used in PCR amplification of cDNA with primers (Oligos Etc., Guilford, CT and Research Genetics, Huntsville, AL) and a GeneAmp kit (Perkin–Elmer/Cetus). Reactions proceeded for 30 cycles of 1 min at 94°C, 1.5 min at 55°C, and 1 min at 72°C for exact primers or five cycles of 1 min at 94°C, 1.5 min at 45°C, and 1 min at 72°C, followed by 25 cycles of 1 min at 94°C, 1.5 min at 55°C, and 1 min at 72°C for degenerate primers. A 1/100th aliquot of an initial reaction was used in sequential rounds of PCR with nested primers.

DNA Subcloning and Sequencing. Standard protocols were used for recombinant DNA manipulations (35). Amplified PCR products were digested with restriction enzymes, isolated from 2–3% agarose gels (NuSieve; FMC), and ligated into Bluescript (Stratagene) or phage M13mp18/19 replicative form vectors. DNA sequence analysis (36) was performed with a Sequenase 2.0 kit (United States Biochemical). Asymmetrically amplified PCR products (37) were purified with Qiagen 5 tips (Diagen GmbH) prior to sequencing.

Sequence Homology Analysis. A sequence homology database search was performed with the PROSEARCH program (38) on an AMT 600 distributed array processor (Active Memory Technology, Reading, U.K.).

Glycosidase Treatment. Two milligrams of Fel dI were boiled for 2 min in 0.2% SDS, 5% (vol/vol) 2-mercaptoethanol, cooled to room temperature, and incubated with 6 units of peptide N-glycosidase F in 50 mM KH_2PO_4, pH 7.1/1% (wt/vol) octyl glucoside (Boehringer Mannheim) for 17 hr at 37°C.

RESULTS

An aliquot of immunoaffinity-purified house dust extract (21) was tested for human allergenic activities *in vitro* prior to protein sequence analysis. Immunoblot analysis of the immunoaffinity-purified protein was performed to assess its IgE-binding capacity. Native protein was subjected to isoelectrophoretic focusing in a pH gradient between 2.5 and 5.0, electroblotted onto nitrocellulose, and probed with the anti-Fel dI mAb 1G9 (21) or with serum from one cat-allergic patient and one nonallergic patient (as determined by skin test challenge with cat pelt extracts). The immunoreactivity of the cat-allergic patient's serum to the native purified protein was readily apparent in contrast to that of the nonallergic patient's serum (Fig. 1*A*). In addition, the isoelectrophoretic mobilities of the molecules recognized by the cat-allergic patient's serum and the anti-Fel dI mAb were identical and approximate the previously reported pI for Fel dI of 3.85 (12). Similarly, only the IgE from cat-allergic patient's serum possessed reactivity to the reduced and denatured purified proteins on the SDS/PAGE immunoblot (Fig. 1*B*).

Blood cells collected from the allergic and nonallergic patients were incubated with various dilutions of the immunoaffinity-purified house dust extract, and the levels of histamine released from basophils were quantitated. Concentrations of purified protein as low as 100 ng/ml elicited a strong response from the cat-allergic patient's basophils, whereas 1000-fold higher levels of protein failed to mediate release of histamine from the nonallergic patient's basophils (Fig. 1*C*). As a positive control, antibody-mediated crosslinking of surface-bound IgE on both patients' basophils provoked equivalent levels of histamine release.

The data from the above *in vitro* analyses strongly suggest that the major species in the purified extract, presumably Fel dI, is allergenic in nature. Moreover, these results are representative data from experiments involving 10 patients (data not shown).

A 2-mg aliquot of the immunoaffinity-purified protein was used for sequence determination. N-terminal sequence analysis of intact protein revealed a major amino acid sequence in conjunction with several minor ones. The major sequence (Fig. 2*A*), which corresponded to the published N-terminal 33 residues of Fel dI (21) with the exception of two residues, was termed chain 1. The most prevalent minor sequence (present at 55% of the major sequence level) was designated chain 2 (Fig. 2*B*). All of the remaining minor sequences could be accounted for as having arisen from N-terminal proteolytic deletions of chain 2. Summation of the sequence signals

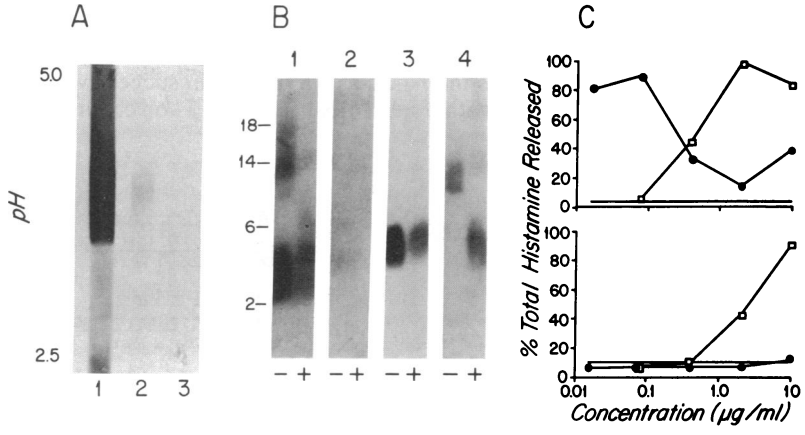

FIG. 1. *In vitro* allergenic activity of immunoaffinity-purified Fel dI. (*A*) Isoelectric focusing/immunoblot analysis of Fel dI with the following primary antibodies in lanes: 1, 1G9 anti-Fel dI mAb; 2, cat-allergic patient serum; 3, nonallergic patient serum. (*B*) SDS/PAGE/Western blot analysis of untreated Fel dI (lanes −) and peptide N-glycosidase F-treated Fel dI (lanes +) with the following primary antibodies in lanes: 1, cat-allergic patient serum; 2, nonallergic patient serum; 3, chain 1 Fel dI anti-peptide antisera; 4, chain 2 Fel dI anti-peptide antisera. Sizes are shown in kDa. (*C*) Fel dI-mediated histamine released from cat-allergic (*Upper*) and nonallergic (*Lower*) patient's basophils. Solid line with no symbols, buffer control; ●, Fel dI; □, HP6061 mouse anti-human IgE mAb.

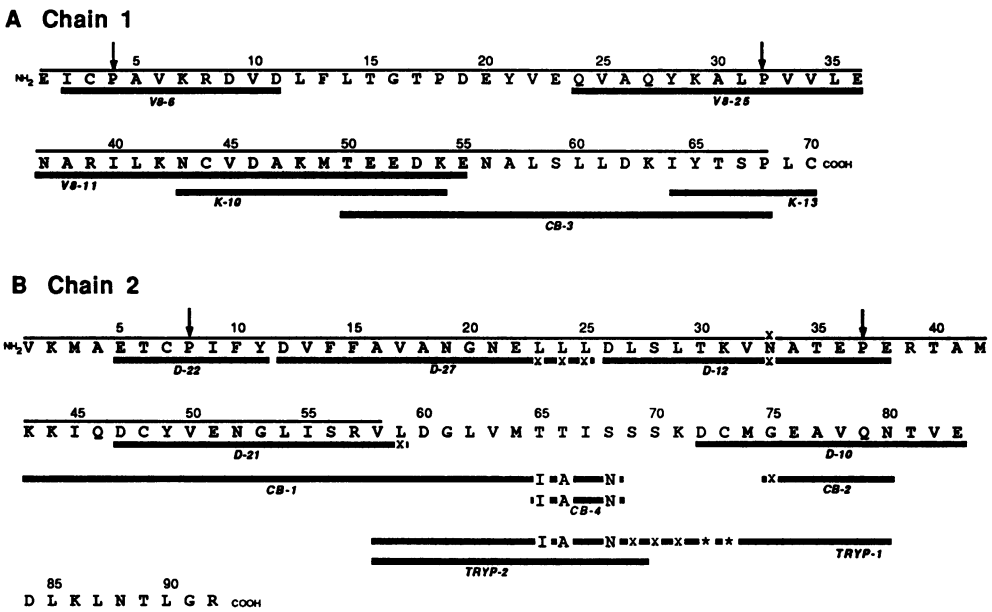

FIG. 2. Protein sequence analysis of Fel dI. (*A*) Chain 1 of Fel dI. (*B*) Chain 2 of Fel dI. The overlined sequence was determined from intact Edman degradation. The underlined sequences were determined from proteolytic fragments generated from digestion by: V8, *Staphylococcus aureus* V8; K, endopeptidase Lys-C; D, endoproteinase Asp-N; TRYP, trypsin; and CB, CnBr. x, Undetermined residue; ★, space in sequence. Arrows mark the site of blockage by OPA treatment.

indicated that chains 1 and 2 were present at equimolar ratios in the Fel dI preparation.

Since residues 4 of chain 1 and 7 of chain 2 were determined to be proline, OPA (which modifies primary but not secondary amines, such as proline) blockage was used to perform isolated sequence analysis of each of the two chains of Fel dI. Applying OPA before the fourth cycle of Edman degradation blocked the N terminus of chain 2, allowing unambiguous sequence data to be obtained for chain 1. Similarly, to acquire chain 2 sequence, OPA was used prior to the seventh sequencing cycle to block chain 1 (note the arrows at proline residues in Fig. 2). OPA blockage at these cycles, as well as at cycle 32 for chain 1 and cycle 37 for chain 2, permitted significant extension of the N-terminal protein sequence for chain 1 (68 amino acid residues) and chain 2 (58 amino acid residues).

Intact N-terminal protein sequence was confirmed and extended by sequence analysis of peptides derived from enzymatic and chemical digests of the purified Fel dI (Fig. 2). After *in situ* CNBr digestion, one major peptide sequence, CB-1 and three minor peptides that were present at 60% (CB-2), 38% (CB-3), and 12% (CB-4) of the signal level of the major peptide were identified. CB-1 was 25 amino acids in length, with an N terminus corresponding to residue 43 of chain 2, thereby extending the sequence of chain 2 to 68 residues.

Sequence from the tryptic peptide TRYP-1 and the endoprotease Asp-N-derived peptide D-10 overlapped and, when applied in conjunction with the CNBr peptides CB-1 and CB-2, extended the sequence of chain 2 to 83 residues (Fig. 2*B*). Similarly, K-13, an endopeptidase Lys-C-generated peptide, was contiguous with residues 64–68 of chain 1 and possessed two additional residues; this indicated chain 1 is comprised of at least 70 amino acids (Fig. 2*A*).

Two incongruities became apparent during protein sequence analysis of Fel dI. When compared to peptide TRYP-2, peptides CB-1, TRYP-1, and D-10 revealed sequence and size polymorphism between residues 65 and 73 of chain 2 (Fig. 2*B*). In addition, sequence signal at residue 33 of chain 2 was not detected during intact or peptide-derived sequence analysis.

Partial cDNAs encoding portions of Fel dI chains 1 and 2 were obtained in three discrete PCR amplifications. Parotid and mandibular glands were used as a source of Fel dI mRNA, since cat saliva harbors high levels of the allergen (7). By using degenerate 5′ sense and 3′ antisense primer pairs (Fig. 3) based on contiguous amino acids of limited codon ambiguity (39), internal portions of chains 1 and 2 were amplified from parotid/mandibular cDNA. DNA fragments of the predicted size were subcloned and sequenced (Fig. 4). The deduced amino acid sequence of the clones confirmed the PCR products' authenticity as portions of the cDNAs for chains 1 and 2 of Fel dI.

The 5′ and 3′ portions of the chain 1 and chain 2 cDNAs were obtained by anchored PCR methods. Two consecutive rounds of PCR amplification were carried out with a nested pair of chain 1- or chain 2-specific 5′ primers in conjunction with a 3′ primer (ED primer, Fig. 3) encoding a tag sequence covalently linked 3′ to the oligo(dT) primer (EDT primer, Fig. 3) used in first-strand cDNA synthesis (40). These cDNA fragments encompassed the 3′ ends of chains 1 and 2, including stop codons, 3′ untranslated sequences, polyadenylylation signals, and poly(A) tracts (Fig. 4).

Similarly, the 5′ regions of the cDNAs were isolated after ligating an "anchor" template (41) onto the 5′ termini of double-stranded parotid/mandibular cDNA; this anchor sequence (42) was then used as a 5′ primer in successive PCR amplifications with a nested pair of chain 1- or chain 2-spe-

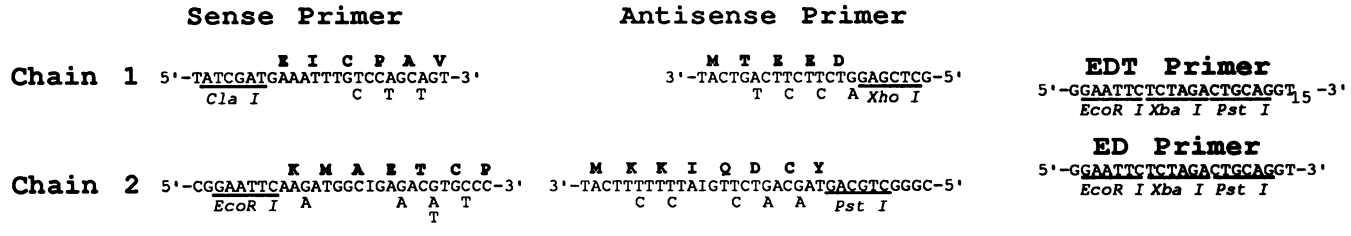

FIG. 3. Primers and probes used to amplify by PCR and subclone internal and 3′ portions of cDNAs of Fel dI chains 1 and 2.

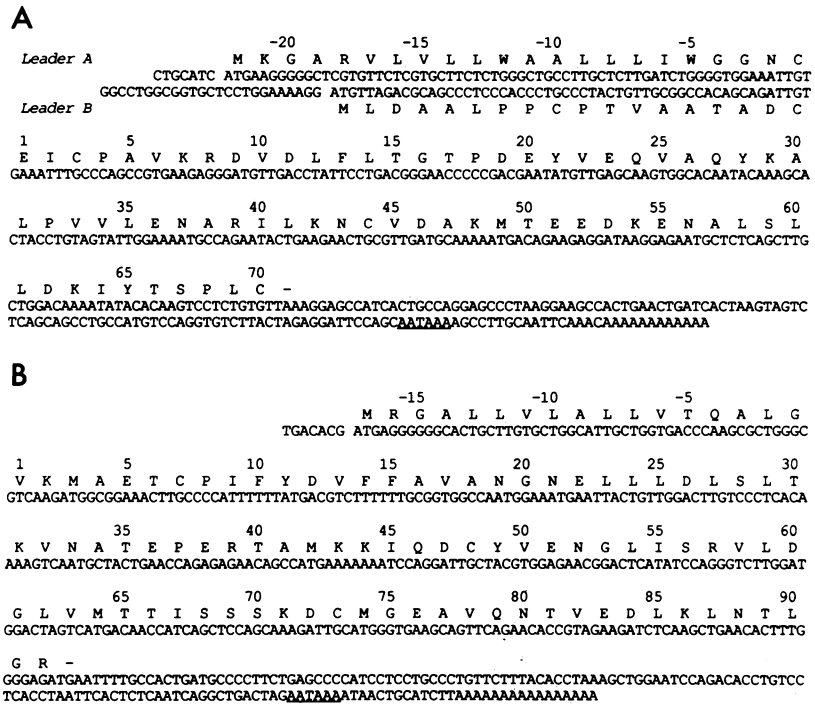

FIG. 4. cDNA sequence of Fel dI. Negative residues compose the leader sequences, and residue 1 represents the amino terminus of the mature proteins. Polyadenylylation signals are underlined. (*A*) Chain 1 of Fel dI including both leader sequences. (*B*) Chain 2 of Fel dI.

cific 3′ primers. Two different 5′ partial cDNAs were identified for chain 1 (leaders A and B in Fig. 4*A*) in contrast to a single one for chain 2 (Fig. 4*B*). Each of the three 5′ partial cDNAs had open reading frames with presumptive methionine initiator codons (43).

Contiguous chain 1 and chain 2 Fel dI cDNAs were generated by amplifying parotid/mandibular cDNA with 5′ and 3′ noncoding primer pairs. These PCR products were reamplified asymmetrically (38) to generate single-stranded template for sequence analysis; direct sequencing of the PCR products confirmed the sequence for chains 1 and 2 obtained from the partial cDNAs.

A search of a protein sequence data base for sequence homology with chains 1 and 2 of Fel dI revealed homology between the rabbit uteroglobin precursor (44) and the "full-length" leader A–chain 1 protein (Fig. 5). The observed homology (25 identities and 25 conservative substitutions with two gaps in 93 residues) is such that the probability of two random polypeptides of this size sharing this degree of similarity is less than 1 in 10^4 (38). Homology of a similar extent was found between chain 1 and a 10-kDa protein secreted from human lung Clara cells (45). No proteins with significant homology to chain 2 were identified.

Protein sequence analysis of Fel dI could not identify the amino acid at position 33 of chain 2. The presence of a consensus sequence for N-linked glycosylation (N-A-T at positions 33–35) in Fel dI chain 2 cDNA suggested glycosylation was responsible for the lack of sequence signal at residue 33. To determine whether chain 2 is modified by N-linked glycosylation, Fel dI was treated with peptide N–glycosidase F and analyzed on SDS/PAGE and Western immunoblots with chain 1- or chain 2-specific anti-peptide antisera (Fig. 1*B*). The electrophoretic mobility of chain 2 increased upon digestion with N-glycosidase, while that of chain 1 remained unaffected. This observation and the lack of a consensus sequence for N-linked glycosylation in chain 1 suggest that solely chain 2 of Fel dI is modified by N-linked oligosaccharides.

DISCUSSION

By use of novel techniques, such as OPA blockage and *in situ* acetylation/CNBr cleavage, the greater part of the protein sequence of Fel dI, the major allergen of the domestic cat, was determined. cDNA cloning was then utilized to complete the primary structure analysis of the allergen.

The deduced amino acid sequence of the chain 1 cDNA is in complete agreement with the protein sequence obtained from immunoaffinity-purified Fel dI. cDNA cloning of chain 2 of Fel dI served to link various peptide-derived sequences and reveal an additional nine C-terminal amino acids. However, the sequence and spacing polymorphism detected by protein sequencing in the vicinity of residues 65–73 was not observed in the chain 2 cDNA.

Analysis of the chain 2 cDNA sequence disclosed that residue 33, which could not be resolved by protein sequence

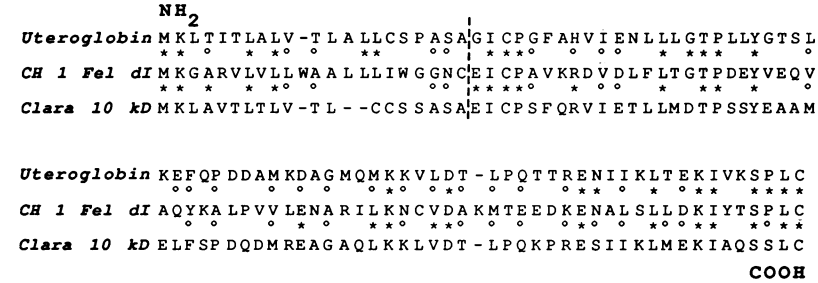

FIG. 5. Protein sequence homology of chain 1 of Fel dI, rabbit uteroglobin, and human Clara cell 10-kDa secretory protein. ☆, Amino acid identities; ∘, conservative amino acid substitutions; vertical dashed line, amino termini of the mature secreted proteins.

analysis, is asparagine. This led to the prediction that chain 2 of Fel dI is a glycoprotein with N-linked oligosaccharides, which was corroborated by an observed increase in the electrophoretic mobility of chain 2 of Fel dI after digestion with Peptide N-glycosidase F. This result is in agreement with a previous report that one of the chains of Fel dI is modified by N-linked oligosaccharides (22).

The isolation of two distinct cDNAs for chains 1 and 2 demonstrates that Fel dI is comprised of polypeptides encoded by different genes. Both cDNAs encode N termini with short stretches of hydrophobic residues, which most likely represent the precursor forms of the individual chains of Fel dI, whereas the N termini determined by protein sequence analysis are probably representative of the mature forms of chains 1 and 2. The two different leaders in the chain 1 cDNAs can be accounted for by alternative splicing of the nascent chain 1 mRNA transcript (unpublished data).

The sequence homology of Fel dI chain 1 with rabbit uteroglobin and the human Clara cell 10-kDa secretory protein is intriguing but inconclusive in establishing a homology of function. All three proteins are synthesized with hydrophobic leader peptides and secreted as mature proteins ≈70 amino acids long. Uteroglobin has been crystallized and shown to exist as a homodimer of antiparallel chains linked by disulfide bonds (46). The 10-kDa secreted protein from human Clara cells is also a disulfide-linked homodimer (45). The tertiary structure of Fel dI appears to be more complex (22), as preliminary analysis indicates chains 1 and 2 are linked by intermolecular disulfide bonds in an antiparallel topology (data not shown).

Uteroglobin is believed to protect the wet epithelia by acting as an immunomodulatory protein, for example, limiting the maternal immune response against the embryo during implantation (47). Uteroglobin possesses both steroid binding (48) and phopholipase A_2 inhibitory (49) activities. The latter activity is shared with lipocortins (50) and mediates an antiinflammatory effect by preventing the formation of arachidonic acid metabolites from membrane phospholipids. Since Fel dI has no known biological function it is interesting to speculate why cat skin epithelia is ubiquitously coated with the protein [either from synthesis *in situ* (20) (unpublished data) or from deposition of salivary proteins during licking]. Perhaps Fel dI is involved in protecting the feline dry epithelia in a manner that is analogous to that by which uteroglobin protects the wet epithelia. Last, if further study demonstrates Fel dI has an inherent ability to modulate immune processes, what role might this activity play in the human allergic response?

The complete primary structure of Fel dI presented in this manuscript should facilitate the mapping of its B- and T-cell epitopes and thereby help to elucidate the mechanism of the human immune response to this clinically important allergen. Ultimately, the knowledge gained from such studies may lead to more effective disease management for cat-allergic patients.

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1. Friedhoff, L. R., Meyers, D. A. & Marsh, D. G. (1984) *J. Allergy Clin. Immunol.* **73**, 490–499.
2. Bryant, D. H. & Burns, M. W. (1976) *Med. J. Aust.* **1**, 918–926.
3. Sarsfield, J. K., Boyle, A. G., Rowell, E. M. & Moriarty, S. C. (1976) *Arch. Dis. Child.* **51**, 186–192.
4. Pollart, S. M., Chapman, M. D., Fiocco, G. P., Rose, G. & Platts-Mills, T. A. E. (1989) *J. Allergy Clin. Immunol.* **83**, 875–882.
5. Sears, M. R., Herbison, G. P., Holdaway, M. D., Hewitt, C. J., Flannery, E. M. & Silva, P. A. (1989) *Clin. Exp. Allergy* **19**, 419–424.
6. Ohman, J. L., Jr., Kendall, S. & Lowell, F. C. (1977) *J. Allergy Clin. Immunol.* **60**, 317–323.
7. Anderson, M. C. & Baer, H. (1981) *J. Immunol.* **127**, 972–975.
8. Ohman, J. L., Jr., Lowell, F. C. & Bloch, K. J. (1974) *J. Immunol.* **113**, 1668–1677.
9. Marsh, D. G., Goodfriend, L., King, T. P., Lowenstein, H. & Platts-Mills, T. A. E. (1988) *Int. Arch. Allergy Appl. Immunol.* **85**, 194–200.
10. Lowenstein, H., Lind, P. & Week, B. (1985) *Allergy* **40**, 430–441.
11. Duffort, O., Carreira, J. & Lombardero, M. (1987) *Int. Arch. Allergy Appl. Immunol.* **84**, 339–344.
12. Leitermann, K. & Ohman, J. L., Jr. (1984) *J. Allergy Clin. Immunol.* **74**, 147–153.
13. de Groot, H., van Swieten, P., van Leeuwen, J., Lind, P. & Aalberse, R. C. (1988) *J. Allergy Clin. Immunol.* **82**, 778–786.
14. Van Metre, T. E., Marsh, D. G., Adkinson, N. F., Jr., Fish, J. E., Kagey-Sobotka, A., Norman, P. S., Radden, E. B., Jr., & Rosenberg, G. L. (1986) *J. Allergy Clin. Immunol.* **78**, 62–75.
15. Luczynska, C. M., Li, Y., Chapman, M. D. & Platts-Mills, T. A. E. (1990) *Am. Rev. Respir. Dis.* **141**, 361–367.
16. Ohman, J. L., Jr., Findlay, S. R. & Leitermann, K. M. (1984) *J. Allergy Clin. Immunol.* **74**, 230–239.
17. Sundin, B., Lilja, G., Graff-Lonnevig, V., Hedlin, G., Heilborn, H., Norrlind, K., Pegelow, K.-O. & Lowenstein, H. (1986) *J. Allergy Clin. Immunol.* **77**, 478–487.
18. Brown, P. R., Leitermann, K. & Ohman, J. L., Jr. (1984) *Int. Arch. Allergy Appl. Immunol.* **74**, 67–70.
19. Anderson, M. C., Baer, H. & Ohman, J. L., Jr. (1985) *J. Allergy Clin. Immunol.* **76**, 563–569.
20. Dabrowski, A. J., Van Der Brempt, X., Soler, M., Seguret, N., Lucciani, P., Charpin, D. & Vervloet, D. (1990) *J. Allergy Clin. Immunol.* **86**, 462–465.
21. Chapman, M. D., Aalberse, R. C., Brown, M. J. & Platts-Mills, T. A. E. (1988) *J. Immunol.* **140**, 812–818.
22. Duffort, O. A., Carreira, J., Nitti, G., Polo, F. & Lombardero, M. (1991) *Mol. Immunol.* **28**, 301–310.
23. O'Hehir, R. E., Garman, R. G., Greenstein, J. L. & Lamb, J. R. (1991) *Annu. Rev. Immunol.* **9**, 67–95.
24. Berzofsky, J. A., Brett, S. J., Streicher, H. Z. & Takahashi, H. (1988) *Immunol. Rev.* **106**, 5–31.
25. Rothbard, J. B. & Gefter, M. L. (1991) *Annu. Rev. Immunol.* **9**, 527–565.
26. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
27. Towbin, H., Staehlin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
28. Morel, A. M. & Delaage, M. A. (1988) *J. Allergy Clin. Immunol.* **82**, 646–654.
29. Andrew, P. C. & Dixon, J. E. (1987) *Anal. Biochem.* **161**, 524–528.
30. Brauer, A. W., Oman, C. L. & Margolies, M. N. (1984) *Anal. Biochem.* **137**, 134–142.
31. Strosberg, A. D., Fraser, K. J., Margolies, M. N. & Haber, E. (1972) *Biochemistry* **11**, 4078–4085.
32. Simpson, F. J. & Edouard, C. N. (1984) *Biochem. Int.* **8**, 787–791.
33. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
34. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
35. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
36. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
37. Gyllensten, U. B. & Ehrlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7652–7656.
38. Collins, J. F., Coulson, A. F. W. & Lyall, A. (1988) *Comput. Appl. Biosci.* **4**, 67–71.
39. Lee, C. C., Wu, X., Gibbs, R. A., Cook, R. G., Muzny, D. M. & Caskey, C. T. (1988) *Science* **239**, 1288–1291.
40. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
41. Roux, K. H. & Dhanarajan, P. (1990) *Biotechniques* **8**, 48–57.
42. Rafnar, T., Griffith, I. J., Kuo, M.-C., Bond, J. F., Rogers, B. L. & Klapper, D. G. (1991) *J. Biol. Chem.* **266**, 1229–1236.
43. Kozak, M. (1986) *Cell* **44**, 283–292.
44. Lipman, D. J. & Pearson, W. R. (1985) *Science* **227**, 1435–1441.
45. Singh, G., Katyal, S. L., Brown, W. E., Phillips, S., Kennedy, A. L., Anthony, J. & Squeglia, N. (1988) *Biochim. Biophys. Acta* **950**, 329–337.
46. Mornon, J. P., Fridlansky, F., Bally, R. & Milgrom, E. (1980) *J. Mol. Biol.* **137**, 415–429.
47. Mukherjee, D. C., Ulane, R. E., Manjunath, R. & Mukherjee, A. B. (1983) *Science* **219**, 989–991.
48. Beato, M. & Baier, R. (1975) *Biochim. Biophys. Acta* **392**, 346–356.
49. Miele, L., Cordella-Miele, E. & Mukherjee, A. B. (1987) *Endocr. Rev.* **8**, 474–490.
50. Miele, L., Cordella-Miele, E., Facchiano, A. & Mukherjee, A. B. (1988) *Nature (London)* **335**, 726–730.