Population Growth and Allergen Content of Cultured *Euroglyphus maynei* House Dust Mites

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Key Words

Euroglyphus maynei · House dust mite · Population growth · Allergen · Endotoxin

Abstract

Background: The house dust mite, *Euroglyphus maynei*, occurs in homes worldwide and is an important source of many allergens. Many patients sensitive to *Dermatophagoides farinae* and *D. pteronyssinus* are also sensitive to *E. maynei*. Extracts to detect sensitivity to *E. maynei* and reagents to detect *E. maynei* allergens in the environment or in cultures are not readily available. Information for the culture of *E. maynei* and for the determination of allergen and endotoxin levels in cultures is limited. **Method:** We mass cultured *E. maynei* at 23 and 30°C and determined the population growth profiles from inoculation until cultures could be harvested for the production of extracts. We also developed an ELISA to measure Eur m 1 and Eur m 2 allergens using mouse monoclonal antibodies directed at cross-reacting epitopes of group 1 and group 2 allergens of *D. farinae* and *D. pteronyssinus*. **Results:** The *E. maynei* populations grew exponentially at both 23 and 30°C; however, the cultures matured more rapidly at 23°C. The Eur m 1 and Eur m 2 allergen concentrations in culture extracts changed independently as the cultures grew and matured. At both temperatures, the Eur m 1 concentrations increased as the cultures matured, while the Eur

m 2 concentrations did not. The endotoxin levels in these cultures were low. **Conclusion:** We report here that *E. maynei* can be cultured at 23 and 30°C. Monoclonal antibodies directed at cross-reacting epitopes on *Dermatophagoides* allergens can be used to measure the associated *E. maynei* allergen levels in these cultures.

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Introduction

The house dust mites, *Euroglyphus maynei*, *Dermatophagoides farinae* and *D. pteronyssinus*, in the family Pyroglyphidae occur in homes worldwide. *E. maynei* frequently co-inhabits with *D. farinae* and *D. pteronyssinus* in some geographical areas, and in some instances *E. maynei* may be the more abundant species [1–12]. Many dust mite allergic patients are skin test positive to all 3 species of these mites [13–16].

E. maynei is the source of many allergens. Some *E. maynei* allergens are species-specific, but some are cross-reactive with allergens of *D. farinae* and *D. pteronyssinus* and of other mite species [14, 17–21]. Few of the many allergens from *E. maynei* have been characterized compared to those from *D. farinae* and *D. pteronyssinus* [7, 22, 23], and no commercial assays for monitoring their levels in mite cultures or extracts are currently commercially available.

D. farinae and *D. pteronyssinus* are widely cultured for research and commercial purposes, but *E. maynei* is not and is generally not commercially available, especially in the US. The population growth and associated allergen and endotoxin profiles of cultured *D. farinae* and *D. pteronyssinus* have been described [24–35]. However, there are little data that characterize the population growth in cultured *E. maynei* mites and the parallel allergen production and endotoxin accumulation in these cultures. Additional allergen and endotoxin data are needed for cultured *E. maynei* in order to develop culture conditions suitable to obtain mite material to make extracts for research, diagnostic and immunotherapeutic purposes.

We report here the development of assays to monitor 2 allergens (Eur m 1 and Eur m 2) in *E. maynei* culture extracts using commercially available reagents developed to detect *Dermatophagoides* group 1 and group 2 allergens. We also studied the population growth characteristics of *E. maynei* cultured at 2 temperatures (23 and 30°C) and used our assays to monitor the associated allergen accumulation in the mite cultures as they matured to the point when mite material for extract preparation could be harvested.

Materials and Methods

Stock Cultures

Laboratory stock cultures that had been maintained for many years were the source of *E. maynei* for these studies. These stock cultures had been grown on media consisting of a mixture (9:1, v/v) of dried raw whole egg powder (Product No. 1190, Bio-Serv Inc., Frenchtown, N.J., USA) and granular baker's yeast (Fleischmann's Active Dry Yeast No. 2192, AB Mauri Foods, Chesterfield, Mo., USA) at 75% relative humidity (RH) and room temperature (20–23°C).

Population Growth at 23 and 30°C (Experiment No. 1)

The methods used for these studies were similar to those previously described for *D. farinae* and *D. pteronyssinus* [24–26]. Briefly, 5 replicate test cultures were started in one-cup glass Ball® jars (Cat. No. 14400–6500, Alltrista Corp., Indianapolis, Ind., USA) by inoculating 30 g of culture medium with 6 g of well-mixed thriving culture for the experiments at 23°C and by adding 3 g of thriving culture to 15 g of diet for the experiments at 30°C. The cultures used for inoculation had previously been grown at room temperature. These inoculated test cultures were thoroughly mixed, and the jars with inoculum and diet were capped with plastic lids (Cat. No. 14400–37000, Jardan Home Brands, Daleville, Ind., USA) and placed in a humidity chamber (33 cup Rubbermaid Servin' Saver No. 8, Rubbermaid Commercial Products, Winchester, Va., USA) maintained at 75% RH using saturated NaCl solution that was held in a temperature-controlled incubator (Model 307C, Fisher Scientific, Pittsburgh, Pa., USA). Both the jar and the chamber lids were ventilated with a 2.5-cm circular hole covered with 35-µm nylon

mesh (Cat. No. B-CMN-35, Small Parts Inc., Miami Lakes, Fla., USA) sealed in place with Shoe Goo (Eclectic Products, Inc., Pin-ville, La., USA). At the time of inoculation ($t = 0$) and every 2 weeks thereafter, the test cultures were mixed, and duplicate weighed samples of the culture material (10–50 mg) were removed from each of the 5 cultures and placed into a gridded petri dish, and the numbers of live mites (all mobile life stages) were counted using a stereomicroscope. All mite counts were normalized to mites/100 mg of culture material.

Population Growth at 30°C after Acclimation at 30°C (Experiment No. 2)

Based on the results of experiment No. 1, we repeated the 30°C culturing experiment using stock cultures that had been acclimated at 30°C for 12 weeks. As before, 5 jars each containing 15 g of food were inoculated with 3 g of thriving culture material from the acclimated stock cultures. At inoculation and at weekly intervals, 1 weighed aliquot of culture material was removed from each of the 5 well-mixed culture jars, and the numbers of live mites were counted and the counts normalized to mites/100 mg culture as before.

Biochemical Analyses (Protein, Endotoxin and Allergen)

At each sampling time during the growth of the cultures in experiment No. 1, aliquots of ~100 mg of whole culture material were removed from each of the well-mixed cultures and frozen in 5-ml microtubes (T2076A, Argos Technologies, Elgin, Ill., USA) for allergen and endotoxin analyses at the end of the experiment. After all samples were collected, aqueous extracts were prepared, and the protein and endotoxin contents were determined as previously described [24–26]. Briefly, aliquots of culture from each sample were extracted overnight at 4°C at 50.0 mg/ml in endotoxin-free water (Cat. No. W50–500, Lonza, Walkersville, Md., USA). The next day, samples were sonicated on ice for 10 min (FS20 Bath Sonicator, Fisher Scientific), and aliquots were transferred to 1.5-ml microfuge tubes (Protein LoBind 022431081, Eppendorf NA, Westbury, N.Y., USA) and then microfuged at 4°C for 10 min at 14,000 g. The supernatants were collected, and a NanoDrop 1000 spectrophotometer (Thermo Scientific, Pittsburgh, Pa., USA) was used to determine the protein concentrations using the 'Abs = 1 mg/ml' settings in the 'Protein A280' module. A QCL-1000 endotoxin test kit (Lonza) was used as prescribed to determine the amount of endotoxin in each sample aliquot.

Enzyme-linked immunosorbent assays (ELISAs) were developed to determine the amounts of group 1 and group 2 allergen in the aqueous culture extracts. Assay plates (Costar 3590, Corning Inc., Corning, N.Y., USA) were coated at 1/1,000 in 50 mM carbonate/bicarbonate buffer, pH 9.6 (Cat. No. C-3041, Sigma-Aldrich Co., St. Louis, Mo., USA) with a mouse monoclonal antibody. Antibody 4C1 (MA-4C1, Indoor Biotechnologies, Charlottesville, Va., USA), that binds to a common epitope on mite group 1 allergens, was used to capture Eur m 1, while an antibody raised against Der p 2 (MA-1D8, Indoor Biotechnologies) was used to capture Eur m 2. The capture antibody was allowed to bind overnight at 4°C, and the plates were washed after this and every step. The wash (PBST) was composed of Dulbecco's phosphate buffered saline (Sigma D5652–50L) containing 0.05% Tween 20 (Fisher BP-337–500). PBST with 1% bovine serum albumin (Fisher BP-1600–100) was used as block and diluent. The samples were serially diluted and incubated on the test plate for 2 h at room temperature. Fol-

Fig. 1. Population growth of *E. maynei* at 2 different growth temperatures. Growth at 30°C was determined twice.

Fig. 2. Protein concentrations in aqueous extracts prepared from *E. maynei* cultures grown at 2 different temperatures.

lowing the washing, the plates were incubated for 1 h with rabbit antiserum to *E. maynei* whole body extract [17] diluted at 1/2,000. Mouse-adsorbed, peroxidase-labeled, goat antirabbit IgG (Cat. No. 4050-05, Southern Biotech, Birmingham, Ala., USA) at 1/5,000 for 1 h was used as detecting antibody. The plates were developed with 100 μ l of 1 mM ABTS (Sigma A1888) in 70 mM citrate phosphate buffer, pH 4.0, with 0.03% hydrogen peroxide (Sigma H1009). Development was stopped at 5:00 min for Eur m 1 and at 7:00 min for Eur m 2 by the addition of 50 μ l of 0.2% sodium azide, and the plates were read immediately using an ELx800 microtiter plate reader (Bio-Tek Instruments, Winooski, Vt., USA). Data are presented as the absorbance at 405 nm of samples diluted at 1/400.

Results

Population Growth

E. maynei populations grew exponentially at both 23 and 30°C until the experiment was stopped or the diet was consumed and became limiting (fig. 1). However, the growth profiles at the 2 temperatures were very different. At 23°C, the population growth began immediately, and the population size had increased about 3 times during the first 2 weeks of population growth, and by 8 weeks it had increased 20-fold compared to the mite density at the time of inoculation ($t = 0$). In contrast, the cultures grown at 30°C exhibited a >90% decline in the numbers of live mites within 2–3 weeks of the inoculation. This was followed by an exponential growth with a lag of 7 weeks.

This was observed both in the cultures inoculated with mites that had been grown at room temperature (30°C, experiment No. 1) and in the cultures inoculated with mites that had been acclimated to 30°C for 12 weeks prior to the inoculation (30°C, experiment No. 2). After 12 weeks of culture at 30°C, the population densities of both sets of cultures also increased 20-fold compared to the starting mite density ($t = 0$). Thus, the peak population densities for all cultures were similar, regardless of the rearing temperature.

Protein and Allergen Concentrations

The amount of water-soluble protein that could be extracted from 50 mg of culture material declined constantly in the cultures held at 23°C, even though thriving mite populations developed (fig. 2). Over this same time, the Eur m 1 concentrations in these extracts steadily increased, while the Eur m 2 concentrations declined slightly (fig. 3).

At 30°C, the protein concentrations in the aqueous extracts initially declined but then returned to the initial levels after 14 weeks when the cultures had reached maturity (fig. 2). The Eur m 1 concentration profile in the 30°C culture extracts paralleled the live mite population growth, increasing over time (fig. 3). The Eur m 2 concentrations in the extracts declined during the first 4 weeks of culturing but then returned to the initial levels when the cultures matured.

Fig. 3. Eur m 1 and Eur m 2 allergen concentrations in aqueous extracts prepared from *E. maynei* cultures grown at 2 different temperatures.

Endotoxin Levels

The endotoxin levels in the aqueous extracts of the cultures grown at both 23 and 30°C were low (<105 EU/ml extract) but did trend upward during the 8 and 14 weeks of mite population growth, even though the cultures were thriving and most media were depleted (fig. 4).

Discussion

Extracts of *E. maynei* for diagnosis and immunotherapy are not commercially available in the US and many parts of the world. Little is known about methods for mass culture and the associated allergen production in *E. maynei* mite cultures. This is in large part due to the fact that no assays are currently available to measure allergen levels in culture extracts. We began our studies by developing ELISAs to monitor the levels of group 1 (Eur m 1) and group 2 (Eur m 2) allergens in aqueous extracts of *E. maynei* cultures.

The *E. maynei* group 1 allergen, Eur m 1, is a 25 kDa protein that shares about 85% sequence homology with Der p 1 and Der f 1, the group 1 allergens of *D. pteronyssinus* and *D. farinae*, respectively [36, 37]. The group 2 allergens of the 3 species are 14 kDa proteins, and Eur m 2 shares about 83% amino acid sequence homology with Der p 2 and Der f 2 [37, 38]. It therefore seemed likely that a monoclonal antibody raised against Der f 1, that recog-

Fig. 4. Endotoxin concentrations in aqueous extracts prepared from *E. maynei* cultures grown at 2 different temperatures.

nizes a common epitope on Der p 1 (Indoor Biotechnologies antibody MA-4C1; [39]) would also bind to Eur m 1. Likewise, an antigroup 2 monoclonal antibody (Indoor Biotechnologies antibody MA-1D8; [40]) would also bind to Eur m 2 from *E. maynei*. Therefore, we used these monoclonal antibodies to capture the *E. maynei* allergens, Eur m 1 and Eur m 2, from aqueous extracts of mite

culture materials. Polyclonal antiserum previously raised against *E. maynei* whole body extract [17], followed by peroxidase-labeled goat antirabbit IgG and development with a chromogenic peroxidase substrate, allowed for the detection and relative quantitation of the 2 individual *E. maynei* allergens.

Population Growth

We observed that establishing thriving laboratory cultures and the population growth of *E. maynei* in these cultures were influenced by temperature, and this needs to be considered when culturing this mite species for research and medical purposes. At 23°C, the mite population immediately expanded exponentially from the time the cultures were inoculated until they reached maturity and were terminated 8 weeks later. Similar growth profiles for *D. farinae* and *D. pteronyssinus* cultures have been reported [24–30].

In contrast, at 30°C, there was a lag in the population growth of *E. maynei* before exponential growth was apparent. It was not clear what caused this lag in the population growth. It could have been due to the fact that the first set of 30°C cultures were initiated with mites from stock cultures that had been maintained at 75% RH and at a room temperature of approximately 21–22°C for years. The switch to 30°C may have required time to select for mites genetically adapted for better survival and reproduction at the higher temperature which occurred over the lag period before the population expanded exponentially. We hypothesized that this lag time might be reduced by inoculating new cultures with mites taken from thriving cultures that had been acclimated at 30°C for several months. This allowed sufficient time for 6 generations of mites to grow under these conditions [36]. Thriving mite material from this acclimated culture was then used to inoculate new cultures that were again held at 30°C (experiment No. 2). The lag in population growth again occurred before exponential growth was apparent. Thus, given the long time required to develop mature cultures of *E. maynei* at 30°C, it appears that at 75% RH, 23°C is the better temperature for the mass culturing of *E. maynei* to produce material for extracts that can be used for research, diagnosis and therapy purposes.

Allergen Levels in *E. maynei* Culture Extracts

Using our newly developed ELISAs, we were then able to monitor the levels of group 1 and group 2 allergen in aqueous extracts of the *E. maynei* cultures. At both culture temperatures, the Eur m 1 levels paralleled the mite population growth profiles. In contrast, the Eur m 2 al-

lergen levels in extracts of the 23°C cultures remained relatively constant over the 8-week culture period, while in extracts of the cultures grown at 30°C the level of this allergen declined before eventually rebounding to $t = 0$ levels. This suggests that allergen degradation may be occurring in the 30°C cultures. This is in agreement with an earlier report that allergen decay was enhanced in a warm, humid environment [41].

Endotoxin Levels

Endotoxin concentrations in the cultures grown at both 23 and 30°C were very low (<105 EU/ml extract) throughout the experiment. This was surprising since *D. farinae* and *D. pteronyssinus* cultures grown on the same diet at room temperature (19.6–21.8°C) had endotoxin concentrations of 526,380 and 8,440 EU/ml extract, respectively, at the peak of culture growth [24].

Conclusions

This study clearly shows that *E. maynei* can be cultured on the same diet and at the same climatic conditions that supports long-term cultures of *D. farinae* and *D. pteronyssinus*. However, thriving cultures of *E. maynei* clearly develop better at 23°C than at 30°C. The population growth, allergen production and endotoxin in the cultures of *E. maynei* presented in this study can now be compared to similar data for the *Dermatophagoides* species [24–30, 42, 43]. The results of our study will be useful to those interested in establishing long-term cultures of *E. maynei* for research and commercial purposes.

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