

Recombinant Allergens

Christophe Grégoire* and Martin D. Chapman

*Asthma and Allergic Diseases Center, Department of Medicine,
University of Virginia, PO Box 801355, Charlottesville, VA 22908*

Introduction

The introduction of molecular biology techniques over the past 15 years has provided systematic approaches to the identification and structural analysis of allergens. Through the application of these techniques, the sequences of an increasing number of allergens have been revealed since 1988, when the first cDNA sequence of Der p 1 was published (1). More than one thousand partial or complete cDNAs encoding allergens or their isoforms have been cloned, sequenced, and registered in the data banks. For over half of these biologic activity has been attributed based on the knowledge of the primary structure and by analogy with proteins of known function.

In order to avoid redundancy and confusion, allergens are named using guidelines developed by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) subcommittee allergen nomenclature. This nomenclature uses the three first letter of the taxonomic name of the source material following by an Arabic numeral. Allergens are classified based on the chronological order of their identification. By extension, allergens from another source with homologous sequences, or with the same expected function, take the same number. For example, all hymenoptera venom allergens that possess a phospholipase activity are classified as Group 1 (Api m 1, Pol a 1, Ves m 1...); those with hyaluronidase activity as Group 2 (Api m 2, Pol a 2, Ves m 2...), and so forth. Isoallergens and variants are defined by their degree of homology: isoallergens have amino acid sequence identity of at least 67%, variants have sequence identities close to 100% (2). The presence of multigenic families that encode for the same protein have been described for number of allergens like pollens (Phl p 5, Hor v 9, Bet v 1...) (3–5).

*Author to whom all correspondence and reprint requests should be addressed.

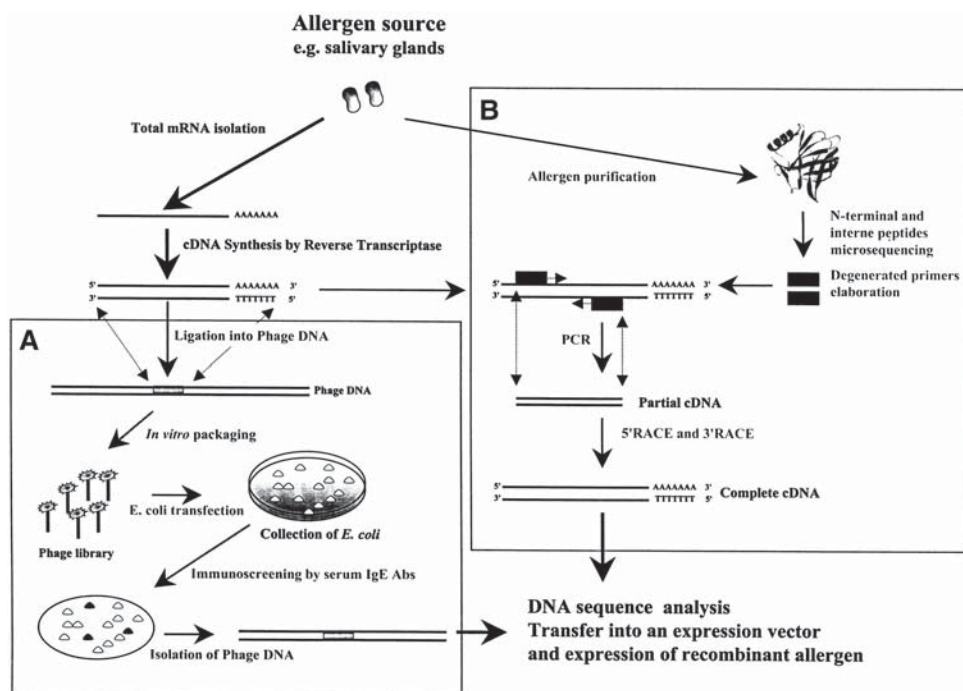


Fig. 1. Principal steps involved in allergen cloning strategies: constitution and screening of DNA library (A) and the RT-PCR cloning method (B). RT-PCR, reverse transcriptase–polymerase chain reaction; RACE, rapid amplification of cDNA ends.

The aims of this chapter are to outline the basic techniques for cloning allergen genes and their expression in relevant hosts. This review discusses rapid progress made in the field of allergen biotechnology and how this technology can improve the quality of diagnostic products or used to develop new therapeutic products.

Allergen Gene Cloning and Expression of Recombinant Forms In Vitro

Cloning Strategies

The aim of cloning and sequencing is to obtain the primary structure of the allergen and to express it in a recombinant form. Several strategies have been employed to obtain cDNA encoding for allergens (Fig. 1A, B). Briefly, these procedures begin with the isolation of the messenger RNA (mRNA) from the natural source. These total mRNA are copied in vitro into complementary DNA (cDNA) using reverse transcriptase and introduced inside bacteriophage genomic DNA. This

library of bacteria infected by phage can in theory express the same proteins as the organ source. It is crucial to ensure that the allergens of interest are actually expressed in the organ source. The cDNA library can be screened by using IgE antibodies from a pool of allergic patients sera or by using polyclonal antibodies (PAb)s from hyper-immunized animals or murine monoclonal antibodies (MAbs). Successive rounds of screening allow the isolation of a single clone of the bacteriophage containing the allergen gene. After these steps of purification, the allergen cDNA can be transferred to another vector system for nucleotide sequence determination (Fig. 1A).

An alternative strategy using reverse transcriptase polymerase chain reaction (RT-PCR) can be employed in absence of biochemical and/or immunological tools to perform the immunologic screening of the expression library. RT-PCR sequencing techniques are useful in the absence of posttranslational modifications or incorrect folding of the recombinant proteins in prokaryote systems. For example, the inability of bacteria to correctly process the cat allergen Fel d 1 heterodimer resulted in unsuccessful cDNA library screening and the Fel d 1 sequence was eventually resolved by RT-PCR (6). This method was also used successfully to sequence the gene of aspergillus allergen Asp f 1, house dust mite allergen Eur m 1, and the horse allergen Equ c 1 (7–9). These strategies use “degenerate” synthetic oligonucleotides as primers, based on the primary amino acid sequence of the allergen. The limitation is the isolation and purification of the natural allergen followed by microsequencing of several internal peptides to construct the degenerate primers. These oligonucleotides are used in PCR steps for amplification of the specific allergen encoding cDNA (Fig. 1B). With both cDNA libraries and PCR, it is common to obtain several clones that encode different isoforms of the same allergen. For example, the major allergen Mus m 1 (*Mus musculus*) or Rat n 1 (*Rattus Norvegicus*) first cited as major urinary proteins (MUP) are encoded by a multigenic family and expressed differentially in several organs (salivary glands, liver, etc.) (10–13).

A direct application of allergen cloning is to determine the potential biological activity by comparing homology with proteins of known sequence. For example, cloning of Der p 1 allowed the thiol protease activity of the Group 1 of the mite allergens to be discovered (1). The binding and transport of small hydrophobic ligands (e.g., steroids, fatty acids, retinoids, pigments) is a potential function of allergens that are members of the lipocalin superfamily (14). Mammalian allergens such as Mus m 1, Rat n 1, Bos d 2, Bos d 5, Can f 1, Can f 2, and Equ c 1... (9,13,15–17) and the cockroach allergen Bla g 4 (18) are known to be lipocalins.

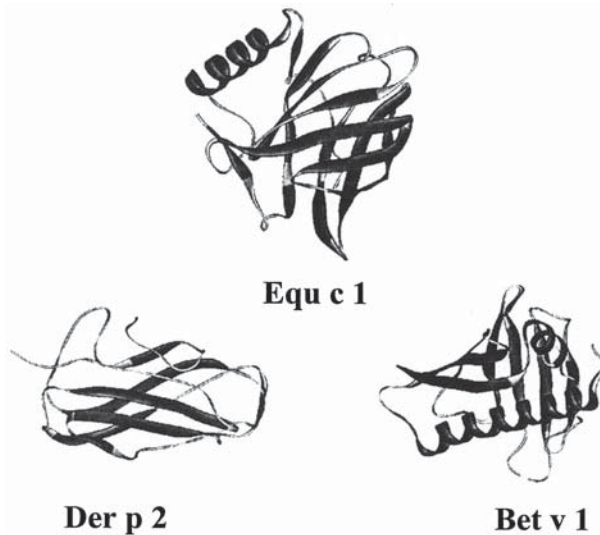


Fig. 2. Tertiary structure obtained by NMR and/or X-ray crystallographic methods for the mite allergen Der p 2 (**1A9V**) (56), for the birch pollen allergen Bet v 1 (**1BV1** and **1BTV**) (20) and for the horse allergen Equ c 1 (**1EW3**)(21). The Brookhaven Protein Databank codes are given in bold.

Expression of Recombinant Allergens

A large number of expression systems for recombinant proteins have been developed and several have proven useful for high-level allergen expression. The choice of expression system will depend on the physicochemical characteristics of the allergen, the original source, and the intended use of the recombinant allergen. The systems the most frequently used for the expression of the recombinant proteins are bacteria (*Escherichia coli*), yeast (*Pichia pastoris* or *Saccharomyces cerevisiae*), insect cells, or plant cells.

The bacterium (*E. coli*) is the first choice as an expression host system. The advantages of *E. coli* are based on the simple composition of the growth media, short generation time, and minimal costs of production. The constraints of prokaryotic systems are the inability of bacteria to perform glycosylation or other post-translational modifications that can be achieved by eukaryotic cells. These modifications may be essential for biological activity. However, bacterial systems are preferred if recombinant allergen is to be used for structural analysis. The tertiary structures of the mite allergen Der p 2 (19), the birch-pollen allergen Bet v 1 (20), and the horse allergen Equ c 1 were obtained by nuclear magnetic resonance (NMR) and/or X-ray crystallographic methods using bacterially expressed protein (21) (Fig. 2). This allows the allergen to be

labeled easily with ^{15}N or ^{13}C amino acids for NMR, or to be obtained in sufficiently high yields without glycosylation for crystallography.

Natural protein with a high percentage of polysaccharides (O and/or N-glycosylation), or recombinants produced in *Pichia* expression system, can prevent the formation of high-quality crystals. Nevertheless, high-resolution structures of natural mouse urinary protein (mMUP) or bovine dander allergen Bos d 2 obtained from *Pichia* have been reported (10,22).

Bacterial-expression systems cannot process pro-enzymes into their mature forms or assemble several sub-units of a protein. Cat allergen Fel d 1 consists of two polypeptide chains linked by disulfide bonds as a heterodimer. Although each of chain has been expressed in bacteria, they do not possess the processing pathways to reassociate the two recombinant chains into a mature protein. This has been accomplished by combining the chains under reducing and denaturing conditions, and allowing them to reassociate (23). Experiments on rChain 1 and rChain 2 of Fel d 1 demonstrated that IgE from cat allergic patients bound these two chains and that both chains contributed to the allergenicity of the heterodimer protein (24,25). Dramatic improvements in the reactivity of rFel d 1 were reported by expressing the allergen in baculovirus. Human IgG and IgE antibodies gave similar binding curves with natural Fel d 1 or baculovirus expressed rFel d 1 (26).

Improvements in allergen expression have also been obtained mite allergen Der p 1. Natural Der p 1 is synthesized as a pre pro-protein with chain lengths of 18, 80, and 223 amino acids, respectively for the pre, pro, and mature forms. The pre pro-protein is processed to a pro protein then to a mature protein with correct folding and enzymatic activity. Der p 1 showed poor reactivity with the IgE antibody when expressed in *E. coli* (27). The incorrect folding in the prokaryote system was partially resolved by expression in *Saccharomyces*, but the protein yield was low (28).

Subsequently, Der f 1 was expressed in insects cells using baculovirus (29) and the recombinant precursor form of Der p 1 produced by *Drosophila* cells (30). Both of these expression systems required acid treatment or endopeptidase digestion to produce the active form of Der f 1. Acid treatment of Der f 1 in *E. coli* has also been reported to result in enzymatically active allergen (31). To overcome protein expression problems in bacteria, high-level expression systems in yeast have been developed. Yeasts (*Pichia pastoris* or *Saccharomyces cerevisiae*) have the advantages that they produce high levels of the protein of interest and also perform protein processing, folding, and post-translational modifications. Less expensive to use than other eukaryotic expression sys-

tem like baculovirus, they can also be used for molecular and genetic manipulations. Yeast vectors may include plasmids that incorporate fusion proteins or poly-histidine tags that will facilitate protein purification. One disadvantage is that both species add carbohydrate to secreted protein. However, the length of the oligosaccharides added by *P. pastoris* are much shorter than those of *S. cerevisiae* and do not have the terminal α 1-3 linked mannose residues characteristic of *S. cerevisiae*. The main difference between the yeast systems are that the highly antigenic sugar from *S. cerevisiae* are unsuitable for therapeutic purposes, but this is not the case with *P. pastoris* products. Most allergens have been expressed in *P. pastoris*. For example, recombinant Bla g 4 was initially produced in bacteria at low yield (18) but could be expressed in *P. pastoris* with a yield around 50 mg/L of culture (32). The difference in yield could be attributed to the presence of rare codons that encode for Arginine (AGA and AGG) which affect synthesis in bacteria but not in yeast. Recently, rDer f 1 and rDer p 1 with excellent immunoreactivity have also been expressed in *Pichia* (33).

Recombinant Proteins as Tools in Investigation

Immediate hypersensitivity reactions are dependant on numerous factors that includes the physicochemical properties of the allergen, exposure frequency and dose, environmental factors, and the genetic subject predisposition of the host (34). With the discovery of the structure and the function of allergens, authors have speculated that the biochemical activity or the presence of substances associated with allergens could be involved directly in the sensitization process as proallergic adjuvants. Use of well-defined recombinant allergens provide useful tools to investigate potential links between biologic function and allergenicity.

The strongest evidence has been obtained for mite allergen Der p 1, which is a cysteine protease. The evidence suggests that the proteolytic activity of Der p 1 facilitates its penetration through mucosal surfaces by action on the inter-cellular junction (35–37). Der p 1 may maintain inflammation through proteolytic action on alpha 1 anti-trypsin (38) and could upregulate IgE synthesis through its ability to cleave CD23 and CD25 (39–42). These data are consistent with the observation of Finkelman and Urban (43) using papain, another plant cysteine-protease allergen. They reported that repeated injections into footpad mice with active papain stimulates an increase in serum IgE levels compared by the enzymatically inactivated form of the allergen. In addition, they reported within 1 h after injection an increase in the expression of mRNA for the cytokines interleukin (IL)-4, IL-5, and IL-9, but not those of interferon- γ (IFN- γ) or IL-2. Similarly, the active form

of bee-venom phospholipase A 2 (Api m 1), but not its catalytic inactive variant, was able to enhance IL-4 production by mast cells in an IgE-independent manner (44). Repeated injection of mice with low doses of both allergen forms gave high levels of specific IgE only in the case of enzymatically active allergen. T-cell epitopes of PLA2 are localized outside the enzyme active site, indicating that the observed results could not be attributed to alteration of T-cell epitopes. These data point to a potential linkage between enzymatic function of the PLA 2 and its strong immunogenicity.

Nowadays, approx 140 allergen families have been characterized with known partial and/or complete amino acid sequences (45). An examination of these data indicate that less of half possess enzymatic activity. Despite the considerable amount of information regarding the molecular structure of many allergens, no unifying feature has been yet discovered. It appears than cases of Der p 1, PLA 2 must be considered more a "case report" than a common rule linking allergenicity to biologic function (46).

Determination of Cross-Reactivity From Sequence Homology

Examples of cross-reactivity are common in food allergy. Among food allergens, crustaceans are a frequent cause of adverse food reactions in allergic individuals. Tropomyosin is a structural protein involved in muscle contraction that was described as a shrimp allergen (47) and also as Group 10 mite allergen (48). Despite the presence of this highly conserved proteins in cells of all species of vertebrates and invertebrates, IgE responses are selective for invertebrates such as crustaceans (shrimp, lobster, crab, crawfish), arachnids (house-dust mites), insects (cockroaches), and molluscs (squid). The sequences of crustacean, cockroach, and house-dust mite tropomyosins share $\pm 80\%$ of sequence identity, in comparison with only 45% homology to human and meat (beef, chicken, pork, lamb, etc.) tropomyosins.

When cross-reactions to fresh fruits and vegetables are involved, most patients are previously sensitized to grass, weed, or tree pollens. There are well-described clinical associations between individuals who are pollen-sensitive, and experience allergic symptoms on eating various fruits and vegetables. These include associations between birch-pollen, apple, carrot, and celery and cherry, between ragweed and melon, or between grass and tomatoes (49,50). In the example of birch pollen, homologs of the major allergens Bet v 1 and Bet v 2 were found in each of the related other species (51). Interestingly, the cross-reactivity included IgE binding as well as T helper (Th) cell epitopes (52–54).

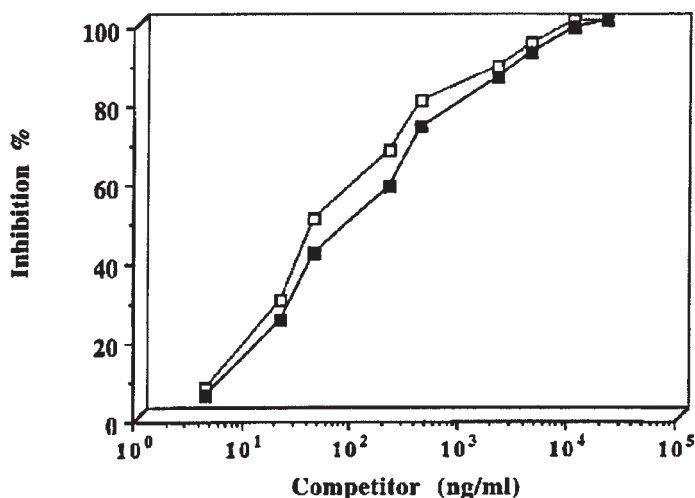


Fig. 3. Competition ELISA performed by pre-incubation of monoclonal antibodies with serial dilutions of rEqu c1 (open symbols) or natural Equ c1 (filled symbols) (9).

Recombinant Allergens to Improve Patient Care

From the Standardization of Natural Allergens to Recombinant Allergen Cocktails

The aim of producing recombinant allergens is to obtain highly purified protein with the same physicochemical and biological characteristics of their natural counterparts. The majority of recombinant allergens (plant, mammal, insect, mite, and fungi) show comparable immunologic and biologic properties to natural allergens. The purification of important allergens led to the development of sensitive immunoassays for the quantification of allergens levels in allergenic products and environmental samples. Improved standardization has been achieved by determining equivalent doses of major allergens in commercial products (55). The problems with the natural extracts are that the absolute potency is difficult to define and some patients give adverse reactions when the extracts are injected. The recombinant allergens can be obtained with a high degree of purity and in the most cases have demonstrated excellent immunoreactivity as well in skin testing and serum IgE antibody assays. The immunoreactivity can be assessed by ELISA (Fig. 3) (9,56), histamine or β -hexosaminidase release assays as well by skin testing (57,58)

Recombinant birch allergens (rBet v 1 and rBet v 2) have been successfully tested for the diagnosis of birch-pollen allergy and could identify more than 95% of pollen-sensitized patients (59). Bet v 1 homologs are found in related tree pollens of the order fagales (60). Sensitization

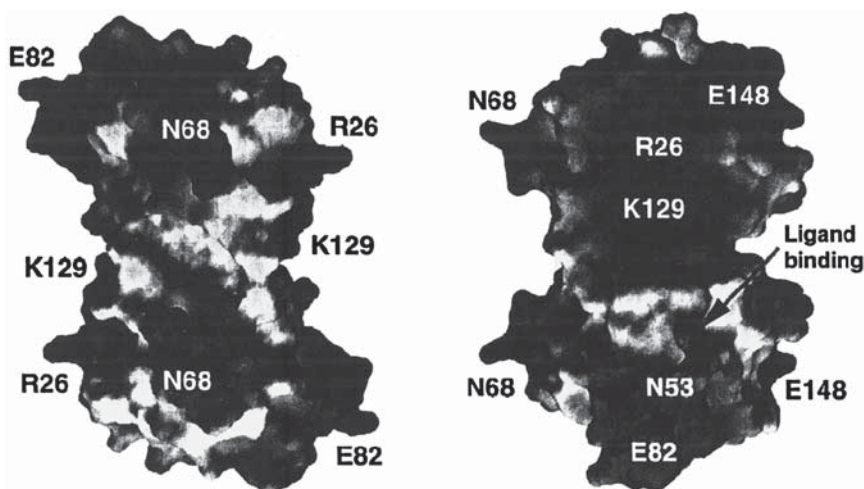


Fig. 4. Front and side view of the molecular surface of the Equ c 1 dimer showing putative IgE-binding targets identified by Lascombe et al. (21). Regions near to the positions of the four amino acid residues substituted by site-directed mutagenesis (Arg²⁶, Glu⁸², Lys¹²⁹, and Glu¹⁴⁸) and the two putative glycosylation sites (Asn⁵³ and Asn⁶⁸) are also indicated.

to Bet v 1 can be used as a general marker of allergy to multiple tree species. Birch pollen-allergic patients often have symptoms following ingestion of fruits and vegetables (oral-allergy syndrome) and the cross-reactive allergens that are found in these foods are profilins, similar to Bet v 2. Thus sensitization to Bet v 2 can help to distinguish patients who may also be allergic to these foods (61).

Hypoallergenic Recombinant Allergens

Natural isoforms occur as a result of post-translational and the presence of a multigenic families coding for isoforms and/or allelic variants. Natural variants of allergen Bet v 1 show difference both in IgE-binding capacity and in T-cell reactivity (62–64). Recombinant allergens have been used to generate “hypoallergenic variants” for more safe and specific immunotherapy. Using site-directed mutagenesis, variants have been produced that show decreased IgE-binding activity, but retain strong T-cell stimulation (5,65). In a recent French study, genetically modified hypoallergenic derivatives of Bet v 1 showed reduced capacity to induce immediate-type skin reactions. These molecules may represent candidates for immunotherapy of birch-pollen allergy with reduced risk of anaphylactic side-effects (61).

The most satisfying method to define amino acids that could be implicated in B epitopes is to determine the tertiary structure of the molecule by X-ray or NMR. A recent study on the horse major allergen Equ c 1 has determined the region that may be involved in the patient

IgE epitopes (Fig. 4) (21). The aim of these determinations was to produce variants with critical amino acid mutation(s) in these IgE epitope(s) and to avoid IgE cross-linking at the surface membrane of the mast cell. Similar mutagenesis techniques have been applied to the Group 2 mite allergens to define the surface amino acids. Mutations of the amino acids that had the highest probability of being involved in B-cell epitopes gave 100-fold less reactivity in skin testing in comparison with the native recombinant form (66). Hypo-reactive allergen or peptide-based therapy is being pursued as an approach when the risk of adverse reactions are too high. Alternative immunotherapy with mutated allergens may also provide a safer treatment for food allergies such as peanut.

Production of enzymatically inactive allergen could be suitable for the development of in vitro diagnosis tests as well as for specific immunotherapy. The production of enzymatically inactive allergens could prevent and lead to the development of more stable allergenic products. Inactive enzymatic forms have been produced for bee-venom phospholipase A 2 (Api m 1) by site-directed mutagenesis of its catalytic domain (44). Another example is the precursor form of Der p 1 expressed by *Drosophyllia* cells, which has similar immuno-reactivity towards IgE, but no cysteine protease activity (30).

Another approach used in the early 1990s was the application of allergen peptides to elicit specific T-cell anergy or hypo-responsiveness. Although Fel d 1 peptides showed symptomatic improvements in clinical trials, high doses of peptides were required (up to 750 µg/ml). Some adverse reactions were reported and the Fel d 1 peptides immunotherapy failed to make phase III clinical trials (67). The application of peptide immunotherapy could find a new start with recent data on induction of late-phase reactions following intradermal injection of short Fel d 1 peptides (14 residues in length) (68). Administration of multiple overlapping peptides spanning the Fel d 1 sequence induced hyporesponsiveness and may provide a viable approach to peptide based immunotherapy (68). Recent observations indicate that the specific T-cell repertoire could be restricted for any allergen. In the family of the lipocalin allergen (Bos d 2), the T-cell epitopes are essentially concentrated and co-localize in the conserved regions of the lipocalins (69). Studies of Bet v 1 indicate that peptides involved in the T-cell repertoire of allergic and nonallergic patients are similar (65).

Conclusion

In the near future, it can be anticipated that recombinant allergens will be used extensively for research purposes, including structural analysis of allergens, determination of T-cell function, and in animal

and human experiments on allergen-induced inflammatory reactions. There is good evidence that recombinant allergen cocktails will be able to substitute or replace natural allergen extracts for diagnostic purposes. They will also be useful for the development of novel, patient-based diagnostic tests. Recombinant allergens will also allow clinical trials of new forms of immunotherapy and may, ultimately, allow prophylactic allergy vaccines to be developed.

Acknowledgment

This work was supported by National Institutes of Health grants AI32557 and AI34607.

References

1. Chua, K. Y., Stewart, G. A., Thomas, W. R., Simpson, R. J., Dilworth, R. J., Plozza, T. M., and Turner, K. J. (1988), *J. Exp. Med.* **167**, 175–182.
2. King, T. P., Hoffman, D., Lowenstein, H., Marsh, D. G., Platts-Mills, T. A., and Thomas, W. (1995), *Allergy* **50**, 765–774.
3. Wurtzen, P. A., Bufer, A., Wissenbach, M., Madsen, H. O., Ipsen, H., Arned, J., and Van Neerven, R. J. (1999), *Clin. Exp. Allergy* **29**, 1614–1625.
4. Astwood, J. D. and Hill, R. D. (1996), *Gene* **182**, 53–62.
5. Ferreira, F., Hirtenlehner, K., Jilek, A., Godnik-Cvar, J., Breiteneder, H., Grimm, R., et al. (1996), *J. Exp. Med.* **183**, 599–609.
6. Morgenstern, J. P., Griffith, I. J., Brauer, A. W., Rogers, B. L., Bond, J. F., Chapman, M. D., and Kuo, M. C. (1991), *Proc. Natl. Acad. Sci. USA* **88**, 9690–9694.
7. Arruda, L. K., Mann, B. J., and Chapman, M. D. (1992), *J. Immunol.* **149**, 3354–3359.
8. Kent, N. A., Hill, M. R., Keen, J. N., Holland, P. W., and Hart, B. J. (1992), *Int. Arch. Allergy Immunol.* **99**, 150–152.
9. Grégoire, C., Rosinski-Chupin, I., Rabillon, J., Alzari, P. M., David, B. and Dandeu, J. P. (1996), *J. Biol. Chem.* **271**, 32,951–32,959.
10. Clark, A. J., Clissold, P. M., Al Shawi, R., Beattie, P., and Bishop, J. (1984), *EMBO J.* **3**, 1045–1052.
11. Shahan, K., Gilmartin, M., and Derman, E. (1987), *Mol. Cell. Biol.* **7**, 1938–1946.
12. Ichiyoshi, Y., Endo, H., and Yamamoto, M. (1987), *Biochim Biophys Acta* **910**, 43–51.
13. Gao, F., Endo, H., and Yamamoto, M. (1989), *Nucleic Acids Res.* **17**, 4629–4636.
14. Flower, D. R., North, A. C., and Attwood, T. K. (1993), *Protein Sci.* **2**, 753–761.
15. Katakura, Y., Totsuka, M., Ametani, A., and Kaminogawa, S. (1994), *Biochim Biophys Acta* **1207**, 58–67.
16. Mantyjarvi, R., Parkkinen, S., Ryttonen, M., Pentikainen, J., Pelkonen, J., Rautiainen, J., et al. (1996), *J. Allergy Clin. Immunol.* **97**, 1297–1303.
17. Novotny, M. V., Ma, W., Wiesler, D., and Zidek L. (1999), *Proc. R. Soc. Lond. B. Biol. Sci.* **266**, 2017–2022.
18. Arruda, L. K., Vailes, L. D., Hayden, M. L., Benjamin, D. C., and Chapman, M. D. (1995), *J. Biol. Chem.* **270**, 31,196–31,201.
19. Mueller, G. A., Benjamin, D. C., and Rule, G. S. (1998), *Biochemistry* **37**, 12,707–12,714.

20. Gajhede, M., Osmark, P., Poulsen, F. M., Ipsen, H., Larsen, J. N., Joost van Neerven, R. J., et al. (1996), *Nat. Struct. Biol.* **3**, 1040–1045.
21. Lascombe, M. B., Grégoire, C., Poncet, P., Tavares, G. A., Rosinski-Chupin, I., Rabillon, J., et al. (2000), *J. Biol. Chem.* **275**, 21,572–21,577.
22. Rautiainen, J., Auriola, S., Rouvinen, J., Kauppinen, J., Zeiler, T., Novikov, D., et al. (1998), *Biochem. Biophys. Res. Commun.* **247**, 746–750.
23. Keating, K. M., Segal, D. B., Craig, S. J., Nault, A. K., Semensi, V., Wasserman, A. S., et al. (1995), *Mol. Immunol.* **32**, 287–293.
24. Van Milligen, F. J., Craig, S., Rogers, B. L., Van Swieten, P., and Aalberse R. C. (1995), *Clin. Exp. Allergy* **25**, 247–251.
25. Rogers, B. L., Morgenstern, J. P., Garman, R. D., Bond J. F., and Kuo, M. C. (1993), *Mol. Immunol.* **30**, 559–568.
26. Ichikawa, K., Sun, W., Wu, Z., Vailes, L. D., Guyre, P., and Chapman, M. D. (1999), *J. Allergy Clin. Immunol.* **103**, S184.
27. Chua, K. Y., Kehal, P. K., Thomas, W. R., Vaughan, P. R., and Macreadie I. G. (1992), *J. Allergy Clin. Immunol.* **89**, 95–102.
28. Greene, W. K., Chua, K. Y., Stewart, G. A., and Thomas W. R. (1990), *Int. Arch. Allergy Appl. Immunol.* **92**, 30–38.
29. Shoji, H., Shibuya, I., Hirai, M., Horiuchi, H., and Takagi, M. (1997), *Biosci. Biotechnol. Biochem.* **61**, 1668–1673.
30. Jacquet, A., Haumont, M., Massaer, M., Daminet, V., Garcia, L., Mazzu, P., et al. (2000), *Clin. Exp. Allergy* **30**, 677–684.
31. Takahashi, K., Takai, T., Yasuhara, T., Yuuki, T., Ohtake, Y., Yokota, T., and Okumura, Y. (2000), *Int. Arch. Allergy Immunol.* **122**, 108–114.
32. Vailes, L. D., Kinter, M. T., Arruda, L. K., and Chapman, M. D. (1998), *J. Allergy Clin. Immunol.* **101**, 274–280.
33. Best, E. A., Stedman, K. E., Bozic, C. M., Hunter, S. W., Vailes, L. D., Chapman, M. D., et al. *Protein Express Purif* **20**, 462–471.
34. Platts-Mills, T. A., Rakes, G., and Heymann P. W. (2000), *J. Allergy Clin. Immunol.* **105**, S503–S508.
35. Wan, H., Winton, H. L., Soeller, C., Tovey, E. R., Gruenert, D. C., Thompson, P. J., et al. (1999), *J. Clin. Invest.* **104**, 123–33.
36. Wan, H., Winton, H. L., Soeller, C., Gruenert, D. C., Thompson, P. J., Cannell, M. B., et al. (2000), *Clin. Exp. Allergy* **30**, 685–698.
37. Herbert, C. A., King, C. M., Ring, P. C., Holgate, S. T., Stewart, G. A., Thompson, P. J., and Robinson, C. (1995), *Am. J. Respir. Cell. Mol. Biol.* **12**, 369–378.
38. Kalsheker, N. A., Deam, S., Chambers, L., Sreedharan, S., Brocklehurst, K., and Lomas, D.A. (1996), *Biochem. Biophys. Res. Commun.* **221**, 59–61.
39. Schulz, O., Sutton, B. J., Beavil, R. L., Shi, J., Sewell, H. F., Gould, H. J., et al. (1997), *Eur. J. Immunol.* **27**, 584–588.
40. Shakib, F., Schulz, O., and Sewell, H. (1998), *Immunol. Today* **19**, 313–316.
41. Schulz, O., Sewell, H. F., and Shakib F. (1998), *J. Exp. Med.* **187**, 271–275.
42. Hewitt, C. R., Brown, A. P., Hart, B. J., and Pritchard, D. I. (1995), *J. Exp. Med.* **182**, 1537–1544.
43. Finkelman, F. D. and Urban, J. F. (1992), *Parasitol Today* **8**, 311–314.
44. Dudler, T., Machado, D. C., Kolbe, L., Annand, R. R., Rhodes, N., Gelb, M. H., et al. (1995), *J. Immunol.* **155**, 2605–2613.
45. Musu, T., Grégoire, C., David, B., and Dandeu, J. P. (1997), *Clin. Rev. Allergy Immunol.* **15**, 485–498.
46. Pomès, A., Smith, A. M., Grégoire, C., Vailes, L. D., Arruda, L. K., and Chapman, M. D. *Allergy Clin. Immunol Int.* In press.
47. Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., and Rao, P. V. (1993), *J. Immunol.* **151**, 5354–5363.

48. Aki, T., Kodama, T., Fujikawa, A., Miura, K., Shigeta, S., Wada, T., et al. (1995), *J. Allergy Clin. Immunol.* **96**, 74–83.
49. Ortolani, C., Pastorello, E. A., Farioli, L., Ispano, M., Pravettoni, V., Berti, C., et al. (1993), *Ann. Allergy* **71**, 470–476.
50. Pastorello, E. A., Incorvaia, C., Pravettoni, V., Farioli, L., Conti, A., Vigano, G., et al. (1998), *Allergy* **53**, 48–51.
51. Daschner, A., Crespo, J. F. and Pascual, C. Y. (1998), *Allergy* **53**, 614–618.
52. Hoffmann-Sommergruber, K., O’Riordain, G., Ahorn, H., Ebner, C., Laimer Da Camara Machado, M., Puhlinger, H., et al. (1999), *Clin. Exp. Allergy* **29**, 840–847.
53. Vanek-Krebitz, M., Hoffmann-Sommergruber, K., Laimer da Camara Machado, M., Susani, M., Ebner, C., Kraft, D., et al. (1995), *Biochem. Biophys. Res. Commun.* **214**, 538–551.
54. Fritsch, R., Bohle, B., Vollmann, U., Wiedermann, U., Jahn-Schmid, B., Krebitz, M., et al. (1998), *J. Allergy Clin. Immunol.* **102**, 679–686.
55. van Ree, R. (1997), *Allergy* **52**, 795–805.
56. Mueller, G. A., Smith, A. M., Williams, D. C., Jr., Hakkaart, G. A., Aalberse, R. C., Chapman, M. D., et al. (1997), *J. Biol. Chem.* **272**, 26,893–26,898.
57. Hoffmann, A., Jamin, A., Foetisch, K., May, S., Aulepp, H., Hausteiner, D., and Vieths, S. (1999), *Allergy* **54**, 446–454.
58. Krebitz, M., Wiedermann, U., Essl, D., Steinkellner, H., Wagner, B., Turpen, T. H., et al. (2000), *FASEB J.* **14**, 1279–1288.
59. Pauli, G., Oster, J. P., Deviller, P., Heiss, S., Bessot, J. C., Susani, M., et al. (1996), *J. Allergy Clin. Immunol.* **97**, 1100–1109.
60. Niederberger, V., Pauli, G., Gronlund, H., Froschl, R., Rumpold, H., Kraft, D., et al. (1998), *Immunol.* **102**, 579–591.
61. Pauli, G., Purohit, A., Oster, J., De Blay, F., Vrtala, S., Niederberger, V., et al. (2000), *Clin. Exp. Allergy* **30**, 1076–1084.
62. Swoboda, I., Scheiner, O., Kraft, D., Breitenbach, M., Heberle-Bors, E. and Vicente, O. (1994), *Biochim Biophys Acta* **1219**, 457–464.
63. Lowenstein, H., Sparholt, S. H., Klysner, S. S., Ipsen, H., and Larsen, J. N. (1995), *Int. Arch. Allergy Immunol.* **107**, 285–289.
64. Ferreira F., Hirthenlehner K., Briza P., Breiteneder H., Scheiner O., Kraft D., et al. (1997), *Int. Arch. Allergy Immunol.* **113**, 125–127.
65. Ebner, C., Schenk, S., Najafian, N., Siemann, U., Steiner, R., Fischer G. W., et al. (1995), *J. Immunol.* **154**, 1932–1940.
66. Smith, A. M. and Chapman, M. D. (1996), *Adv. Exp. Med. Biol.* **409**, 391–394.
67. Norman, P. S., Ohman, J. L., Jr., Long, A. A., Creticos, P. S., Geffer, M. A., Shaked, Z., et al. (1996), *Am. J. Respir. Crit. Care Med.* **154**, 1623–1628.
68. Haselden, B. M., Kay, A. B., and Larche, M. (1999), *J. Exp. Med.* **189**, 1885–1894.
69. Zeiler, T., Mantyjarvi, R., Rautiainen, J., Rytönen-Nissinen, M., Vilja, P., Taivainen, A., et al. (1999), *J. Immunol.* **162**, 1415–1422.