Experimental & Applied Acarology, 10 (1991) 167–186
Elsevier Science Publishers B.V., Amsterdam

House-dust-mite allergens: A review

Larry G. Arlian
Department of Biological Sciences, Wright State University, Dayton, OH 45435, U.S.A.

(Accepted 26 September 1990)

ABSTRACT

Arlian, L.G., 1991. House-dust-mite allergens: A review. *Exp. Appl. Acarol.*, 10: 167–186.

The house-dust mites, *Dermatophagoides farinae, D. pheronyssinus* and *Euroglyphus maynei* are cosmopolitan inhabitants of the homes of humans worldwide. These mites are the sources of multiple potent allergens that trigger allergic reactions in house-dust-mite-sensitive individuals. Many laboratories using widely varied mite materials and allergic sera, and biochemical and immunological assays, have isolated and characterized, to varying degrees, some of the allergens produced by these mites. The resulting large body of literature is difficult to interpret and relate. This review briefly summarizes the progress made in isolating and characterizing mite-derived antigens and allergens, the relationship between antigens isolated in different laboratories, and the patients' reactivity to these allergens. A brief summary of the allergic reaction and the role of IgE are provided as background.

INTRODUCTION

It has long been recognized that house dust triggered allergic reactions such as asthma and rhinitis (Kern, 1921; Cooke, 1922; Spivacke and Grove, 1925; Tuft, 1949). House dust is a complex mixture containing many substances, primary among which are synthetic and natural fibers, food detritus, fungi, algae, pollens, animal dander and saliva, human dander, and insects and mites, along with their secretions and degradation products. Many of these are potentially allergenic. As early as 1928 mites were reported to be the source of house-dust allergens, but this study received little notice (Dekker, 1928). More recently, Voorhorst et al. (1964, 1967, 1969) and others (Spieksma, 1967a,b; Spieksma and Spieksma-Boezeman, 1967) reported that house dust contained mites of the genus *Dermatophagoides* and suggested that these were the major source of allergens in house dust. Many subsequent investigations have confirmed these findings. Mites are now viewed by many as the most important source of allergens in dust.

Mite-derived allergy is particularly important because it is perennial, rather than seasonal like many other aeroallergens. Three mite species of the family Pyroglyphidae are primarily involved (Arlian, 1989): *Dermatophagoides farinae* (DF); *D. pteronyssinus* (DP); and *Euroglyphus maynei* (EM). These species, in particular *Dermatophagoides* spp., are cosmopolitan inhabitants of human dwellings in many parts of the world. Many other species of mites

0168-8162/91/$03.50

may occur in small numbers in homes, but generally these play a lesser role in house-dust allergy — primarily *D. microceras, Blomia tropicalis*, and a few species of storage mites.

Understanding the biology of dust mites, the immune responses they trigger, and isolation and characterization of mite allergens has been of great interest since it became known that these organisms were a major source of allergens in house dust. Knowledge of the biology of the mite, physicochemical properties of the allergens, and the immune response in dust-sensitive individuals is necessary to understanding the mechanisms of allergic responses and to proper diagnosing and treating dust-mite allergy. During the last 15 years, great strides have been made in understanding the physicochemical nature and allergenicity of mite antigens and the immune response. Likewise, much has been learned about the biology, ecology, and prevalence of the mites as a result of research by acarologists. Because of its dependence on mite-allergic patients, most of the immunological research has been done by medical researchers, with little involvement by acarologists. Therefore, the research has been published in medical journals and geared toward clinical researchers and the practicing allergist. On the other hand, much of the basic research on mite biology and ecology has been done by acarologists and published in acarological/entomological journals. Unfortunately, there has been limited interaction between the two groups, largely because of the widely different focus of the respective disciplines. However, the physicochemical and immunological tools and techniques used by medical researchers and some entomological specialities are similar. Thus, there is an opportunity for greater future interaction between individuals of the acarological and medical disciplines in understanding and managing house-dust-mite allergy.

Several excellent detailed reviews on the biology and ecology of these mites are available to both the entomological and the clinical audiences (Van Bronswijk and Sinha, 1971; Van Bronswijk et al., 1971; Wharton, 1976; Arlian, 1989). However, there are fewer reviews available on the immunology of house-dust-mite allergy, particularly for the entomologist/acarologist (Mosbeck, 1985; Platts-Mills and Chapman, 1987). Therefore, the purpose of this paper is to review the progress made in isolating and characterizing the mite allergens and the patient's reactivity to these allergens. A brief summary of the allergic reactions, the role of IgE in allergic reactions, and the immunoassays used for diagnostic and research purposes is also provided as background information.

ALLERGIC REACTION TO MITES AND THE ROLE OF IgE

It is assumed that the humoral immune response to mite antigens is similar to that of other antigens (Platts-Mills and Chapman, 1987). Mite antigen enters the body, where it binds to an immunoglobulin surface receptor on B-

lymphocytes that fits its antigenic determinant. Each lymphocyte has many copies of a single receptor that is specific for an antigenic determinant, or epitope, on the mite antigen. However, a mite antigen may have many different epitopes (Chapman et al., 1984, 1987a; Heymann et al., 1986; Platts-Mills et al., 1986). Therefore, because of its different antigenic determinants, the same antigen may bind to different B-lymphocytes with receptors for one of the antigen's epitopes. Binding of antigen to the lymphocyte ultimately leads to clonial selection, expansion, and production of memory cells and antibody-secreting plasma cells.

Some antigens bind to and activate B-lymphocytes directly without the aid of T-cells (T-independent antigen). Other antigens require T-cell help to activate the B-lymphocyte (T-dependent antigen). Mite allergens are thought to be T-cell-dependent and involve cooperation between helper T-cells and B-lymphocytes (Platts-Mills and Chapman, 1987).

After a T-dependent mite antigen binds to surface receptors on B-cells, it is taken up and degraded (Cohen, 1988). Fragments of the antigen reappear on the B-cell surface. These antigen fragments, in association with class II MHC (major histocompatibility complex) on the B-cell surface, bind T-cells (T-helper cells) that have receptors for this combination of antigen fragment and class II MHC molecule. This binding activates the T-cell. Activated T-cells secrete a variety of growth and differentiation factors that stimulate B-cell growth, division, and secretion of large quantities of antibodies (IgM, IgE, IgG, etc.) by the daughter B-cells (plasma cells).

The immunoglobulin (antibody) IgE is the mediator of allergic reactions (Type-I hypersensitivity) as described below (Cohen, 1988). IgE is present in very small amounts in the serum of normal individuals (0.004% of total serum immunoglobulin, or 0–0.002 mg/ml; Li, 1988). In individuals predisposed to allergy (genetically), plasma cells produce IgE in response to their first introduction to an allergen from such biologicals as mites or pollens. This IgE binds by means of the Fc region to the surface of mast cells (most concentrated in the respiratory and gastrointestinal tracts and skin) and basophils (in the blood) (Cohen, 1988). Upon subsequent encounters with allergenic molecules, the allergens bind with IgE that is attached to mast cells. This interaction of allergen and IgE triggers release of chemical mediators such as histamines, prostaglandins and leukotrines from the mast cell. These mediators cause itching, vasodilation, constriction of smooth muscle, and changes in membrane permeability and leakiness of blood vessels that contribute to the allergic reaction. The clinical signs include: runny and itchy nose; sneezing; tearing, red, itchy eyes; hives; eczema; asthma; abdominal cramps, and diarrhea. Because of the central role of IgE antibodies in the allergic reactions, most diagnostic and research immune assays directly or indirectly measure bound or serum levels of IgE.

Skin tests measure an allergen's interaction with IgE on mast cells in the

skin. RAST (radioallergosorbent test), ELISA (enzyme linked immunosorbent assay), and CRIE (crossed radioimmunoelectrophoresis) measure circulating IgE levels in human serum via its binding to allergens bound to a solid substrate (RAST, ELISA) or an antigen/antibody precipitin complex (CRIE). Leukocyte histamine release (LHR) assays indirectly measure IgE on human leukocytes as a function of histamine released when the cells are challenged with an allergen. Immunoblotting measures serum IgE binding to protein/peptide allergens following fractionation by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and IEF (isoelectric focusing) and transfer to nitrocellulose membranes. Single and double immunodiffusion (Ouchterlony), rocket immunoelectrophoresis and CIE (crossed immunoelectrophoresis) measure the ability of rabbit IgG to recognize a molecule as an antigen when it is reacted with the rabbit antiserum produced to the allergen. Details for these procedures are not given here, but they can be found in many immunological publications including reviews by Lowenstein (1978) and Platts-Mills et al. (1981).

NOMENCLATURE SYSTEM FOR NAMING ALLERGENS

Several labs were concurrently isolating and characterizing allergens in mite extracts using many physicochemical separation techniques and immunoassays. A variety of nomenclatures resulted to identify mite allergens that corresponded to the technique (such as CRIE, SDS-PAGE and immunoblotting, and gel filtration) that was used to isolate or demonstrate the allergen. Similar things were happening with naming specific allergens isolated from other

TABLE 1

Purified allergens from house-dust mites

Source	Designation		Molecular weight[b]	pI
	IUIS[a]	Previous		
D. pteronyssinus				
	Der p I	P_1, Ag 42 (Dp 42), Dpt 12	24	4.6–7.4
	Der p II	Ag x (Dp X)	15	5.0–6.4
D. farinae				
	Der f I	Ag 11 (A 60), Ag 6 (Df 6), F_1 (P_{25}), Me 1?	24	5.7–6.95
	Der f II	Ag 19/20, DF2	15	5.5–8.2
	Der f III	None	29	4.1–4.7
D. microceras				
	Der m I	None		

[a]International Union of Immunological Societies.
[b]KiloDaltons.

materials that were also complex allergenic mixtures (e.g., pollens, animal danders, foods, fungi, and insects). To standardize this situation, the International Union of Immunological Societies (IUIS) recommended a new unified nomenclature system (Marsh et al., 1986, 1988a,b). In brief, under this system the designation for a highly purified allergen is "the first three letters of the genus (italicized); space; first letter of the species name (italicized); space; and a Roman numeral to designate the chronologic order in which the allergen was isolated" if the source material contains multiple allergens. Therefore, following this system, allergen P_1 (the first allergen isolated from *D. pteronyssinus*) officially became *Der p* I. The proper nomenclature for the allergens isolated and characterized from house-dust mites is listed in Table 1. In the following sections, details are given for the isolation and characterization of these named allergens and also those not yet sufficiently characterized to be named.

MITES AS THE SOURCE OF MULTIPLE ANTIGENS AND ALLERGENS

Many studies show that DF and DP are the sources of a large number of distinct and important antigens. Many of these antigens are allergens – that is, they trigger an IgE-mediated immune response (Stewart and Turner, 1980a; Stewart, 1982; Krilis et al., 1984a,b; Arlian et al., 1984a,c, 1987a,b,c; Lind, 1985, 1986b; LeMao et al., 1985; Tovey and Baldo, 1985, 1987; De la Hoz and Carreira, 1986). Therefore, allergenicity is not confined to a single major allergen as was initially thought, although a high percentage of dust-mite-sensitive individuals may react to a few specific allergens (Platts-Mills and Chapman, 1987). Allergens that trigger strong IgE responses in a large percentage of a mite-allergic population have been referred to as 'major allergens' in some literature. However, the reference to a major allergen in this context is a misnomer and, in our opinion, this terminology should not be used. An allergen that triggers a strong allergic response in only a small percentage of individuals from a mite-allergic population is an important (major) allergen to these individuals.

The exact numbers of allergens that originate from DF, DP, and other mites still remain to be determined. The numbers currently reported are highly variable (Stewart and Turner, 1980a,b; Lind and Lowenstein, 1983; Arlian et al., 1984a,c, 1987a,b; Lind et al., 1984; Dale and Landmark, 1984; Lind, 1985; Wahn et al., 1985). Only a few of these have been isolated and adequately characterized to be named in accordance with the new nomenclature recommended by the International Union of Immunological Societies (IUIS) (Marsh et al., 1986, 1988a,b).

Variations in the number of reported allergens and their importance have resulted from the widely varied methodologies and mite materials used in various research laboratories. Therefore, in selecting techniques for physicochemical and immunologic studies or for evaluating data the quality of the

mite extracts and the limits of the techniques must be considered. Some of these variations and considerations are discussed below.

Mite extracts have been prepared from thriving cultures, whole spent cultures, mite bodies (mites collected as they crawled from cultures or separated from culture media by other means), and fecal material. The concentrations of mite components and mite antigens vary greatly in different source materials. Extracts may contain culture material that could be antigenic and/or diluted protein of mite origin. The relative proportions of culture material, mite life stages, exoskeletons, secretions, feces, etc., may vary and may affect potency or completeness of the extract. Also, aqueous collection processes may wash away some soluble antigens and allergens. Studies have shown that the composition of the extract is important (Siraganian et al., 1979; Tovey and Vandenberg, 1979; Lind, 1980; Tovey et al., 1981; Arlian et al., 1984a,b,c, 1987a,b,c; Ford et al., 1985a,b). However, few studies have fully characterized the source material used.

In addition, the various physicochemical separation techniques used have widely different resolving power (Lowenstein, 1978; Platts-Mills et al., 1981). Generally, Sephadex and Sephacryl gel filtration, ion exchange DEAE-cellulose chromatography, and saturated ammonium sulfate (SAS) or acetone precipitation give only crude separations of the multiple proteins in mite extracts. SDS-PAGE fractionation generally dissociates protein molecules into peptides, so that individual antigen identity may not be seen even after preliminary purification steps. Variations in IgE affinity for antigenic epitopes on allergens, number of epitopes or their availability for IgE binding in an assay (CRIE, immunoblot etc.), allergen quantity, and radioactivity influence the results of radioimmunoassays using ^{125}I-labelled antihuman-IgE (Platts-Mills et al., 1981). Some immune assays provide visualization of specific antigens (e.g., CIE, immunodiffusion) and allergens (CRIE), while another may give total IgE binding to a protein or complex of proteins (RAST).

RESOLUTION AND ORIGIN OF ANTIGENS AND ALLERGENS OF DF AND DP EXTRACT

Early studies, primarily using gel filtration and ion exchange chromatography, called attention to the fact that DF and DP were likely to be the sources of multiple allergens spread over a molecular-weight range between 12.7 and 67 kiloDaltons (Ishii et al., 1973, 1982; Woodiel et al., 1974; Biliotti et al., 1975; Ishii and Miyamoto, 1975; Romagnani et al., 1976; Nakagawa et al., 1977; Kabasawa and Ishii, 1979). Subsequent studies using more-refined biochemical and immunological methods have verified these results and further characterized the antigens and allergens from these mites.

CIE and CRIE studies have been very useful in studying allergenic extracts. The CIE technique is particularly attractive because it allows the visualiza-

tion of individual antigens in mite extracts containing mixtures of antigens. CRIE is especially suited for determining which antigens in a complex mixture are allergens. That is, it is possible to see which antigens bind IgE in sera from allergic individuals, and to determine the antigenic specificity of allergic patients' IgE response.

The CIE system has shown that DF and DP mites are the sources of large antigenic pools. The exact numbers reported vary between labs because the CIE technique is dependent upon the qualities of the extract used and the rabbit anti-mite sera produced. However, 15–49 antigens have been visualized in various extracts produced from both DF and DP (body and fecal material) with many of these binding IgE in mite-sensitive patients' sera (Lind et al., 1979, 1984; Stewart and Turner, 1980a; Lind, 1980, 1985, 1986a; Lind and Lowenstein, 1983; Arlian et al., 1984a, 1986, 1987a,c; Dale and Landmark, 1984; Wahn et al., 1985; Holck et al., 1986).

CIE reactions of extracts, made from well-characterized culture-medium-free DF bodies with rabbit antisera produced to the extract, revealed the presence of 35+ antigens (Arlian et al., 1984a, 1987a,b). Thirty and five antigens gave anodic and cathodic migration, respectively. Fourteen of these antigens bound IgE in sera of six reference patients that were skin-test positive to DF. Thus, they were allergens. Binding of mite-specific IgE to the various allergens in DF body extract varied significantly both between and within patients. For example, individual sera recognized from four to nine allergens to varying degrees. However, nine of the 14 allergens exhibited strong IgE binding in one or more of the reference patients. Because all nine allergens exhibited strong IgE binding by at least one patient, they were considered to be major allergens. However, some patients were strong reactors to more allergens than other patients. These results indicate that the potency, and thus importance, of specific allergens varies between patients. Similar results have been obtained for CIE and CRIE analyses of highly purified DF feces, DP body, and DP feces extracts reacted with the corresponding rabbit anti-sera and sera from mite-sensitive patients (Arlian et al., 1984a, 1987a,b,c).

Heterologous and prospective intermediate gel CIE studies clearly showed antigenic and allergenic differences between extracts prepared from culture-medium-free mite bodies and purified mite feces (Arlian et al., 1987a,b,c). Generally, these studies indicated that: (1) body and fecal extracts contain some common antigens; (2) most antigens in feces extracts are also present in body extracts; (3) some antigens common to both body and feces extracts were more concentrated in the feces extract; and (4) extracts prepared from mite bodies contain antigens and allergens not associated with mite feces. It is not known if common antigens in body and fecal extracts are due to fecal material and associated products (enzymes, bacteria, etc.) remaining in the mites' bodies when the extract was prepared, or if it was due to other common molecules in the body. Evidence that supports the conclusion that some anti-

gens are specifically of body origin include: (1) CIE demonstrates that mite body extracts contain more antigens than mite feces extracts; (2) heterologous CIE antigen profiles of feces extract reacted with anti-body sera were similar to homologous reactions of feces extract and anti-feces sera; (3) CIE reactions of body extract into anti-feces sera gave many fewer antigens than reactions of mite body extract and antibody sera; and (4) mite-sensitive patients may exhibit IgE binding to antigens in body extracts but not to antigens in feces extracts.

ISOLATION AND CHARACTERIZATION OF SPECIFIC ALLERGENS

Dermatophagoides pteronyssinus

Platts-Mills and colleagues were the first to isolate and characterize a major mite allergen (Chapman and Platts-Mills, 1978). They first fractionated DP extract on a Sephadex G-100 column. The eluted fractions yielded two protein peaks. A large peak was eluted over the 5–65 000-Dalton molecular-weight range, and a small peak was eluted with the void volume. The skin-test reactivity of six different fractions spanning the entire elution profile was determined. Maximal skin sensitivity was shown with fractions 3 and 4 which eluted as part of the large peak. These fractions were 10–100 times more potent than the other fractions. However, RAST testing using sera from allergic persons showed maximal binding toward fraction 4. Two protein peaks resulted when fraction 4 from the Sephadex G-100 column was further separated by Pevikon block electrophoresis. The two proteins were designated F_4P_1 and F_4P_2, respectively. By immunodiffusion, F_4P_2 gave no precipitate (reactivity) when reacted with rabbit antiserum built to DP and was assumed to contain culture medium. F_4P_1 had a molecular weight of 15–25 kD and was strongly reactive on immunodiffusion and Laurell electrophoresis and was skin-test positive. There was excellent correlation between the F_4P_1 fraction and crude DP extract when compared using skin testing, but the F_4P_1 fraction gave a five-fold increase in skin reactivity.

The F_4P_1 fraction, when further analyzed by IEF, showed multiple bands (Chapman and Platts-Mills, 1980). Antigenic activity was primarily limited to two peaks with pIs of 4.7–5.4 and 6.6–7.1. The protein in the two peaks had similar amino-acid compositions and molecular weights (24 000), and were completely cross-reactive by RAST inhibition and immunodiffusion assays. These data suggested the antigen existed in multiple isoelectric forms. The common antigen in the two IEF fractions of F_4P_1 from DP was designated P_1. When the unified nomenclature system was adopted, allergin P_1 became *Der p* I (Table 1).

This group later used an alternate scheme for purification of P_1 (Chapman et al., 1982). In this case, the extract was first fractionated with 50% saturated

ammonium sulfate (SAS). The precipitate resulting from the 50% SAS cut contained most of the P_1. This was then redissolved, fractionated by gel filtration (Sephadex G-100) and then Pevikon block electrophoresis.

Stewart and Turner (1980a) similarly fractionated DP (whole mites) on a Sephacryl S-300 column. The eluent was pooled into four fractions, designated 1–4 with descending molecular weights. Fractions 3 and 4 showed the greatest allergenicity as determined by RAST. Fractions 1 and 2 were one-tenth as potent but exhibited some activity. CIE showed this same DP extract contained 15 antigens (arbitrarily numbered 1–15). Four antigens, 4, 7, 12, and 13, bound IgE on CRIE reaction with a serum pool from patients allergic to DP. These allergens (antigens) were designated Dpt 4, Dpt 7, Dpt 12, and Dpt 13, respectively. Most of antigen 4 was in fraction 2, but small amounts were also in fraction 1 and 3. Antigen Dpt 7 was in fraction 3, antigen Dpt 13 was in fraction 2 and antigen Dpt 12 was predominantly in fraction 3 but also in fraction 4. Antigens Dpt 4 and Dpt 12 were the most potent allergens of both the whole-mite extract and the fractions.

Antigen Dpt 12 of fraction 4, which had a molecular weight of 24 kD and a pI of 6.6, was also obtained by 50% SAS precipitation of the spent growth medium (SMM) or whole-mite culture (HDM). The precipitated material was redissolved then applied to a Sephacryl S-300 column and eluted. The fraction from the S-300 column that contained Dpt 12 was further fractionated on Sephadex G-50. The eluted fraction containing Dpt 12 was analyzed by chromatofocusing and radioimmunoassay. With these techniques, the pI of the allergen (Dpt 12) was 5.86–6.95. Antigen Dpt 12 isolated from both SMM and HDM reacted strongly with anti-P_1 serum, indicating that Dpt 12 and P_1 (*Der p* I) characterized by Chapman and Platts-Mills (1978, 1980) were the same (Table 1).

A striking aspect of the Stewart and Turner (1980a) results was the large molecular weight of the allergens. They suggested that these molecules may have contained large amounts of carbohydrate and thus did not give true molecular-weight values with the methods used. Antigen Dpt 4 had an apparent molecular weight of 274 kD and a pI of 4.5. Antigen Dpt 4 was antigenically identical to material with a molecular weight of 531 kD, and was thought to be a subunit of this material. SDS-PAGE showed that there were six other components of fraction 2 in addition to Dpt 4, and these ranged in molecular weight from 104 to 537 kD. The weight of Dpt 13 was 510 kD.

Lind et al. (1984) characterized extracts prepared from whole-mite cultures (WMC) of DP by CIE, CRIE, and rocket immunoelectrophoresis. Forty-nine antigens were visualized by CIE, with nine originating from the culture medium. CRIE autoradiograms identified two additional precipitates. Nine of the mite antigens were allergens and bound IgE using sera from 30 reference patients. Antigens labelled 42 (Dp 42 or Ag 42), X (Dp X or Ag X), Y (Dp Y or Ag Y), and 23 (Dp 23 or Ag 23) showed the most frequent and

intense IgE binding among the reference sera. Those labelled X and Y were designated because they were not visible in CIE.

Heterologous CIE reactions of antigen P_1 (*Der p* I) with the same polyclonal rabbit antisera (anti-WMC) resulted in one major precipitate (also a few weak precipitates) that corresponded to the former Ag 42. This precipitate demonstrated intense radiostaining. Tandem CIE reaction of WMC extract and P_1 (*Der p* I) gave complete identity using the polyclonal rabbit antisera built to WMC. Ag 42 (Dp 42) was further purified and its molecular weight and isoelectric point determined by gel filtration (G-75), SDS-PAGE, IEF, and immunoelectrophoresis (Lind, 1985). The values for Dp 42 corresponded well with P_1 (*Der p* I). Thus, *Der p* I (antigen P_1 in older literature) and Ag 42 designate the same antigen (Table 1).

Similarly, Dp X has a molecular weight of 18–20 kD and multiple pIs in the range of 5–7 (Lind, 1985). Its equivalent has not been purified by other investigators. However, Dp X identified by CRIE has been designated *Der p* II (Table 1).

Dermatophagoides farinae

LeMao et al. (1981) isolated DF antigens from a whole DF culture extract (DF+culture medium). This group obtained a precipitate with a 50–80% (*v/v*) acetone cut of the whole-culture extract, which was designated Df 80. The Df 80 precipitate was isolated, redissolved, dialyzed against neutral distilled water and then lyophilized. This lyophilized fraction (precipitate) was designated as Df 80d. The elution profile of redissolved Df 80d on Sephadex G-100 showed two main peaks, designated F_I and F_{II}, of approximately 25 kD and 8 kD, respectively. The Df 80 d, F_I and F_{II} were 62, 80 and 6% protein, respectively. The protein-rich 25-kD fraction (F_I) was called P_{25}, and the carbohydrate-rich 8 kD fraction (F_{II}) was called GP_8 (glycoprotein). CIE of Df 80d using rabbit anti-Df 80d revealed eleven antigens. The antigen numbered 11 (Ag 11) bound IgE on CRIE analysis. CRIE using Df 80d antisera showed Ag 11 present in P_{25} extract but not in GP_8 extract. The allergenic activity (determined by RAST inhibition) was only slightly higher for P_{25} than for Df 80d, suggesting that the two contained similar major allergens. However, GP_8 (direct RAST) reacted with most of the test patients' sera, suggesting it also contained at least one major allergen.

Dandeu et al. (1982) subsequently also obtained Ag 11 in a 60% SAS precipitate of the partially purified *D. farinae* extract, Df 80; this fraction was referred to as A 60. SDS-PAGE and Sephadex G-100 indicated the molecular weight of A 60 was approximately 28 kD. It gave only one band on SDS-PAGE, which indicated it contained only one peptide chain. It also contained 5% reducing sugar and 3.7 hexosamines. Its amino-acid composition was determined, and cystine, glycine and alanine residues were numerous. The cys-

tine residues suggested the peptide chain was extensively cross-linked and folded. The majority of their test patients' sera reacted with it. Ag 11 (A 60, also F_1 or P_{25}) is now officially known as *Der f* I according to the new nomenclature system (Table 1).

Dale and Landmark (1984), by means of CRIE, demonstrated eleven allergens in an extract prepared with isolated and crushed *D. farinae* mites. Based on radiostaining, they were characterized as three major, three intermediate and five minor allergens. Antigen-antibody precipitates numbered 19/20 and 22 (based on electrophoretic mobility) bound IgE in a high percentage of reference sera. Allergen 19/20 was further purified and characterized by Holck et al. (1986). The charge of antigen 19/20 was heterogenic, with pIs in the range of 3.5–7.0. A component of 19/20 was isolated and designated 19/20 IIa. Amino-acid analysis suggested antigen 19/20 IIa was a glycoprotein containing one residue of galactosamine. Antigen 19/20 IIa appears to be equivalent to *Der f* II that was later purified by Heymann et al. (1989), using monoclonal-antibody-affinity chromatography (Table 1). Allergen *Der f* II is a 15-kD protein.

Haida et al. (1985), using radioimmunoelectrophoresis, identified four different proteins in crude DF extract that reacted with human anti-mite IgE. From the slowest to the fastest electrophoretic mobilities, these were designated Me 1, Me 2, Me 3, and Me 4. The Me 1 protein was the most prevalent allergen, and was recognized by antibodies (formed radioprecipitin bands) in 75.2% of mite-sensitive patients' sera. Protein Me 2 reacted with 56.4% of the reference sera. Protein Me 1 gave a single peak and band on HPLC and SDS-PAGE, respectively, which showed to to be 17 kD molecular weight; the pI was 8.0.

In a subsequent analysis of crude mite DF extract, an SAS (60%) precipitate was subjected to Sephacryl S-200 gel filtration (Yamashita et al., 1989). The fraction containing Me 2 gave a clear band on SDS-PAGE that corresponded to 27 kD. The molecular weights of Me 3 and Me 4 were estimated to be 45 kD and 80 kD, respectively, by SDS-PAGE and immunoblotting. On the basis of similarity in molecular weights, this group suggests that Me 2 and Me 1 are similar to DF1 and DF2, respectively, described by Yasueda et al. (1986). Protein Me 1 appears to be the same as Ag 6 isolated by Dandeu et al. (1982).

Yasueda et al. (1986) separated DF extract (made from whole-mite culture) into two fractions with 0–60 and 60–95% SAS cuts. These were designated DF1 and DF2, respectively. The DF1 fraction had a pI of 4.6–7.2, was 25 kD, and was a heat-sensitive acidic protein. Fraction DF2 had a molecular weight of 15 kD and a pI of 7.8–8.3 and was a heat-stable basic protein. Both DF1 and DF2 showed allergenic activity by RAST and LHR. Little or no allergenic cross-reactivity was found between DF1 and DF2, which may be equivalent to antigens *Der f* I and *Der f* II, respectively (Table 1).

Heymann et al. (1989), using monoclonal-antibody-affinity chromatography, purified a third allergen, *Der f* III, from DF extract (Table 1). This allergen was isolated from a 50%–80% SAS fraction of DF spent-growth medium (Heymann et al., 1989). Redissolved precipitate from the SAS cut was fractionated on a Sephacryl S-200 column. The eluted material was collected in 5-ml samples and those rich in protein were pooled into four fractions. Fraction 2 was rechromatographed with Sephacryl S-200 to obtain purified *Der f* III, a 29-kD antigen with a pI of 4.1–4.7 (Table 1).

Dermatophagoides microceras (Dm)

Lind (1986a) initially described the purification of an allergen from *D. microceras* (Dm). Acetone-precipitated fractions of the extract prepared from whole-mite culture (Dm-WMC) were dialyzed and freeze-dried. Reconstituted Dm-WMC was fractionated by Sephadex G-75 gel. Fractions 123–140 contained an allergen designated as Dm 6 that was approximately 185 kD by gel filtration and 29 kD by SDS-PAGE with a pI of 4.9 (sucrose gradient) or 6.5 (isoelectric focusing). The molecular weight and pI for Dm 6 are similar to that of Df 6 (*Der f* I) and Dp 42 (*Der p* I), which suggest a common molecular structure.

Group I and II Allergens

Based on amino-acid composition and N-terminal amino-acid sequence, *Der f* I and *Der p* I are structurally homologous (Heymann et al., 1986) and have been designated group-I allergens. The two allergens exhibit extensive cross-reactivity (Heymann et al., 1986) as do group II allergens (Heymann et al., 1989). *Der f* II and *Der p* II also demonstrate extensive structural and antigenic homology and are designated as group II allergens (Heymann et al., 1989). Allergen *Der f* II has a similar amino-acid composition to *Der p* II. The N-terminal amino-acid sequence of *Der f* II and *Der p* II differed in only four of the first 35 residues. Allergens *Der p* I, *Der f* I and *Der m* I exhibit immunochemical differences despite similar physicochemical properties (Lind, 1986a). Group-I allergens are more concentrated in extract rich in mite feces, while group-II allergens are more concentrated in whole-mite bodies than in fecal material (Ford et al., 1985a; Heymann et al., 1989). A high percentage ($\approx$80%) or mite-sensitive patients have IgE directed at group-I allergens (Platts-Mills and Chapman, 1987). Allergen *Der f* III is structurally unlike *Der f* I and *Der f* II (Heyman et al., 1989).

Monoclonal antibodies have been produced against *Der f* I, *Der f* II, *Der f* III, *Der p* I and *Der p* II (Chapman et al., 1987b; Van der Zee et al., 1988; Heymann et al., 1989). These may be used to quantitate antigen levels in dust or extracts.

CROSS-REACTIVITY BETWEEN DF, DP AND EM

Many dust-mite-sensitive patients are skin-test-positive to both DF and DP (Biliotti et al., 1972; Sarsfield, 1974; Van Hage-Hamsten and Johansson, 1989). A positive skin test to both species could indicate that the patient was sensitized to allergens of both mite species, or it could indicate sensitization to one species but reaction to both due to cross-reacting allergens between mite species. It is now clear that DF and DP demonstrate extensive cross-reactivity, but each is also the source of species-specific antigens and allergens (Chapman et al., 1984, 1985, 1987a,b; Lind, 1986a,b, Platts-Mills et al., 1986; Arlian et al., 1987b; Heymann et al., 1989). Heterologous CIE reactions of DF and DP indicate the two species are the sources of at least 21 cross-reacting antigens (Arlian et al., 1987b). Nine of the cross-reacting antigens bound IgE in dust-mite-allergic sera. Extracts prepared from the fecal material of each species also contained 13 cross-reacting antigens, with eight of these being allergens. In spite of the high level of cross-reactivity, CRIE studies have shown that a patient skin-test-positive to body extracts of both species may exhibit circulating IgE to feces antigens directed at one species but not the other (Arlian et al., 1987b).

When the allergen *Der p* I was compared with *Der f* I, the two antigens were found to have both species-specific and common antigenic determinants (Chapman et al., 1987a,b). Monoclonal-antibody studies have mapped six antigenic sites (determinants) on *Der p* I and *Der f* I (Chapman et al., 1987a). These consist of four non-repeated, non-overlapping epitopes on *Der p* I, a single species-specific epitope on *Der f* I, and a cross-reacting epitope on *Der p* I and *Der f* I. Interestingly, this group found that BALB/c mice almost exclusively recognize species-specific epitopes on *Der p* I and *Der f* I. Conversely, humans show 80–95% of IgE directed against cross-reacting sites on *Der p* I and *Der f* I (Heymann et al., 1986). However, the differences between human and murine antibody responses may be attributed to genetic differences or to different modes of immunization (Chapman et al., 1987a).

Allergens *Der p* II and *Der f* II demonstrate complete cross-reactivity by immunodiffusion (using polyclonal antisera from A/J mice) and give similar end points on human intradermal skin tests (Heymann et al., 1989).

CROSS-REACTIVITY BETWEEN HOUSE DUST MITES, STORAGE MITES, AND PARASITIC MITES

Many species of nonpyroglyphid 'stored-product' or 'storage' mites are commonly found in livestock feed, barns, hay, straw, granaries, and commercial grain storage/transfer facilities (Krantz, 1961; Sinha, 1963, 1964, 1968; Brady, 1970; Griffiths et al., 1976; Hughes, 1976; Jeffrey, 1976; Hallas, 1981; Terho et al., 1982). Some of these mites are the sources of important allergens

for farmers or for workers in grain storage/transfer facilities (Cuthbert et al., 1979; Woodcock and Cunnington, 1980; Arlian et al., 1984a,b,c; Terho et al., 1985; Van Hage-Hamsten et al., 1985, 1987, 1988; Van Hage-Hamsten and Johansson, 1989). The more common genera involved are *Tyrophagus, Glycophagus, Acarus, Lepidoglyphus, Chortoglyphus* and *Gohieria.* Several species regularly occur in small numbers in homes along with the dominant mites DP, DF, and EM (Maunsell et al., 1968; Mumcuoglu, 1977; Wraith et al., 1979; Arlian et al., 1984b,c). Because of the dual occupational and domestic exposure in some populations, the allergenicity of storage mites and their cross-allergenic relationship to house-dust mites is of interest.

There appears to be some but limited cross-reactivity between *Dermatophagoides* spp. or *E. maynei* and other mites found in stored products/grain/hay. CIE studies demonstrate that an extract produced from *T. putrescentiae* bodies was the source of 20 antigens, at least five of which were allergens (Arlian et al., 1984b,c). When TP body or TP feces extract was reacted by CIE with anti-DF sera, two cross-reacting antigens were evident. Both cross-reacting antigens were allergens.

RAST inhibition studies suggest DP and EM share some allergenic components but generally possess unique allergens (Van Hage-Hamsten and Johansson, 1989). This group also found little cross-reactivity between *Lepidoglyphus destructor* (LD) and DP or between LD and EM. However, a previous study showed cross-reactivity between LD and DP (Van Hage-Hamsten et al., 1987). RIA showed little or no cross-reactivity between *Der p* I and the storage mites, *Tyrophagus* sp., *Acarus* sp., or with the tick, *Dermacentor* sp. (Platts-Mills et al., 1986).

Recent studies have also demonstrated cross-reactivity between antigens of the mange mites *Psoroptes cuniculi* (PC) and *Psoroptes ovis* and antigens of DP (Stewart and Fisher, 1986). Rabbits infested with PC exhibited antibodies directed at both DP and PO. Extracts of PC and PO reacted with a sheep anti-DP serum detected at least eight cross-reacting antigens. Since PC is a common parasite of laboratory rabbits, one must be careful that rabbits used to produce anti-DP or DF serum were not previously infested with PC or PO. It has also been shown by CIE and CRIE that *Sarcoptes scabiei* is cross-reactive with both DF and DP (Falk et al., 1981; Arlian et al., 1988, 1990a,b).

ACKNOWLEDGEMENT

I am grateful to DiAnn Vyszenski-Moher for editorial assistance, Chris Rapp for assembling the references, and Nancy Stephens for typing the manuscript.

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